

RESEARCH ARTICLE

# Value of component based diagnostics in IgE-mediated hymenoptera sting reactions

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## Abstract

**Background:** Stings by insects can precipitate many signs and symptoms of dermatological and ocular diseases. Of particular importance is the anaphylaxis after Hymenoptera stings. Selection of the appropriate venom for immunotherapy requires a precise diagnosis, which is frequently difficult to confirm since the history presented by the patient is many times not conclusive and diagnostic tests are often positive for bee venom (BV) and vespula venom (VV). This double positivity is either caused by true double sensitization or by antibodies cross-reactive to homologous peptide sequences or to cross-reactive carbohydrate determinants (CCDs). In this study, we analyzed in 39 patients, tested positive for specific immunoglobulin E (sIgE) against BV and VV and CCDs whether the routine detection of sIgE against the recombinant species-specific major allergens (SSMAs) rApi m1 and rVes v5 enables the discrimination between genuine double sensitization and cross reactivity and therefore may be superior to other in vitro assays such as IgE-inhibition test or the basophil activation test.

**Materials and methods:** Thirty-nine patients each with allergic reactions to vespula and/or honey bee stings and tested positive for sIgE antibodies against CCDs were analyzed for sIgE against BV, VV, CCDs (MUF3) and SSMAs by UNICAP (CAP) and to BV, VV, bromelain, horseradish peroxidase and ascorbat oxidase by Immulite 2000 (IMMU). In 12 cases results from a basophil activation test, in nine cases results from IgE-inhibition assays and in 10 cases an unambiguous history of the patient were taken into consideration.

**Results:** A definite diagnosis could be assigned to each patient: sensitization to BV  $n = 7$ , sensitization to VV  $n = 29$  and true double sensitization to both venoms  $n = 3$ . Detection of sIgE against BV and VV by CAP leads in three cases to the diagnosis BV allergy, in 35 cases to the diagnosis double sensitization and in one case to the diagnosis VV allergy. Detection of sIgE against BV and VV by IMMU leads in five cases to the diagnosis BV allergy, in 27 cases to the diagnosis double sensitization and in seven cases to the diagnosis VV allergy. Detection of sIgE against rApi m1 and rVes v5 by CAP leads in six cases to the diagnosis BV allergy, in eight cases to the diagnosis double sensitization, in 21 cases to the diagnosis VV allergy and in four cases to a false double-negative result implicating no allergy.

**Discussion and conclusion:** Detection of sIgE to rApi m1 and rVes v5 by CAP is the most reliable diagnostic procedure to discriminate between true double sensitization and cross reactivity in patients with double-positive IgE results to venom extracts in the presence of sIgE against CCDs. In this study, however, we demonstrate that in nine of 39 patients tested positive for sIgE against CCDs, even the allergen component based diagnostic produces false double-positive and also false double-negative test results. Thus, we conclude that especially in hard to diagnose CCD positive patients beside the detection of sIgE, in vitro assays such as the IgE-inhibition test or the basophil activation test are still of importance. Detection of sIgE against only two SSMAs is not sufficient for a precise diagnosis. We propose inclusion of further SSMAs in diagnostic procedures.

**Keywords:** Hymenoptera venom allergy, venom-specific immunotherapy, recombinant species-specific major allergens, component based diagnostics, cross-reactive carbohydrate determinants

## Introduction

Stings by insects can precipitate many signs and symptoms of dermatological and ocular diseases. The most severe ones are IgE-mediated conjunctivitis, urticaria,

angioedema and even anaphylaxis after Hymenoptera stings (1). A single Hymenoptera sting can induce an allergic reaction and occasionally fatal anaphylaxis. In Europe, the responsible insects are the honey bee (*Apis mellifera*)

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and wasps (*Vespula vulgaris* et *Vespula germanica*). Bumble bees (*Bombus* spp.) are mainly of importance for workers in greenhouses. In the United States stinging Hymenoptera also include the fire ant (*Solenopsis invicta*). Recently, also wasps of the polistes and dolichovespula species may play a role beside *Vespula vulgaris* (2). The prevalence of Hymenoptera sting systemic reactions in the general population has been the subject of various studies which estimate the phenomenon to be between 0.15% and 3.3% (3). A venom-specific immuno therapy (VIT) can prevent allergic reactions and anaphylaxis and is successful in 80–85% of the patients sensitized to BV and in 90–95% of the patients sensitized to VV. For the selection of the right venom for VIT the identification of the stinging insect and a definite diagnosis is essential (2). However, patients often fail to identify the stinging insect and diagnostic procedures frequently produce double-negative (skin testing) or double-positive (sIgE detection) results for BV and VV. Double-positive tests can be caused by true double sensitization or by cross-reactive IgE antibodies which recognize similar epitopes of different venoms, homologous peptide sequences in protein allergens (e.g. hyaluronidases and dipeptidyl peptidase IV homologs) or cross-reactive carbohydrate determinants (CCDs) (4–6). Double positivity to both BV and VV in patients who failed to identify the stinging insect requires additional *in vitro* assays such as the IgE-inhibition test or the basophil activation test to distinguish true double sensitization from cross reactivity (7–9). If a diagnostic procedure existed that detects sIgE without producing multiple double-positive results especially in patients tested positive for antibodies against CCDs, it could save time and reduce the costs for diagnostic procedures. Here, we assessed whether the routine use of recombinant SSMA of BV (rApi m 1) and VV (rVes v 5), in an ImmunoCAP solid-phase assay (Phadia, Uppsala, Sweden), improves the diagnosis of Hymenoptera venom allergy and enables the correct discrimination between true double sensitization and cross reactivity. We compared this method to the conventional detection of sIgE against BV or VV with the two different detection techniques ImmunoCAP (CAP) and Immulite 2000 (IMMU) and focused especially on those hard to diagnose patients tested positive for sIgE against CCDs or CCD rich proteins.

## Patients and methods

### Patients

All patients had 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. Some of the patients failed to identify the stinging insect, others had a clear history for either BV or VV allergy. We focused on the patients that were tested positive for sIgE against MUF3 (CAP) and/or bromelain (IMMU) and/or horseradish peroxidase (IMMU) and/or ascorbat oxidase (IMMU), to assess in

what way diagnostic procedures are hampered by antibodies against CCDs.

### Specific serum IgE antibodies (sIgE)

We detected in all patients sIgE to BV, VV, CCDs and the SSMA rVes v5 and rApi m1 by UNICAP (CAP) (Phadia, Uppsala, Sweden). sIgE to BV, VV and the strongly glycosylated marker allergens bromelain, horseradish peroxidase and ascorbat oxidase were also detected with the Immulite 2000 (IMMU) for comparison of the two methods (Siemens, Munich, Germany). A value of >0.35 kU/l was considered positive.

### Inhibition assay

For the inhibition assays three serum samples of each patient were blended with BV at a ratio of 1:10, 1:100 and 1:1000 and another three samples were blended with VV. In each of these samples sIgE against BV and VV was detected as described in the last preceding paragraph.

### Basophil activation test (BAT)

The BAT (Flow-Cast, Bülmann Laboratories, Switzerland) is based on the flow cytometric detection of activated basophils, which are characterized by an enhanced surface expression of CD 63 upon allergen stimulation. In all patients, BATs were performed within the first 6 months, but not earlier than 6 weeks after the original sting reaction.

The leukocyte fraction of whole blood was isolated by centrifugation within 30 min after sample collection and then resuspended with a) 50 ng of BV and VV in stimulation buffer (ALK-Abelló, Frederiksberg, Denmark), b) the heparin and interleukin 3 containing stimulation buffer (negative control) and c) a monoclonal anti-Fc  $\epsilon$  RI antibody (positive control). The obtained cells were incubated with an antibody reagent containing monoclonal phycoerythrin (PE)-marked anti-CD63 antibodies as well as monoclonal fluoresothiocyanate (FITC)-marked anti-IgE-antibodies. The fraction of activated (CD63 +) basophils within the total number of all (IgE +) basophils was determined with a flow cytometer (FACScan, Beckton Dickinson Immunocytometry System, San Jose, CA). A percentage of >10% CD63 + cells was considered positive.

## Results

A clear diagnosis for all 39 patients could be presented, although, the high grade of interference of the sIgE antibodies against CCDs with diagnostic tests hampered part of the test results. In some cases, therefore, also results from basophil activation tests, sIgE-inhibition tests and the patient's history had to be taken into consideration to confirm the diagnosis.

Table 1 shows the test results and the clinical information collected for all 39 patients. Despite many inconsistent test results a precise diagnosis could finally be assigned to

Table 1. Demographic, serologic and clinical information about patients.

| Patient |   | Specific IgE against CCDs or CCD rich proteins [CAP Class] |      |                  |             |              | Specific IgE against Bee Venom or Component [CAP Class] |         | Specific IgE against Vesputa Venom or Component [CAP Class] |        |         | Additional Information that indicates B/W and confirms diagnosis |                 |         |           |
|---------|---|------------------------------------------------------------|------|------------------|-------------|--------------|---------------------------------------------------------|---------|-------------------------------------------------------------|--------|---------|------------------------------------------------------------------|-----------------|---------|-----------|
|         |   | Bromelain                                                  | HRPO | Ascorbat oxidase | CCD (MUFX3) | BV Immultite | BV CAP                                                  | rApi mI | VV Immultite                                                | VV CAP | rVes v5 | Baso Test CD63+                                                  | RAST Inhibition | History | Diagnosis |
| 1       | m | 39                                                         | 0    | 3                | 2           | 2            | 2                                                       | 0       | 4                                                           | 3      | 3       |                                                                  |                 |         | W         |
| 2       | m | 14                                                         | 0    | 2                | 2           | 4            | 2                                                       | 2       | 0                                                           | 1      | 1       |                                                                  | B               |         | B         |
| 3       | m | 65                                                         | 0    | 2                | 1           | 1            | 2                                                       | 0       | 5                                                           | 4      | 5       |                                                                  |                 |         | W         |
| 4       | m | 58                                                         | 1    | 3                | 2           | 2            | 2                                                       | 0       | 4                                                           | 3      | 3       |                                                                  |                 |         | W         |
| 5       | f | 58                                                         | 4    | 6                | 5           | 4            | 3                                                       | 2       | 6                                                           | 3      | 3       | BW                                                               | BW              | ?       | BW        |
| 6       | m | 24                                                         | 0    | 2                | 1           | 4            | 3                                                       | 2       | 0                                                           | 0      | 0       |                                                                  |                 |         | B         |
| 7       | m | 45                                                         | 2    | 3                | 2           | 2            | 2                                                       | 0       | 5                                                           | 4      | 4       |                                                                  |                 |         | W         |
| 8       | f | 48                                                         | 0    | 0                | 0           | 4            | 4                                                       | 3       | 0                                                           | 0      | 0       |                                                                  |                 |         | B         |
| 9       | m | 17                                                         | 2    | 0                | 2           | 0            | 0                                                       | 0       | 4                                                           | 3      | 3       |                                                                  | W               |         | W         |
| 10      | m | 53                                                         | 2    | 4                | 3           | 3            | 3                                                       | 1       | 4                                                           | 3      | 4       |                                                                  | W               |         | W         |
| 11      | f | 46                                                         | 2    | 3                | 3           | 3            | 3                                                       | 1       | 3                                                           | 4      | 5       | W                                                                | ?               |         | W         |
| 12      | f | 54                                                         | 1    | 2                | 1           | 2            | 2                                                       | 0       | 4                                                           | 3      | 3       |                                                                  |                 |         | W         |
| 13      | m | 65                                                         | 1    | 2                | 2           | 2            | 1                                                       | 1       | 4                                                           | 3      | 2       |                                                                  | W               |         | W         |
| 14      | m | 46                                                         | 3    | 5                | 4           | 3            | 3                                                       | 0       | 6                                                           | 4      | 5       | W                                                                | ?               |         | W         |
| 15      | f | 77                                                         | 2    | 3                | 3           | 3            | 2                                                       | 1       | 3                                                           | 3      | 3       |                                                                  | W               |         | W         |
| 16      | f | 52                                                         | 2    | 4                | 3           | 3            | 3                                                       | 3       | 3                                                           | 3      | 2       | BW                                                               | BW              | ?       | BW        |
| 17      | m | 75                                                         | 2    | 4                | 3           | 3            | 2                                                       | 0       | 3                                                           | 2      | 1       |                                                                  |                 |         | W         |
| 18      | m | 53                                                         | 2    | 3                | 2           | 1            | 1                                                       | 2       | 4                                                           | 3      | 3       |                                                                  |                 |         | W         |
| 19      | f | 21                                                         | 0    | 0                | 0           | 0            | 1                                                       | 0       | 4                                                           | 4      | 3       |                                                                  |                 |         | W         |
| 20      | m | 8                                                          | 0    | 2                | 0           | 2            | 1                                                       | 3       | 0                                                           | 0      | 3       | -                                                                |                 |         | W         |
| 21      | f | 61                                                         | 1    | 3                | 2           | 1            | 2                                                       | 0       | 3                                                           | 3      | 0       |                                                                  |                 |         | B         |
| 22      | f | 65                                                         | 1    | 2                | 2           | 2            | 1                                                       | 0       | 3                                                           | 3      | 3       |                                                                  | W               |         | W         |
| 23      | m | 42                                                         | 0    | 3                | 2           | 6            | 6                                                       | 1       | 0                                                           | 2      | 0       | B                                                                | B               | ?       | B         |
| 24      | m | 17                                                         | 0    | 2                | 1           | 6            | 5                                                       | 5       | 2                                                           | 2      | 2       |                                                                  | BW              |         | BW        |
| 25      | m | 31                                                         | 0    | 1                | 0           | 4            | 4                                                       | 3       | 2                                                           | 2      | 0       | B                                                                | B               |         | B         |
| 26      | f | 64                                                         | 2    | 3                | 3           | 3            | 3                                                       | 0       | 3                                                           | 3      | 4       | W                                                                | W               | ?       | W         |
| 27      | f | 16                                                         | 0    | 2                | 0           | 0            | 2                                                       | 0       | 4                                                           | 4      | 1       |                                                                  |                 |         | W         |
| 28      | m | 60                                                         | 2    | 1                | 1           | 0            | 2                                                       | 0       | 3                                                           | 2      | 2       |                                                                  |                 |         | W         |
| 29      | m | 39                                                         | 4    | 5                | 3           | 5            | 5                                                       | 4       | 2                                                           | 3      | 0       |                                                                  |                 |         | B         |
| 30      | m | 41                                                         | 4    | 5                | 4           | 4            | 4                                                       | 0       | 5                                                           | 4      | 2       | W                                                                | BW              | W       | W         |
| 31      | f | 40                                                         | 2    | 2                | 2           | 0            | 1                                                       | 0       | 4                                                           | 3      | 4       |                                                                  |                 |         | W         |
| 32      | m | 56                                                         | 0    | 3                | 2           | 2            | 2                                                       | 0       | 6                                                           | 4      | 4       |                                                                  |                 |         | W         |
| 33      | f | 60                                                         | 3    | 2                | 3           | 3            | 3                                                       | 0       | 5                                                           | 4      | 4       |                                                                  |                 |         | W         |
| 34      | m | 66                                                         | 0    | 2                | 0           | 0            | 1                                                       | 0       | 3                                                           | 2      | 0       |                                                                  |                 | W       | W         |
| 35      | m | 46                                                         | 3    | 3                | 3           | 3            | 2                                                       | 0       | 3                                                           | 2      | 0       | W                                                                | -               | W       | W         |
| 36      | m | 41                                                         | 1    | 4                | 2           | 2            | 2                                                       | 0       | 5                                                           | 3      | 3       |                                                                  |                 |         | W         |
| 37      | m | 17                                                         | 3    | 3                | 3           | 3            | 2                                                       | 0       | 3                                                           | 4      | 1       | W                                                                | W               |         | W         |
| 38      | m | 59                                                         | 1    | 2                | 2           | 1            | 1                                                       | 0       | 4                                                           | 4      | 5       | W                                                                | W               |         | W         |
| 39      | f | 70                                                         | 0    | 2                | 2           | 0            | 1                                                       | 0       | 3                                                           | 2      | 2       | W                                                                |                 |         | W         |

The test results and the clinical information collected for all 39 patients. A precise diagnosis could be assigned to each patient.

m: male, f: female, B: bee venom sensitization, W: vesputa venom sensitization, HRPO: horseradish peroxidase, CCD: cross-reacting carbohydrate determinant, BV: bee venom, VV: vesputa venom, ?: insect unknown, -: negative for B and W.

each patient. Seven of 39 patients were sensitized to BV. 29 of 34 patients were sensitized to VV. Three of 39 patients presented a true double sensitization to BV and VV.

Figure 1 demonstrates how precise these diagnoses could have been confirmed by performing only one of the three sIgE detection procedures: (1) detection of sIgE against BV and VV by CAP; (2) detection of sIgE against BV and VV by IMMUN and (3) detection of sIgE against rApi m1 and rVes v5 by CAP. It becomes obvious that the detection of sIgE against SSMA is the most accurate procedure to discriminate between true double sensitization and cross reactivity, while the detection of sIgE against BV and VV by CAP produces the most false double-positive results. Detection of sIgE against BV and VV by IMMUN leads to intermediate results.

In Figure 2 specificity and sensitivity values for the three detection procedures are given in regard to their ability to confirm the correct diagnosis.

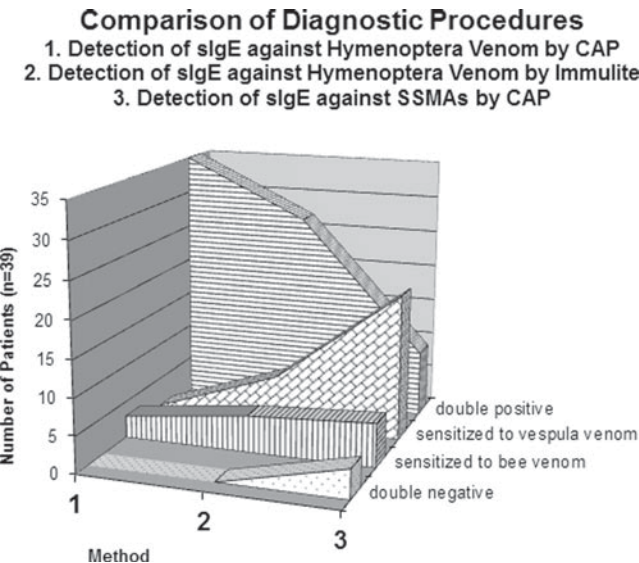


Figure 1. Comparison of diagnostic procedures. (1) Detection of sIgE against Hymenoptera venom by CAP. (2) Detection of sIgE against Hymenoptera venom by Immulite. (3) Detection of sIgE against SSMA by CAP.

| Patients tested positive for sIgE against CCDs (n=39)<br>MUF3 and/or Bromelain and/or Horseradish Peroxidase and/or Ascorbat oxidase |                                                        |                                                       |                                                                 |
|--------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|-------------------------------------------------------|-----------------------------------------------------------------|
| True Sensibilization                                                                                                                 | CAP                                                    | Immune                                                | CAP SSMA                                                        |
| BV n=7                                                                                                                               | BV: 3/7<br>Sensitivity: 43%<br>Specificity: 100%       | BV: 5/7<br>Sensitivity: 71%<br>Specificity: 100%      | rApi m1: 6/7<br>Sensitivity: 85%<br>Specificity: 100%           |
| BV+VV n=3                                                                                                                            | BV +VV: 35/3<br>Sensitivity: 100%<br>Specificity: 8.5% | BV +VV: 27/3<br>Sensitivity: 100%<br>Specificity: 11% | rApi m1 + rVes V5: 8/3<br>Sensitivity: 100%<br>Specificity: 38% |
| VV n=29                                                                                                                              | VV: 1/29:<br>Sensitivity: 3.4%<br>Specificity: 100%    | VV: 7/29:<br>Sensitivity: 24%<br>Specificity: 100%    | rVes v5: 21/29:<br>Sensitivity: 72%<br>Specificity: 100%        |
|                                                                                                                                      |                                                        |                                                       | rApi m1 - rVes V5 -: 4/0<br>Sensitivity: -<br>Specificity: -    |

Figure 2. Sensitivity and specificity of different sIgE detection methods. Specitivity and sensitivity values for the three detection procedures are given in regard to their ability to confirm the correct diagnosis (true sensitization).

Detection of sIgE against BV and VV by CAP leads in 3 cases to the diagnosis BV allergy (sensitivity 0.43 and specificity 1.0), in 35 cases to the diagnosis double sensitization (sensitivity 1.0 and specificity 0.085) and in one case to the diagnosis VV allergy (sensitivity 0.034, specificity 1.0). Detection of sIgE against BV and VV by IMMUN leads in 5 cases to the diagnosis BV allergy (sensitivity 0.71 and specificity 1.0), in 27 cases to the diagnosis double sensitization (sensitivity 1.0 and specificity 0.11) and in 7 cases to the diagnosis VV allergy (sensitivity 0.24, specificity 1.0). Detection of sIgE against rApi m1 and rVes v5 by CAP leads in 6 cases to the diagnosis BV allergy (sensitivity 0.85 and specificity 1.0), in 8 cases to the diagnosis double sensitization (sensitivity 1.0 and specificity 0.38), in 21 cases to the diagnosis VV allergy (sensitivity 0.72, specificity 1.0) and in 4 cases to a false double-negative result implicating no sensitization.

Of special interest are the 9 patients in which the component based diagnostic failed to discriminate between true double sensitization and cross reactivity. Therefore, the results for these patients are summarized in Figure 3. A box indicates false positive results and a circle indicates false negative results that are responsible for misdiagnosis in these 9 cases. The remarkable fact that false negative results only occur in the detection of sIgE against rVes v5 and almost all false positive results are related to the detection of sIgE to rApi m1 is reflected in the specificity and sensitivity values for the detection of the single SSMA as outlined in Figure 4.

Discussion

Double positivity to BV and VV in sIgE detection was first described shortly after the introduction of Phadebas RAST and confirmed in several publications since (6,8,10–13). A prevalence of 20–50% of double positivity for BV and VV was recorded and a significant increase

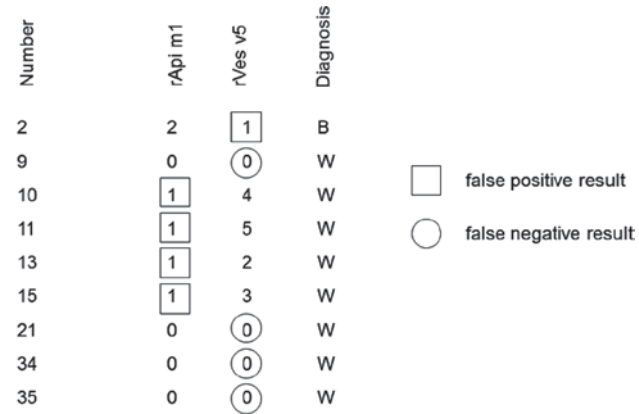
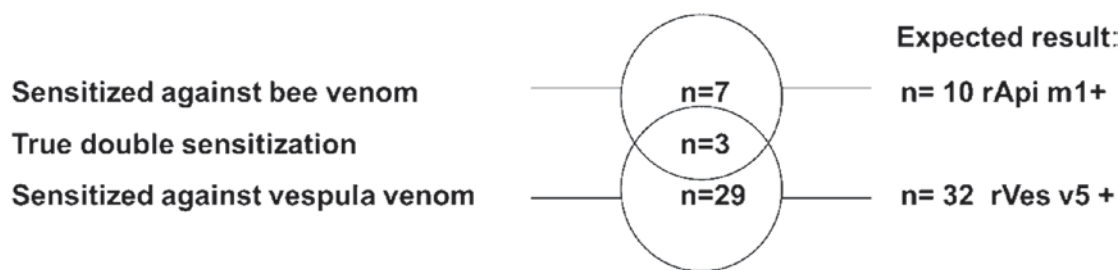


Figure 3. Molecular component based diagnosis failed in 9 CCD positive patients. The results of the 9 patients in which the component based diagnostic failed to discriminate between true double sensitization and cross reactivity. A box indicates false positive results and a circle indicates false negative results.



|                |                    |              |             |
|----------------|--------------------|--------------|-------------|
| <b>rApi m1</b> | <b>Sensitivity</b> | <b>10/10</b> | <b>100%</b> |
|                | <b>Specitivity</b> | <b>10/14</b> | <b>71%</b>  |
| <b>rVes V5</b> | <b>Sensitivity</b> | <b>27/32</b> | <b>84%</b>  |
|                | <b>Specitivity</b> | <b>28/29</b> | <b>97%</b>  |

Figure 4. Specitivity and sensitivity values for the detection of the single SSMAAs.

in double positivity was observed after the introduction of the CAP-platform for the measurement of sIgE. Double positivity may be caused by true double sensitization indicating immunotherapy with both venoms or by cross reactions between the two venoms indicating treatment only with the relevant one. Cross-reactions can be caused by partial sequence homology between peptide allergens (e.g. hyaluronidases and dipeptidyl peptidase IV homologues) in both venoms or by cross-reacting carbohydrate determinants (CCDs) (4,5). They are constituents of several allergens in Hymenoptera venoms and have been shown to be responsible for part of the double positivity (6,8,10–13). The relevant carbohydrate epitope was identified as an  $\alpha$ -1-3 fucosylated N-glycan (14). Definite *in vitro* cross reactions caused by these CCDs were described between insect allergens and pollen of grass, trees, rape and weeds as well as natural rubber latex, bromelain from pine apple, tomatoes and horseradish peroxidase (11,15).

Recently, several publications postulate that the majority of double positivity for BV and VV in Hymenoptera venom allergic patients are caused by cross reactivity based on sIgE to CCDs and that the recombinant allergen based sIgE detection in BV and VV allergic patients can reduce the need for expensive inhibition tests or basophil activation tests and thus also lower the costs of analysis of double positivity with regard to the choice of venoms for immunotherapy (9,11,16).

This novel method of detecting sIgE only against single constituents of venom allergens is rendered possible since different components of Hymenoptera venoms can be produced and modified by molecular biologic techniques. The products are recombinant allergens that for example lack the carbohydrate epitopes that are responsible for part of the cross reactivity. Beside the missing susceptibility to cross-reacting antibodies another advantage compared to the whole venom is the species specificity of some recombinant allergens. The

VV components Ves v1 and Ves v5 and the BV components Api m 1, Api m 3, Api m 4 and Api m 10 are regarded as species specific. In the nomenclature the r stands for recombinant in comparison to the natural (n) protein and is followed by three letters denominating the genus and one letter denominating the species the protein is isolated from. A following Arabic numeral indicates the order of first description (9,16–18).

A diagnostic procedure that detects sIgE without producing multiple double-positive results especially in patients tested positive for antibodies against CCDs, would also be of special value since alternative test methods like the basophil activation test can also be hampered by antibodies against CCDs (19).

In comparison to the other studies that analyzed larger groups of not especially selected BV and/or VV allergic patients we picked only the hard to diagnose patients tested positive for antibodies against CCDs. Our intention is the evaluation of the influence of these particular antibodies on diagnostic procedures.

The detection of sIgE to BV and VV with IMMU and CAP is compared to the recombinant allergen based diagnostic, that is the detection of the SSMAAs rApi m 1 and rVes v 5 by CAP.

Unsurprising the detection of sIgE against Venom by CAP produces the most false double-positive results. In CAP, the allergen is bound to the immunosorbent which is incubated in the patient's serum. All venom-specific antibodies, beside IgE also antibodies of other immunoglobulin classes, are bound and finally labeled with an anti-IgE antibody. To minimize competition between IgE and non-IgE specific antibodies, a very high amount of allergen is bound to the immunosorbent. In IMMU, the anti-IgE is bound to paramagnetic particles and then incubated with the patient's serum. Here only IgE is bound and then labeled allergen is added. The advantage of this approach is 1) the lack of interference from non-IgE antibodies and 2) the smaller amount of required allergen giving low-affinity cross-reacting sIgE like those

to CCDs a smaller chance to bind in comparison to the sIgE against venom (11,20).

Our results (Figures 1 and 2), the higher sensitivity for the diagnosis BV or VV sensitization together with the smaller number of false double-positive results in IMMU support this hypothesis.

As anticipated, the recombinant allergen based diagnostic produces the least false double-positive results due to its species specificity. It was rather unexpected that it produced double-positive results at all. Figure 3 shows that no false double-positive result measured higher than CAP class 1 and that 4 of 5 double-positive results were related to rApi m1. False negative results, responsible for double negativity, in contrast, only appeared in the detection of sIgE against rVesV 5. Double negativity can easily be explained by the fact that the patients are sensitized to one or more of the other allergens contained in VV but not to Ves v5. The percentage of patients sensitized to VV and presenting sIgE against VV but not against rVes v5 amounts to 4% in a study by Müller et al. (11) Our data would even suggest a higher rate. In contrast to Ves v5, natural Api m1 is glycosylated, but not the recombinant preparation. Therefore, cross-reacting antibodies against CCDs shouldn't be responsible for these cases of double positivity. Partial sequence homology between peptide allergens in both venoms shouldn't play a role here, either, since phospholipase A2 from BV and phospholipase A1 from VV are structurally not related (21). It remains open if there is a weak true sensitization without clinical consequence or an additional mechanism for false double-positive results. Thus, the presence of sIgE to both Ves v5 and Api m1 doesn't prove in every case true double sensitization that requires immunotherapy with both venoms. Notably a CAP class 1 result for Api m1 should be questioned and further diagnostic procedures like *in vitro* assays such as the IgE-inhibition test or the basophil activation test should be performed to prevent unnecessary immunotherapies with both venoms in patients sensitized only to one venom.

Especially in patients tested positive for sIgE against CCDs, the implementation of these additional *in vitro* assays is recommended at least as long as rVes v5 and rApi m1 are the only SSMAs available for routine use. After the introduction of further species-specific recombinant allergens into routine diagnostic of BV and VV sensitization (e.g. Api m 2, Api m 3, Api m 4, Api m 10, or Ves v 1), the assumed benefits of the recombinant allergen based diagnostic in comparison to other diagnostic procedures will have to be re-evaluated.

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## Declaration of interest

The authors report no declarations of interest.

## References

1. Pinghong Lai, JYHCHX. Prognosis of corneal wasp sting: case report and review of the literature. *Cutaneous and Ocular Toxicology* 2011;1-3. Early online: DOI: 10.3109/15569527.2011.579930.
2. Biló BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN; EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005;60:1339-1349.
3. Antonicelli L, Bilò MB, Bonifazi F. Epidemiology of Hymenoptera allergy. *Curr Opin Allergy Clin Immunol* 2002;2: 341-346.
4. Blank S, Seismann H, Bockisch B, Braren I, Cifuentes L, McIntyre M et al. Identification, recombinant expression, and characterization of the 100 kDa high molecular weight Hymenoptera venom allergens Api m 5 and Ves v 3. *J Immunol* 2010;184:5403-5413.
5. Jin C, Focke M, Léonard R, Jarisch R, Altmann F, Hemmer W. Reassessing the role of hyaluronidase in yellow jacket venom allergy. *J Allergy Clin Immunol* 2010;125:184-90.e1.
6. Hemmer W, Focke M, Kolarich D, Wilson IB, Altmann F, Wöhrle S et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J Allergy Clin Immunol* 2001;108:1045-1052.
7. Bonifazi F, Jutel M, Biló BM, Birnbaum J, Muller U; EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. *Allergy* 2005;60:1459-1470.
8. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hübsch-Müller C, Enk A. *In vitro* hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy* 2006;61:1220-1229.
9. Hofmann SC, Pfender N, Weckesser S, Huss-Marp J, Jakob T. Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 2011;127:265-267.
10. Hemmer W, Focke M, Kolarich D, Dalik I, Götz M, Jarisch R. Identification by immunoblot of venom glycoproteins displaying immunoglobulin E-binding N-glycans as cross-reactive allergens in honeybee and yellow jacket venom. *Clin Exp Allergy* 2004;34:460-469.
11. Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy* 2009;64:543-548.
12. Straumann F, Bucher C, Wüthrich B. Double sensitization to honeybee and wasp venom: immunotherapy with one or with both venoms? Value of FEIA inhibition for the identification of the cross-reacting ige antibodies in double-sensitized patients to honeybee and wasp venom. *Int Arch Allergy Immunol* 2000;123:268-274.
13. Egner W, Ward C, Brown DL, Ewan PW. The frequency and clinical significance of specific IgE to both wasp (*Vespula*) and honey-bee (*Apis*) venoms in the same patient. *Clin Exp Allergy* 1998;28:26-34.
14. Tretter V, Altmann F, Kubelka V, März L, Becker WM. Fucose alpha 1,3-linked to the core region of glycoprotein N-glycans creates an important epitope for IgE from honeybee venom allergic individuals. *Int Arch Allergy Immunol* 1993;102:259-266.
15. Foetisch K, Westphal S, Lauer I, Retzek M, Altmann F, Kolarich D et al. Biological activity of IgE specific for cross-reactive carbohydrate determinants. *J Allergy Clin Immunol* 2003;111:889-896.
16. Mittermann I, Zidarn M, Silar M, Markovic-Housley Z, Aberer W, Korosec P et al. Recombinant allergen-based IgE testing to distinguish bee and wasp allergy. *J Allergy Clin Immunol* 2010;125:1300-1307.e3.
17. Habermann E. Bee and wasp venoms. *Science* 1972;177:314-322.

18. Seismann H, Blank S, Cifuentes L, Braren I, Bredehorst R, Grunwald T et al. Recombinant phospholipase A1 (Ves v 1) from yellow jacket venom for improved diagnosis of hymenoptera venom hypersensitivity. *Clin Mol Allergy* 2010;8:7.
19. Mertens M, Amler S, Moerschbacher BM, Brehler R. Cross-reactive carbohydrate determinants strongly affect the results of the basophil activation test in hymenoptera-venom allergy. *Clin Exp Allergy* 2010;40:1333-1345.
20. Fromberg J. IgE as a marker in allergy and the role of IgE affinity. *Allergy* 2006;61:1234.
21. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. *Int Arch Allergy Immunol* 2000;123:99-106.