Diagnosis of Hymenoptera Venom Allergy using component resolved molecular approach and the clinical utility of serologic IgE testing

Laurent SAMSON, Pharm.D
1. Introduction to Hymenoptera venom Allergy

2. Diagnosis
   - Skin tests
   - In Vitro testing
     - Extract Specific IgE (Cross-reactivity and CCD)
     - Recombinant allergens

3. Therapy and Immunotherapy
Agenda

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Introduction

- Hymenoptera venom allergies affect around 1/4 of the population.

- 1 to 5% show systemic and sometimes life threatening symptoms.

- Even if the incidence for fatal events is low (0.09 to 0.48 per 1 million population per year), it remains one of the main causes for fatal allergic reactions and the quality of live of affected individuals is significantly reduced.

- In Europe the main threat is coming from the western honeybee (Apis mellifera) and the common wasp (Vespula Vulgaris).

- The paper wasps (Polistes) are widespread especially in Southern Europe and Mediterranean areas.

- Several major allergens, usually glycoproteins have been identified in venoms of bees, vespids and ants.
Cause of anaphylaxis

In Israel, a study of 92 patients under the age of 18 years admitted to a single pediatric medical center for anaphylactic reaction during 1993–2004 found that food allergy was the cause in 43%, drug allergy in 22%, and hymenoptera sting allergy in 14%

V. Hoffer 2011

An other study which recruited 141 children and adolescents who attended the allergy clinic of the Schneider Children’s medical center of Israel gives the following percentages: food allergy 83%, 11% hymenoptera sting allergy and other 6%

Nirit Segal, IMAJ vol 14 January 2012

<table>
<thead>
<tr>
<th>Causes of anaphylaxis</th>
<th>Food</th>
<th>Insect bite</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of children, n (%)</td>
<td>118</td>
<td>15 (11)</td>
<td>8 (6)</td>
<td>141</td>
</tr>
<tr>
<td>Male/female</td>
<td>79/39</td>
<td>10/5</td>
<td>4/4</td>
<td>03/48</td>
</tr>
<tr>
<td>Allergic trigger, n*</td>
<td>Milk 75 Peanut 34 Nut 19 Sesame 17 Egg 13 Fish 6 Soy 1 Almond 1 Peach 1</td>
<td>Honey bee 7 Yellow jacket 5 Wasp 3 Drugs (penicillin) 1 Pollens 2 Cold 1 Idiopathic 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at presentation (yrs, mean ± SD)</td>
<td>1.13 ± 1.82</td>
<td>10.07 ± 4.17</td>
<td>0.33 ± 6.05</td>
<td>2.55 ± 4.09</td>
</tr>
<tr>
<td>Age at study (yrs, mean ± SD)</td>
<td>2.67 ± 3.19</td>
<td>12.99 ± 4.98</td>
<td>12.01 ± 5.06</td>
<td>7.2 ± 4.39</td>
</tr>
</tbody>
</table>
## Taxonomy of Hymenoptera

<table>
<thead>
<tr>
<th>Family subfamily</th>
<th>Genus</th>
<th>Species</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apidae</strong></td>
<td>Apis</td>
<td>A. Mellifera</td>
<td>Honeybee</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Terrestris</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Medius</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Agrorum</td>
<td></td>
</tr>
<tr>
<td><strong>Bombus</strong></td>
<td></td>
<td></td>
<td>Bumblebee</td>
</tr>
<tr>
<td><strong>Vespidae</strong></td>
<td>Vespula</td>
<td>V. Germanica</td>
<td>Yellow jacket or Wasp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V. Vulgaris</td>
<td></td>
</tr>
<tr>
<td><strong>Vespinae</strong></td>
<td>Dolichovespula</td>
<td>D. Maculata</td>
<td>White faced hornet or Wasp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D. Arenaria</td>
<td></td>
</tr>
<tr>
<td><strong>Vespa</strong></td>
<td></td>
<td>V. Crabo</td>
<td>Hornet</td>
</tr>
<tr>
<td><strong>Polistinae</strong></td>
<td>Polistes</td>
<td>P. Dominulus</td>
<td>Paper wasp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P. Gallicus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P. Nimpha</td>
<td></td>
</tr>
<tr>
<td><strong>Formicidae</strong></td>
<td>solenopsis</td>
<td>S. Invicta</td>
<td>Ant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Images

- *Apis mellifera* (Honeybee)
- *Bombus* (Bumblebee)
- *Vespula Vulgaris* (Yellow jacket or Wasp)
- *Vespa Crabo* (Hornet)
- *Polistes dominulus* (White faced hornet or Wasp)
- *S. invicta* (Yellow hornet or Wasp)
Venom allergens and venom dose per sting

- 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

- venom dose per sting:
  - Varies from species to species and even within the same species
  - Bee stings release an average of 50 µg up to 140 µg of venom protein
  - Bumblebee stings release 10-31 µg of venom
  - Vespinae are capable of repeated stings but generally inject less venom per sting.
  - Vespula (wasp) stings release from 1.7-5 µg and Dolichovespula to 4.2 - 17 µg and Polistes stings from 4.2 to 17 µg

Helbling, Allergo J 2013;22(4) 265-73
Clinical Features in Hymenoptera Stings

sting reaction categories

- **Normal local**
  Swelling < 10 cm in diameter, fades again within several hours. The pruritus at. The sting site can continue several days

- **Severe local**
  Swelling > 10 cm, duration > 24H-1 week, associated with venom-specific IgE

- **Systemic toxic**
  Occur after multiple Hymenoptera stings. Primarily due to the cytotoxic effect of melittin, phospholipases and kinins that lead to hemolysis, kidney and liver damages. (children 10-50 stings adults after 50-100 stings)

- **Systemic anaphylactic reactions**
  The prevalence of an allergy systemic reaction after a Hymenoptera sting is estimated at. Between 1 and 7% in Europe. Risk increases with the frequency of stings.

- **Unusual reactions**
  Those that are not based on an IgE mediated nor on a toxic mechanism such as lymphadenopathy, peripheral neuropathies...

Helbling, Allergo J 2013;22(4) 265-73
Classification of systemic reactions to insect stings
(L.Mueller & J Ring & Messmer)

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

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Generalized urticaria, itching, malaise and anxiety</td>
</tr>
<tr>
<td>II</td>
<td>Any of the above plus two of more of the following: angioedema, chest constriction, nausea, vomiting, diarrhea, abdominal pain, dizziness</td>
</tr>
<tr>
<td>III</td>
<td>Any of the above plus two or more of the following: dyspnea, wheezing, stridor, dysarthria, hoarseness, weakness, confusion, feeling of impending disaster</td>
</tr>
<tr>
<td>IV</td>
<td>Any of the above plus two or more of the following: fall in blood pressure, collapse, loss of consciousness, incontinence, cyanosis</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Generalised skin symptoms (e.g. flush, generalised urticaria, angioedema)</td>
</tr>
<tr>
<td>II</td>
<td>Mild to moderate pulmonary, cardiovascular, and/or gastrointestinal symptoms</td>
</tr>
<tr>
<td>III</td>
<td>Anaphylactic shock, loss of consciousness</td>
</tr>
<tr>
<td>IV</td>
<td>Cardiac arrest, apnoea</td>
</tr>
</tbody>
</table>
Venom allergens

- The venom is a complex mixture of active amines, lipids, amino acids, peptides and proteins.
- Peptides and protein are responsible for the binding of IgE and, therefore, for the allergic reactions.
- The protein composition of hymenoptera venoms is considered elucidated in rough form with the most prominent compounds being identified.
- This relates primarily to the phospholipases as well as the hyaluronidases, found throughout all venoms in significant amounts.
- Further components that have been identified are the antigen 5 in different wasp venoms and an acid phosphatase in honeybee and bumblebee venom.
- In honeybee venom some additional allergens have been described, however, the IgE prevalence for most of them is either initially or uncharacterized.
- Another allergen found in both honeybee and wasp venom is a dipeptidylpeptidase IV like enzyme which was designated Api m 5 and Ves v 3, respectively, and showed a high IgE prevalence.
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3. Therapy and Immunotherapy
Diagnosis of Hymenoptera venom allergy

introduction

Three crucial questions for successful treatment of for prevention of future allergic events:

- First to define the sensitization pattern
- Next the severity of sensitization
- Last the significance of the sensitization

Initially the primary sensitization is assessed by skin prick test or intra-dermal skin testing. Moreover the assessment of the sIgE level is of importance, since it may predict of future allergic reaction.

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.
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Skin Prick test

- **Patient history**
  - Honeybees are nearly always leaving their stinger in the skin of the victim.
  - But frequently patients are not able to discriminate between honeybee and vespid.
  - To be considered: number and date of sting reactions, severity of symptoms, time between sting and the onset of symptoms and potential additional risk factors (medication, cardiovascular risk...)

- **In general primary sensitization assessed by skin prick testing or intra-dermal skin testing**
  - Tiny amounts of allergens are applied to the skin
  - In case of a sensitisation degranulation of effector cells leads to local wheals.
  - Commercial honeybee and vespid venom extract used at least 2 weeks after the sting reaction to avoid false-negative reaction during the refractory period.
Skin Prick test

• The sensitivity of the skin prick test is lower than that of the intra-dermal test (up to 1.0 µg/ml), which has to be used in order to confirm the negative result.

• Standardised hymenoptera venom products, including Yellow Jacket and Polistes wasp venoms, are commercially available in many countries, being mixtures of the clinically relevant species for YJ (Vespula vulgaris, V. flavopilosa, V. germanica, V. maculifrons, V. pensylvania, V. squamosa) as well as American Polistes (Polistes annularis, P. exclamans, P. fuscatus, P. metricus) venom extracts.

• No recombinant venom allergens are commercially available for skin testing.

• There is no correlation between the severity of sting reactions and skin-test reactivity to whole venom extracts
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In vitro testing : Total IgE (tIgE)

• Several studies investigated the specific/total IgE ratio in the context of atopy and of allergen-specific immunotherapy (reviewed in Hamilton et al).
• In 54% of Hymenoptera venom-sensitized individuals, the ratio of sIgE/tIgE was >4%).

• Thus, in the clinical management of bee venom allergy, the measurement of tIgE can provide guidance to the clinician in the context of the ratio sIgE/tIgE, although it is not generally recommended in the guidelines.

*Hamilton, J Allergy Clin Immunol 2010;126:33-38*
Allergen-specific IgE – extracts

Honeybee venom (i1) and vespid venom extract (Yellow Jacket i3, Paper Wasp i4):

• Specific IgE measurements to venom extracts might show multiple positive test results due to sensitization to multiple venoms or to cross-reactivity of cross-reactive carbohydrate determinants (CCD) or homologous allergens present in different venoms.
• Results might be negative due to the underrepresentation of particular allergens in the extract.

Bumblebee venom extract (i205):

Although, allergy to bumblebee venom is rare, sIgE detection to bumblebee venom could be useful in patients heavily exposed to bumblebee stings since bumblebee venom contains proteins not found in honeybee venom. Although major allergens of bumblebee and honeybee venom are partially cross-reactive, additional species-specific epitopes are present due to an incomplete sequence identity.
Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract and caveats

- Even if standardized, **batch to batch variation** in terms of concentration and composition of particular components is the major limitation in allergy diagnosis.

- The result in unit definition does **not** implicate **interchangeable results** between different assays.

- Another concern about allergen extracts arises from homologous proteins resulting in **cross-reactivity of these extracts**:
  - CCD Cross-reactive carbohydrate
  - Homologous peptides sequence:
    - Hyaluronidase
    - Dipeptidyl peptidase IV homologs

**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**

*B.M.Bilo Allergy 2005:60:1339-1349  (*) under development*
Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract and cross reactive carbohydrate determinants

* Placement and types of carbohydrates are purely illustrative and not intended to be accurate

Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract and cross reactive carbohydrate determinants

- In Hymenoptera venom allergy the rate of double-positive patients which have cross-reactive IgE for both honeybee and wasp venom is up to 30-40%.

- 70-80% are exclusively reactive on a carbohydrate level: IgE against cross-reactive carbohydrate determinants (CCDs, alpha1,3-fucosylated N-glycans) with low clinical relevance.

- Only a minority shows a true double-sensitization or cross-reactivity on protein level

Hautarzt. 2008
sIgE to CCD can cause False Positive in vitro results

Cross-reacting Carbohydrate Determinants (CCDs)

MMXF
(mannose, mannose, xylose, fucose)

Mannose

N-Acetyl glucosamine

Fucose

Xylose
CCDs in Insect

Recombinant Allergens without CCDs
(sf9 insect line)

## Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract and cross reactive carbohydrate determinants

<table>
<thead>
<tr>
<th>True sensitization</th>
<th>IMMUNOCAP assay</th>
<th>Immulite 3G</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i1) BV n=7</td>
<td>BV : 3/7</td>
<td>BV : 5/7</td>
</tr>
<tr>
<td></td>
<td>Sensitivity : 43%</td>
<td>Sensitivity : 71%</td>
</tr>
<tr>
<td></td>
<td>Specificity : 100%</td>
<td>Specificity : 100%</td>
</tr>
</tbody>
</table>

(i1 + i3)BV+VV n=3  

<table>
<thead>
<tr>
<th>True double Sensitization</th>
<th>IMMUNOCAP assay</th>
<th>Immulite 3G</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV +VV : 35/3</td>
<td>BV +VV : 27/3</td>
<td></td>
</tr>
<tr>
<td>Sensitivity : 100%</td>
<td>Sensitivity : 100%</td>
<td></td>
</tr>
<tr>
<td>Specificity : 8.5%</td>
<td>Specificity : 11%</td>
<td></td>
</tr>
</tbody>
</table>

(i3) VV n=29  

<table>
<thead>
<tr>
<th>IMMUNOCAP assay</th>
<th>Immulite 3G</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV : 1/29</td>
<td>VV : 7/29</td>
</tr>
<tr>
<td>Sensitivity : 3.4%</td>
<td>Sensitivity : 24%</td>
</tr>
<tr>
<td>Specificity : 100%</td>
<td>Specificity : 100%</td>
</tr>
</tbody>
</table>

Higher sensitivity has been seen for the diagnosis of BV and VV sensitization together with the smaller number of false double positive results with IMMULITE 3G.

- Patients with a well-documented history of systemic allergic reactions to honey bee and/or vespula stings but inconsistent test results in skin tests or in sIgE detection.

- 39 Patients positive again MUFX3 and/or Bromelain and/or Horseraddish peroxidase and/or ascorbat oxidase.

- True sensitization could be confirmed taking in account basophil activation tests, sIgE inhibition tests and patient’s history and component based diagnostics in IgE-mediated hymenoptera sting reaction.

Mark M. Neis cutaneous and Ocular Toxicology, 2011, 1-7
Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract sIgE method comparison

- Serum from 70 patients with a history of systemic reaction (SR) to yellow jacket (YJ) and paper wasp (WA) were tested using CAP and IMMULITE.
- 50 patients from this group had negative results in CAP.

- To assess specificity, 71 participants who had never experienced either a WA or YJ sting were tested using immunoCAP and Immulite.
- 50 patients from this group tested positive using immunoCAP.

Fig. 1. Study design. SR, systemic reactions; CAP, ImmunoCAP.

M. Watanabe, Asia Pac Allergy 2012;2:195-202
Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract sIgE method comparison

**Fig. 2.** Quantitative results for Hymenoptera sIgE according to IMMULITE 3gAllergy (IMMULITE) using its 0.1 IU/mL cutoff among the 50 participants who had a history of Hymenoptera stings but who tested negative according to ImmuNoCAP (CAP) (<0.35 IU/mL). Results demonstrate IMMULITE's sensitivity relative to CAP.
## Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract sIgE method comparison

<table>
<thead>
<tr>
<th>A. IMMULITE</th>
<th>IMMULITE</th>
<th>IMMULITE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Systemic reaction</td>
<td>Yes</td>
<td>30'/27(^\d)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>63'/60(^\d)</td>
<td>78'/81(^\d)</td>
</tr>
</tbody>
</table>

*Using 0.1 IU/mL as the cutoff. "Using 0.35 IU/mL as the cutoff.

<table>
<thead>
<tr>
<th>B. CAP</th>
<th>CAP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic reaction</td>
<td>Yes</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>78</td>
</tr>
</tbody>
</table>

Sensitivity 28.5%
Specificity 39.4%
Agreement 34.0%

<table>
<thead>
<tr>
<th>A. IMMULITE</th>
<th>IMMULITE</th>
<th>IMMULITE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Systemic reaction</td>
<td>Yes</td>
<td>41'/33(^\d)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>77'/69(^\d)</td>
<td>64'/72(^\d)</td>
</tr>
</tbody>
</table>

*Using 0.1 IU/mL as the cutoff. "Using 0.35 IU/mL as the cutoff.

<table>
<thead>
<tr>
<th>B. CAP</th>
<th>CAP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic reaction</td>
<td>Yes</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>78</td>
</tr>
</tbody>
</table>

Sensitivity 28.5%
Specificity 47.9%
Agreement 38.3%

M. Watanabe, Asia Pac Allergy 2012;2:195-202
Diagnosis of Hymenoptera venom allergy in vitro tests: allergen extract sIgE method comparison

Fig. 3. Correlation of quantitative results for participants who were positive for Hymenoptera specific IgE on both immunoassay systems (n = 20). R values were determined by linear regression analysis for results ≥0.35 IU/mL (ImmunoCAP) or 0.1 IU/mL (IMMULITE 3gAllergy).

In conclusion, we found that the overall positive rate for sIgE to both WA and YJ from the participants who had history of anaphylactic reactions and negative CAP tests was 20-42% when evaluated using the IMMULITE quantitative assay using the manufacturer’s reported LoD of 0.1 IU/mL, and was 14-26% when the CAP cutoff of 0.35 IU/mL was used. Additionally, sensitivity, specificity and agreement were higher with the IMMULITE than with the CAP, regardless of the cutoff. Thus, the IMMULITE proved useful in detecting sIgE to Hymenoptera venom and may in fact be better for diagnosing patients with suspected Hymenoptera anaphylaxis.

M. Watanabe, Asia Pac Allergy 2012;2:195-202
Guidelines For Investigation of CCD Interference

Detection of CCD sigE is recommended:

- Symptoms/history do not agree with in-vitro results
- **Honeybee & Wasp**: positive tests for both venoms

Distinguish clinically relevant IgE reactivity from CCD interference

- A positive test for a CCD marker doesn’t rule out allergy
- **Molecular allergy**: component identification
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3. Therapy and Immunotherapy
Extract and specific allergen component

- Antigène 1: Protein cross reactivity
- Antigène 2: degraded
- Antigène 3: CCD Cross-reactivity
- Antigène 4: underrepresented
- Antigène 5: masked

Homologous allergen: Available in large amount
Available stable form
Free CCD reactivity
Accessible epitopes
Molecular allergen: Nomenclature

King TP, Hoffman D, Lowenstein H, Marsh DG, Platts-Mills TA, Thomas W
Allergen nomenclature. WHO/IUIS Allergen Nomenclature Subcommittee.
Int Arch Allergy Immunol. 1994;105:224-233

Apis mellifera

Apis mellifera

Api m 1

(Phospholipase A2)
# Component of the Venom (Apis Mellifera)

## European, western or common honeybee

<table>
<thead>
<tr>
<th>Venom</th>
<th>Enzymatic function/ common name</th>
<th>Allergen</th>
<th>Molecular weight (kDa)</th>
<th>Comment / prevalence among patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apis mellifera</td>
<td>Phosphalipase A2</td>
<td>Api m1</td>
<td>16</td>
<td>Most important, no cross reactivity with wasp PLA2 53% identical (HoneyBee/Bumblebee) / 57-97%</td>
</tr>
<tr>
<td></td>
<td>Hyaluronidase</td>
<td>Api m2</td>
<td>45</td>
<td>Major allergen, 50% sequence identity with vespid venom hyaluronidase (ves v2) / 46-52%</td>
</tr>
<tr>
<td></td>
<td>Acid phosphatase</td>
<td>Api m3</td>
<td>49</td>
<td>Major allergen (honeybee, bumblebee) / 50%</td>
</tr>
<tr>
<td></td>
<td>Melittine</td>
<td>Api m4</td>
<td>2.8</td>
<td>Major component, 50% of dry weight but only few patients have specific IgE / 22.9-42.5%</td>
</tr>
<tr>
<td></td>
<td>dipeptidylpeptidase</td>
<td>Api m5</td>
<td>102</td>
<td>Common with Wasp</td>
</tr>
<tr>
<td></td>
<td>Protease inhibitor</td>
<td>Api m6</td>
<td>8</td>
<td>Bee venom, 1-2%, Minor</td>
</tr>
<tr>
<td></td>
<td>CUB-serine protease</td>
<td>Api m7</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carboxyesterase</td>
<td>Api m8</td>
<td>70</td>
<td>Minor</td>
</tr>
<tr>
<td></td>
<td>Carboxypeptidase</td>
<td>Api m9</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Icarapin/venom protein 2</td>
<td>Api m10</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MRJP8/MRJP9</td>
<td>Api m11</td>
<td>?/?</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vitellogenin</td>
<td>Api m12</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>
# Component of the Venom (Vespula vulgaris)

<table>
<thead>
<tr>
<th>Venom</th>
<th>Enzymatic function/ common name</th>
<th>Allergen</th>
<th>Molecular weight (ksD)</th>
<th>Comment/ prevalence among patient %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vespula vulgaris</td>
<td>Phospholipase A1</td>
<td>Ves v1</td>
<td>35</td>
<td>Major allergen / 33.3-54%</td>
</tr>
<tr>
<td></td>
<td>Hyaluronidase</td>
<td>Ves v2a</td>
<td>38</td>
<td>Major allergen / 5%-25%</td>
</tr>
<tr>
<td></td>
<td>Hyaluronidase (inactive)</td>
<td>Ves v2b</td>
<td>47</td>
<td>Major allergen / 20-25%</td>
</tr>
<tr>
<td></td>
<td>dipeptidylpeptidase</td>
<td>Ves v3</td>
<td>100</td>
<td>Common with Honey Bee / 50-62.8%</td>
</tr>
<tr>
<td></td>
<td>CUB-protease</td>
<td>Ves v4</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Antigen 5</td>
<td></td>
<td>Ves v5</td>
<td>23</td>
<td>In different Wasp venom no cross reactivity with Bee / 84.5-100%</td>
</tr>
<tr>
<td>Vitellogenin</td>
<td></td>
<td>Ves v6</td>
<td>200</td>
<td>39%</td>
</tr>
<tr>
<td>Polistes dominulus</td>
<td>Antigen 5</td>
<td>Pol d5</td>
<td>23</td>
<td>69-72% (native) 44% (recombinant)</td>
</tr>
</tbody>
</table>

*Bilo, Allergy 2005: 60: 1339–1349*<sup>®</sup>

*EAACI Molecular Allergology User’s guide 2016*
Molecular allergen: specific allergen component

Allergenic source:
- Bee/Wasp

Allergenic extract:
- Bee i1, Wasp i3

Cross-reactive allergen components:
- Api m2 / Ves v2

Specific allergen component:
- Bee Api m1
- Wasp Ves V5
Molecular allergen: specific allergen component

- **Api m 1**
  - Phospholipase A2

- **Ves v 1**
  - Phospholipase A1

- **Api m 2**
  - Hyaluronidase

- **Ves v 5**
  - Antigen 5

References:
Using rApi m 1, rApi m 2, rVes v 1, and rVes v 5 Can Differentiate Between Single and Double Positivity
Recombinant Api m1, Api m2, Ves v1 and Ves V5 Allergens for measurement of specific IgE to Hymenoptera Venom

- **Phospholipase A2 (Api m1)** and Hyaluronidase (Api m2) are major allergens from honeybee venom (HBV).

- The recombinant forms of these two allergens rApi m1 and rApi m2 do not contain cross-reactive carbohydrate determinants (CCD).

- rApi m1* and rApi m2* allergenic proteins from HBV were expressed in Sf9 insect cells, purified, biotinylated and used on the IMMULITE 2000 systems to measure HBV-specific IgE.

- Phospholipase A1B (Ves v1) and **antigen 5 (Ves v5)** are major allergenic components of yellow Jacket venom.

- The recombinant forms of these two allergens from Yellow Jacket venom (YJV) were expressed in insect cells, affinity-purified, and biotinylated.
Internal data molecular allergen/Extract

Specific IgE measurements for Honey Bee Extract (HBV), rApi m1, rApi m2, rApi m3, sApi m4, for Yellow Jacket extract (YJE), rVes v1 and rVes v5.

Patient Clinical histories are indicated by dual positively to HBV and YJV (dual), mono-sensitized to HBV (HBV), mono-sensitized to YJV (YJV) and no known of history of insect sting allergy (unknown)

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Yellow Jacket Extract</th>
<th>rVes v1</th>
<th>rVes v5</th>
<th>rApi m2</th>
<th>rApi m1</th>
<th>rApi m3</th>
<th>sApi m4</th>
<th>Honeybee Extract</th>
<th>Clinical History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.30</td>
<td>1.78</td>
<td>1.61</td>
<td>51.10</td>
<td>7.99</td>
<td>9.25</td>
<td>10.10</td>
<td>24.50</td>
<td>dual</td>
</tr>
<tr>
<td>2</td>
<td>0.29</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.91</td>
<td>0.15</td>
<td>&lt;0.10</td>
<td>2.63</td>
<td>HBV</td>
</tr>
<tr>
<td>3</td>
<td>0.52</td>
<td>0.47</td>
<td>0.41</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.10</td>
<td>YJV</td>
</tr>
<tr>
<td>4</td>
<td>0.76</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>7.24</td>
<td>4.25</td>
<td>0.32</td>
<td>&lt;0.10</td>
<td>7.17</td>
<td>HBV</td>
</tr>
<tr>
<td>5</td>
<td>0.45</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.18</td>
<td>0.30</td>
<td>0.25</td>
<td>&lt;0.10</td>
<td>4.27</td>
<td>HBV</td>
</tr>
<tr>
<td>6</td>
<td>1.52</td>
<td>1.79</td>
<td>1.04</td>
<td>6.88</td>
<td>1.49</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>1.43</td>
<td>dual</td>
</tr>
<tr>
<td>7</td>
<td>1.11</td>
<td>1.85</td>
<td>1.39</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>YJV</td>
</tr>
<tr>
<td>8</td>
<td>0.37</td>
<td>0.18</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>unknown</td>
</tr>
<tr>
<td>9</td>
<td>0.22</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.11</td>
<td>&lt;0.10</td>
<td>0.11</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>unknown</td>
</tr>
<tr>
<td>10</td>
<td>5.76</td>
<td>6.94</td>
<td>5.79</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>YJV</td>
</tr>
<tr>
<td>11</td>
<td>1.65</td>
<td>0.37</td>
<td>1.30</td>
<td>0.28</td>
<td>0.34</td>
<td>0.25</td>
<td>&lt;0.10</td>
<td>1.27</td>
<td>dual</td>
</tr>
<tr>
<td>12</td>
<td>0.24</td>
<td>0.11</td>
<td>0.42</td>
<td>79.90</td>
<td>19.58</td>
<td>1.18</td>
<td>0.10</td>
<td>38.80</td>
<td>dual</td>
</tr>
<tr>
<td>13</td>
<td>0.21</td>
<td>0.27</td>
<td>0.36</td>
<td>0.21</td>
<td>0.18</td>
<td>0.25</td>
<td>&lt;0.10</td>
<td>0.28</td>
<td>dual</td>
</tr>
<tr>
<td>14</td>
<td>9.31</td>
<td>6.92</td>
<td>1.60</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>YJV</td>
</tr>
<tr>
<td>15</td>
<td>0.18</td>
<td>0.11</td>
<td>0.15</td>
<td>0.19</td>
<td>&lt;0.10</td>
<td>0.84</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>dual</td>
</tr>
<tr>
<td>16</td>
<td>1.51</td>
<td>0.58</td>
<td>0.46</td>
<td>0.27</td>
<td>0.35</td>
<td>&lt;0.10</td>
<td>0.24</td>
<td>0.82</td>
<td>dual</td>
</tr>
<tr>
<td>17</td>
<td>12.20</td>
<td>10.26</td>
<td>&lt;0.10</td>
<td>2.23</td>
<td>1.04</td>
<td>1.02</td>
<td>&lt;0.10</td>
<td>19.73</td>
<td>dual</td>
</tr>
<tr>
<td>18</td>
<td>9.56</td>
<td>15.36</td>
<td>&lt;0.10</td>
<td>0.11</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>0.72</td>
<td>YJV</td>
</tr>
</tbody>
</table>
Benefits to use the molecular allergens

- **In case of multiple positive test results** with different venoms to discriminate between true sensitization and cross-reactivity.

- **For differential diagnosis in patients with inconclusive patient history to identify the culprit insect(s).**

- **In case of negative test results** with different venoms despite a convincing clinical history due to enhanced sensitivity of the component-resolved diagnostic approach.

  Api m 2 seems to be an important allergen to diagnose honeybee venom allergy in certain patients, it might show cross-reactivity with vespid allergen Ves v 2, and thus is no specific marker allergen.

- **In patients with mastocytosis.**

  Api m 2 seems to be an important allergen to diagnose honeybee venom allergy in certain patients, it might show cross-reactivity with vespid allergen Ves v 2, and thus is no specific marker allergen.

- **CCD markers (MUXF3, horseradish peroxidase, bromelain, ascorbate oxidase):** To confirm the presence of CCD-specific IgE antibodies as reason of multiple positive test results.
Diagnostic algorithm in honeybee and Vespid venom

BAT: Basophil activation test

EACCI Molecular User’s guide
2016
Comparison of rApi m1 and rVes v5
ImmunoCap and IMMULITE

Table 1. Sensitivity of both techniques with rVes v 5 and rApi m 1 in subsamples of patients with different reaction severities (LR = local reaction, SR = systemic reaction, AR = anaphylactic reaction)

<table>
<thead>
<tr>
<th>Reaction severity</th>
<th>Sensitivity (CAP) (%)</th>
<th>Sensitivity (LITE) (%)</th>
<th>Difference in sensitivity, %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee venom-allergic patients (n = 95)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>9/13 (69)</td>
<td>10/13 (76)</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>SR</td>
<td>18/34 (53)</td>
<td>26/34 (76)</td>
<td>23</td>
<td>0.08</td>
</tr>
<tr>
<td>AR</td>
<td>40/48 (83)</td>
<td>48/48 (100)</td>
<td>17</td>
<td>0.01</td>
</tr>
<tr>
<td>Wasp venom-allergic patients (n = 110)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>10/12 (83)</td>
<td>11/12 (92)</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>SR</td>
<td>31/36 (86)</td>
<td>35/36 (97)</td>
<td>11</td>
<td>0.2</td>
</tr>
<tr>
<td>AR</td>
<td>49/62 (79)</td>
<td>56/62 (90)</td>
<td>11</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Table 2. Number of patients positive/negative for rApi m 1/rVes v 5 allergen with each of the techniques

<table>
<thead>
<tr>
<th>LITE positive</th>
<th>LITE negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bee venom-allergic patients (n = 95; [rApi m 1])</td>
<td></td>
</tr>
<tr>
<td>CAP positive</td>
<td>67</td>
</tr>
<tr>
<td>CAP negative</td>
<td>17</td>
</tr>
<tr>
<td>Wasp venom-allergic patients (n = 110; [rVes v 5])</td>
<td></td>
</tr>
<tr>
<td>CAP positive</td>
<td>90</td>
</tr>
<tr>
<td>CAP negative</td>
<td>12</td>
</tr>
</tbody>
</table>

J:Selb, Clinical & Experimental Allergy, 46, 621-630 (2015)
The combination of rApi m 1 and rApi m 2 (LITE) and the combination of rVes v 5 (LITE) and rVes v 1 (CAP) almost matched the sensitivity of native venoms (95% and 97%, respectively), whereas the diagnostic sensitivity of the combination of rVes v 5 and rVes v 1 (CAP) did not reach the sensitivity of rVes v 5 (LITE) alone (90% vs. 93%). IgE levels to venom recombinants and total IgE did not correlate with the severity of sting reaction.

“Conclusions & Clinical Relevance The use of rApi m 1 and rVes v 5 with the IMMULITE system significantly enhanced diagnostic utility of venom recombinants and should improve the dissection of bee and yellow jacket venom allergy.”
Diagnosis of Bee venom Comparison ImmunoCap and IMMULITE

- 40 bee venom allergic patients with clear history of bee anaphylactic sting-reaction and positivity of skin test and/or bee venom IgE extract were included.
- Control group of 25 persons with no history of anaphylactic sting-reaction

<table>
<thead>
<tr>
<th>Test combination</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE rApi m1 (CAP) + rApi m10 (CAP)</td>
<td>79.5</td>
<td>96.5</td>
</tr>
<tr>
<td>IgE rApi m1 (CAP) + rApi m1 (IMMULITE)</td>
<td>84.6</td>
<td>96.9</td>
</tr>
<tr>
<td>IgE rApi m1 (CAP) + rApi m2 (IMMULITE)</td>
<td>92.3</td>
<td>84.6</td>
</tr>
<tr>
<td>IgE rApi m1 (IMMULITE) + rApi m10 (CAP)</td>
<td>89.7</td>
<td>96.9</td>
</tr>
<tr>
<td>IgE rApi m1 + rApi m2 (both IMMULITE)</td>
<td>94.9</td>
<td>83.0</td>
</tr>
<tr>
<td>IgE rApi m1 + rApi m2 (both IMMULITE) + rApi m10 (CAP)</td>
<td>94.9</td>
<td>83.0</td>
</tr>
<tr>
<td>IgE rApi m1 (CAP) + rApi m2 (IMMULITE) + rApi m10 (CAP)</td>
<td>92.3</td>
<td>83.0</td>
</tr>
<tr>
<td>IgE rApi m1 (CAP) + rApi m1 (IMMULITE) + IgE rApi m2 (IMMULITE)</td>
<td>94.9</td>
<td>83.0</td>
</tr>
<tr>
<td>IgE rApi m1 (CAP) + rApi m10 (CAP) + IgE rApi m1 + rApi m2 (both IMMULITE)</td>
<td>94.9</td>
<td>83.0</td>
</tr>
</tbody>
</table>

The IMMULITE IgE rAPI m1 + rAPI m2 represent currently the optimal IgE combination for diagnosing of bee venom allergy.

EACCI Congress 2016, poster M.Vachova
Easy Algorithm for Differentiation
Honey bee and Vespula allergy

Unclear Patient History

Specific IgE extract i1 (Honey bee) + i3 (Vespula) +
Double positivity

Specific IgE molecular allergen rApi m1 & vrVes v5 + rApi m2

rApi m1 - rVes V5 -  
rVes V5 -

rApi m1 + rVes V5 +  
rApi m1 - rVes V5 +

rApi m2 +

Honey bee venom  
Double sensitization Honeybee & Vespula venom  
Vespula venom
Diagnosis of IgE-mediated Allergy
How can molecular allergy help the clinicians?

- Negative sIgE and/or skin test despite convincing history of anaphylaxis/allergy
- Selection of patients for immunotherapy
- Double-positive sIgE and/or skin test
Agenda

1. Introduction to Hymenoptera venom Allergy

2. Diagnosis
   - Skin tests
   - In Vitro testing
     - Extract Specific IgE (Cross-reactivity and CCD)
     - Recombinant allergens

3. Therapy and Immunotherapy
Prevention and Therapy Honey-Bee

**Preventive measures**
A series of recommendations have been formulated

**Aim: substantially minimizing the risk of field honeybee re-sting**
- Avoidance of perfumes
- Avoidance of floral or bright colored clothing
- Careful outdoor eating and drinking
- Wearing shoes outside
- Avoidance of swatting to bees
- Keeping windows of the vehicle closed
- Staying away from beehives

**Pharmacotherapy (emergency kit)**
Due to the risk of severe reactions patients allergic to bee venom should carry an emergency kit including an adrenaline auto-injector for self-administration, especially during the bee season. Although, this is a highly debated issue, according to current guidelines, also patients who have successfully undergone immunotherapy are recommended to carry an emergency kit to eliminate a remaining risk.
In this study, the prescription of adrenaline auto-injector to Japanese outdoor workers who had a positive result of sIgE to either Hymenoptera venom was approximately 6-33%. In addition, the prescription of adrenaline auto-injector in these workers who had experienced systemic reactions to a Hymenoptera sting with a positive result of sIgE to either Hymenoptera venom was approximately 23-57%.

The percentage of outdoor workers who usually carry their auto-injector during work was approximately 52-78%.

Masamitsu Tatewaki, letter to the editor, Allergology International (March 2016) 1-4
Specific Immunotherapy Honey Bee

Honeybee venom immunotherapy is indicated both in children and adults with a history of a severe systemic reaction

- including respiratory and cardiovascular symptoms
- Documented sensitization to honeybee venom with either skin test and/or specific serum IgE tests.

- Immunotherapy is not indicated when neither skin testing nor serum specific IgE indicate a sensitization as well as for large local reactions or unusual reactions.

Venom immunotherapy with honeybee venom also seems to be sufficient in nonprofessionally exposed bumblebee-allergic patients who most likely react on the basis of cross-reactivity and a primary sensitization to honeybee venom.
  - Indeed, in heavily exposed greenhouse workers who are frequently stung by bumblebees an immunotherapy with bumblebee venom would be preferable. However, bumblebee venom for routine therapeutic approaches is commercially not generally available and such approaches have only been reported in case reports.

- The success of specific immunotherapy may be monitored by a sting challenge test with a live insect
Prevention and Therapy Wasp

Preventive measures

A series of recommendations have been also formulated
the aim is also to minimize the risk of field re-sting, but no evidence based studies support this.

Untreated patients with anaphylaxis should not be given β-blockers, except when the administration of
these drugs is urgently required as in the case of certain cardiac arrhythmias. If possible, angiotensin
converting enzyme inhibitors (ACEI) should also be avoided.

Pharmacotherapy (emergency kit)

Hymenoptera venom allergic patients should carry an emergency kit for self-administration at. All times,
especially during the insect season.

Self-injectable adrenaline should be considered for all patients with a history of a Systemic Reaction
Taking in account the risk of future exposure (forestry workers, beekeepers, gardeners, waste management
workers)
Venom specific Therapy Vespid

- The efficacy of subcutaneous venom immunotherapy has been confirmed by both sting challenge and infield sting in prospective controlled and uncontrolled studies, in one meta-analysis and systematic reviews demonstrating that **the protection rate of vespid VIT is greater than that of honeybee VIT**.

- The repeatedly **observed difference in the success rates in bee and vespid venom-allergic patients is not completely clear**.

- The fact that **the amount of venom delivered** by a bee sting during a sting challenge is much larger and more consistent may partially explain this difference in the reaction rate to sting challenges.

- **The composition of bee venom**, which is a mixture of proteins and other pharmacologically active molecules, including melittin, and/or the absence or underrepresentation of major allergens in commercially available venom preparations may be another alternative explanation.

Finally, **venom-allergic patients with mast cell diseases will benefit from VIT**, albeit to a lesser extent than patients without mastocytosis.
Specific Venom IgG?

- The immunological mechanisms underlying SIT still remain incompletely understood.
- There is increasing evidence that clinically effective SIT is associated with an increase in allergen-specific IgG antibodies, particularly the IgG4 subclass.
- Several studies, involving either sublingual immunotherapy (SLIT) with aeroallergens or subcutaneous immunotherapy with aeroallergens and hymenoptera venoms have documented an induction of allergen-specific IgG and IgG4 in sera.
- It is still a matter of debate whether the efficacy of SIT could depend on allergen-specific IgG induction.
- Although IgG antibodies, especially the subclass IgG4, are certainly of importance in allergy and tolerance induction, they are nowadays still not of value for clinical practice.

S. Hofmaier, Eur Ann Allergy Clin Immunol Vol 46, N 1, 6-11, 2014
Conclusions

**Improved sensitivities of rApi m1 and rVes v5** recombinant venom allergens on IMMULITE system should facilitate more accurate serologic dissection of bee and yellow jacket allergy..

(J.Selb 2015)

**Molecular venom allergens are additional tools to help the Clinicians:**
- Negative sIgE and/or skin test despite convincing history of anaphylaxis/allergy
- Selection of patients for immunotherapy
- Double positive
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Siemens Healthcare Diagnostics  
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