

## Component-resolved diagnosis in selecting patients for yellowjacket venom immunotherapy



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### ABSTRACT

**Background:** Venom immunotherapy is effective in preventing systemic allergic reactions (SARs), but the diagnosis of venom allergy is problematic.

**Objective:** To compare the performance of component-resolved diagnosis and conventional tests in patients referred for venom immunotherapy.

**Methods:** We measured serum-specific immunoglobulin E to yellowjacket and honeybee venoms (Ves v 1 and Ves v 5 and Api m 1), cross-reactive carbohydrate determinants, serum basal tryptase (ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden), and skin prick test reactions in 84 patients referred to receive venom immunotherapy. History of SAR and its severity were evaluated.

**Results:** Of the 78 patients with suspected yellowjacket venom (YJV) allergy, a history of SAR was confirmed in 47 (60%) and 31 (40%) had a non-SAR reaction. The most accurate tests to confirm venom allergy after a SAR were serum-specific immunoglobulin E to yellowjacket whole-venom extract spiked with Ves v 5 (area under the curve 0.87, 95% confidence interval 0.77–0.97,  $P < .001$ ) and Ves v 5 (area under the curve 0.86, 95% confidence interval 0.76–0.96,  $P < .001$ ). Sensitization to Ves v 1 was infrequent and its area under the curve was low (0.62, 95% confidence interval 0.47–0.76,  $P = .106$ ). Sensitivity of the YJV skin prick test was 86%, but its specificity was low at 54%. Double sensitization to yellowjacket and honeybee occurred frequently in skin prick tests. Of the patients without a SAR, 26% showed a positive reaction to YJV in any serum test and 46% showed a positive reaction in skin tests.

**Conclusion:** Specific immunoglobulin E to the YJV spiked with Ves v 5 confirmed the allergy after a SAR. A history of SAR should be confirmed before testing, because venom sensitization is frequent in other types of reactions.

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### Introduction

Hymenoptera venom allergy is a potentially life-threatening condition after an insect sting. Hymenoptera stings cause anaphylaxis more frequently in adults (3%) than in children (0.34%) and account for one fourth of fatalities caused by anaphylaxis.<sup>1</sup> Systemic mastocytosis increases the risk for severe anaphylaxis to Hymenoptera stings.<sup>2–4</sup> The risk of a systemic allergic reaction (SAR) to Hymenoptera re-stings is 25% to 75% in adults with a previous SAR. The

risk depends on the severity of previous reactions and other known high-risk factors such as age and medication.<sup>5</sup> Children have lower risk of a SAR at re-stings and their SARs are mild in 60%.<sup>6,7</sup>

Venom immunotherapy (VIT) with extracted Hymenoptera venoms is effective in preventing a SAR to insect stings: fewer than 3% of patients treated with vespid VIT have a subsequent SAR. VIT can prevent fatal reactions, and it effectively improves quality of life.<sup>8</sup> The prerequisite for efficacious VIT is a history of a SAR to an insect sting and immunoglobulin E (IgE) sensitization to the culprit venom.

The assessment of IgE sensitization can be problematic because honeybee (*Apis mellifera*; Api m) and yellowjacket (*Vespa vulgaris*; Ves v) venom extracts are complex mixtures of proteins including venom-specific and cross-reactive components. The major allergens are hyaluronidases (Api m 2, Ves v 2), phospholipase A2 (Api m 1), phospholipase A1 (Ves v 1), acidic phosphatase (Api m 3), icarapin (Api m 10), and antigen 5 (Ves v 5).<sup>6,9</sup>

Double sensitization to yellowjacket and honeybee venoms as determined by skin prick tests and serum-specific IgE tests is common; up to 50% of patients show a positive reaction to the 2

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venoms. The major cross-reactive components in honeybee and yellowjacket venoms are hyaluronidases with 50% sequence identity. Furthermore, cross-reactive carbohydrate determinants (CCDs) cause double-positive test results.<sup>6</sup> In addition to clinically insignificant positive test results, venom extracts might lack important low-abundance allergens, resulting in false-negative test results.

The distinction between cross-reactivity and true sensitization is important for the choice of VIT, because treating patients who are not sensitized to the allergens in the VIT extract can cause de novo sensitizations, missing protection, and unnecessary costs.<sup>9,10</sup>

The current diagnostic framework in Hymenoptera venom allergy includes skin prick tests, stepwise intradermal testing, and serum-specific IgE to whole-venom extracts and to venom-specific allergen components.<sup>11</sup> The conventional yellowjacket venom (YJV) extract in ImmunoCAP alone has shown lower sensitivity than the combination of Ves v 5 and Ves v 1. Therefore, it is spiked (sYJV) with Ves v 5. When using the sYJV instead of the conventional YJV, the sensitivity increased from 83% to nearly 97%.<sup>12</sup> In the US guidelines, venom testing usually requires intradermal testing for optimal sensitivity, and prick tests are optional.<sup>5</sup> In addition, basophil activation tests and in-house immunoblot tests have been used. Component-resolved diagnostics has improved for the diagnosis of Hymenoptera venom allergy. Measuring specific IgE to allergen components instead of venom extracts helps to distinguish true sensitization from cross-reactivity. We studied the performance of diagnostic tests, including skin prick tests, serum-specific IgE, in-house immunoblot, and serum baseline tryptase, in patients with a strong suspicion of venom allergy referred for VIT.

## Methods

### Study Population and Classification of Allergic Symptoms

The study population of this case-control study consisted of children and adults referred for VIT for suspected Hymenoptera venom allergy. The cases had a SAR to a Hymenoptera sting and the controls had a large local reaction or other type of reaction. The study took place at a single center, a tertiary care hospital. We recruited 30 patients retrospectively from March 2010 through December 2013 and 54 prospectively in 2014. An allergist examined the patients and recorded the history of hymenoptera stings: date, symptoms, signs, emergency visits, and medication. The stinging insect was identified by the patient or by the caregiver. We defined a SAR as a sudden-onset reaction with rapid progression of signs and symptoms involving at least 2 organs including dermatologic (generalized urticaria or erythema, angioedema, or generalized pruritus and skin rash), cardiovascular (hypotension or clinical diagnosis of uncompensated shock), and respiratory (wheeze, stridor, upper airway swelling, or respiratory distress) symptoms. Generalized urticaria without any other symptoms was defined as a SAR in adults. Minor criteria included milder dermatologic, cardiovascular, respiratory, and gastrointestinal signs and symptoms. The definition was based on the Brighton Collaboration Anaphylaxis Working Group suggestion described in detail by Ruggeberg et al.<sup>13</sup> Severity of the reaction was graded as suggested by Mueller,<sup>14</sup> referenced in Bilo et al:<sup>6</sup> grade I was generalized urticaria, itching, malaise, and anxiety; grade II was any grade I reaction plus at least 2 of the following: angioedema, chest constriction, nausea, vomiting, diarrhea, abdominal pain, and dizziness; grade III was any grade II reaction plus at least 2 of the following: dyspnea, wheezing, stridor, dysarthria, hoarseness, weakness, confusion, and feeling of impending disaster; and grade IV was any grade III reaction plus at least 2 of the following: decrease in blood pressure, collapse, loss of consciousness, incontinence, and cyanosis. A large local reaction was defined as a swelling exceeding 10 cm lasting at least 24 hours.

### Allergy Tests

Skin prick tests were carried out on the patient's inner forearm with a disposable single-use lancet. The yellowjacket and honeybee venoms were tested at 100 and 300 µg/mL, respectively (ALK-Abello, Hørsholm, Denmark). Histamine chloride (10 mg/mL) was the positive control, and the solvent was the negative control. A reaction wheal of at least 3 mm was considered positive. We measured serum basal tryptase and specific IgE to sYJV (i3), honeybee venom (i1), CCD (o214), Ves v 1 (i211), Ves v 5 (i209), and Api m 1 (i208; ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden). We defined sensitization conventionally as a specific IgE level of at least 0.35 kU/L. Since 2012, the conventional YJV ImmunoCAP has been spiked with Ves v 5.<sup>12</sup> All samples taken before September 2012 were re-analyzed using the sYJV ImmunoCAP. In addition, an in-house Immunospot method<sup>15</sup> was used to evaluate serum IgE antibodies to YJV and honeybee venom. For the test, 300 µg/mL of *Vespula* species (ALK-Abelló), 20,000 µg/mL of *Vespula* species European mix (Allergon, Ängelholm, Sweden), and 300 µg/mL of *A mellifera* (ALK-Abelló) venoms were used. A nonatopic serum served as a control.

### Statistical Methods and Ethics

The main outcome measure was the accuracy of any diagnostic test in confirming venom allergy in a patient with SAR. We applied receiver-operating characteristics and area under the curve (AUC) to evaluate the performance of the tests in SAR and non-SAR categories. We calculated sensitivity, specificity, and likelihood ratios with 95% confidence interval (CI). We calculated positive and negative predictive values for specific IgE concentrations. Spearman rank correlation served to correlate severity with the specific IgE concentrations. Mann-Whitney *U* test was used to compare non-normally distributed data, and Pearson  $\chi^2$  or Fisher exact test was used for categorical data. SPSS 21 (SPSS, Inc, Chicago, Illinois) was used for the analyses. The local ethics committee approved the study protocol, according to which the patient (or 1 parent of the child) signed a written informed consent. The study followed the principles of the Declaration of Helsinki.

## Results

Of the 84 patients, 78 were referred for VIT after a yellowjacket sting, 5 after a honeybee sting, and 1 after a sting by an unknown insect. The median age was 44 years (range 1–75); 24 (28%) were children or adolescents. All children with urticaria (13 of the 23) had other symptoms (dyspnea, vomiting, or angioedema). The performance of the allergy tests was evaluated for yellowjacket allergy in the 78 individuals. Of these patients, 47 (60%) had experienced a SAR and 31 (40%) had a non-SAR. The study included only 4 patients with a SAR triggered by honeybee venom. The demographic data and test results of the 78 subjects with a yellowjacket sting are presented in Table 1.

Patients with a SAR to yellowjacket sting were more frequently sensitized to sYJV ( $P < .001$ ) and Ves v 5 ( $P < .001$ ), and they had higher specific IgE to sYJV ( $P < .001$ ) and Ves v 5 ( $P < .001$ ) than those with a non-SAR. The best accuracy was obtained using specific IgE to sYJV (AUC 0.87, 95% CI 0.77–0.97,  $P < .001$ ) and to Ves v 5 (AUC 0.86, 95% CI 0.76–0.96,  $P < .001$ ). The AUC of specific IgE to Ves v 1 was lower (0.62, 95% CI 0.47–0.76,  $P = .106$ ; Fig 1).

Specific IgE to sYJV had the best likelihood ratio (3.2), with 89% sensitivity and 74% specificity. Sensitivity of the skin prick test was 86%, but its specificity was lower at 54%. Accordingly, of the patients with a non-SAR to yellowjacket, 46% were sensitized to skin prick tests and 26% were sensitized to the serum sYJV test. Sensitivity of the immunoblot was the lowest at 62%. The performances

**Table 1**  
Baseline Characteristics and Results of Diagnostic Tests of the 78 Patients With Suspected Yellowjacket Venom Allergy

	Non-SAR (n = 31)	SAR (n = 47)	P value <sup>a</sup>
Age (y), mean (SD)	39 (5–72)	47 (1–75)	.702
Men, n (%)	7 (22)	22 (47)	.034
Time from sting to blood sampling (mo)	18 (0–175)	3 (0–387)	.007
Asthma, n (%)	12 (41)	11 (23)	.098
Other allergy, n (%)	19/31 (61)	7 (15)	.000
Objective symptoms, n (%)			
Large local reaction	23 (74)	21 (45)	.010
Hypotension	0	13 (28)	.001
Loss of consciousness	0	10 (23)	.011
Vomiting or diarrhea	0	11/44 (25)	.005
Airway obstruction	0	7/44 (16)	.043
Angioedema	0	18 (38)	.000
Urticaria	0	28 (60)	.000
Anaphylactic shock	0	9 (19)	.010
Subjective symptoms, n (%)			
Tightness of chest	14/25 (56)	18/44 (41)	.227
Dizziness	9/25 (36)	19/44 (43)	.617
Treatment of latest reaction, n (%)			
Received glucocorticoids	11 (28)	28 (72)	.037
Received epinephrine	4 (13)	24 (51)	.001
Received rescue fluid therapy	0	12 (86)	.002
Emergency department visit	14/30 (48%)	37 (79)	.006
Serum sIgE			
sYJV (kU/L), median (range)	0.08 (0.01–100)	2.76 (0.01–100)	.000
Honeybee venom (kU/L), median (range)	0.04 (0.01–3.88)	0.05 (0.03–36.9)	.031
Ves v 1 (kU/L), median (range)	0.01 (0–100)	0.02 (0.01–19.7)	.108
Ves v 5 (kU/L), median (range)	0.07 (0–96.7)	3.48 (0.01–100)	.000
Api m 1 (kU/L), median (range)	0.01 (0–0.11)	0.01 (0–0.48)	.850
CCD (kU/L), median (range)	0.01 (0–2.49)	0.01 (0–3.09)	.92
sYJV > 0.35 kU/L, n (%)	8 (26)	41/46 (89)	.000
Honeybee venom > 0.35 kU/L, n (%)	2/29 (7)	9/46 (20)	.186
Ves v 1 > 0.35 kU/L, n (%)	2 (7)	5/45 (11)	.694
Ves v 5 > 0.35 kU/L, n (%)	10 (32)	39/46 (85)	.000
Api m 1 > 0.35 kU/L, n (%)	0	1/46 (2)	1.00
Double sensitization to sYJV and honeybee whole venom extracts, n (%)	2/28 (7)	9/46 (20)	.190
CCD > 0.35 kU/L, n (%)	2/23 (9)	4/40 (10)	1.00
Tryptase (μg/L), median (range)	4.63 (1.10–257)	3.9 (1.40–75.8)	.191
Tryptase > 13 μg/L, n (%)	3/30 (10)	5/46 (11)	1.00
Skin prick test (titrated) reaction wheal ≥ 3 mm, n (%)			
YJV	11/24 (46)	30/35 (86)	.002
Honeybee venom	12/24 (50)	17/34 (50)	1.00
Double sensitization	7/24 (29)	16/34 (47)	.188
Positive immunoblot test reaction, n (%)			
YJV	8/30 (27)	28/45 (62)	.004
Honeybee venom	0	4/44 (9)	.147
Started venom immunotherapy	5 (18)	23 (55)	.012

Abbreviations: CCD, cross-reactive carbohydrate determinant; SAR, systemic allergic reaction; sIgE, specific immunoglobulin E; sYJV, spiked yellowjacket venom; YJV, yellowjacket venom.

<sup>a</sup>P values were determined using Pearson  $\chi^2$ , Fisher exact, or Mann-Whitney *U* test.

of the diagnostic tests are presented in Table 2. Fig 2 shows the sensitization patterns of the patients with a SAR to yellowjacket sting.

Of the 15 serum samples that were negative to the conventional YJV-specific IgE, 7 (47%) were positive in the sYJV test spiked with Ves v 5.

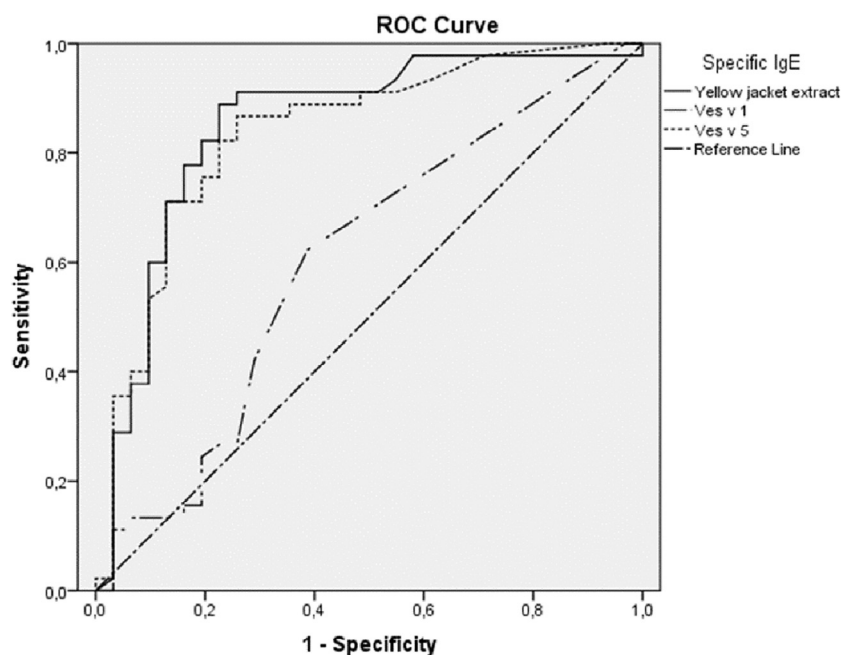
Double sensitization to yellowjacket and honeybee whole-venom extracts was more common to skin prick tests than to serum tests. Of all patients with suspected YJV or honeybee venom allergy, 24 of 61 (39%) were doubly sensitized to titrated skin prick tests, and 13 of 82 (16%) were doubly sensitized to serum tests. Sensitization to the CCD did not explain double sensitization in serum tests. Of the double-positive serum tests, 10 (77%) were positive only to Ves v 5, 3 (23%) were positive to Ves v 5 and Api m 1, and 8 (62%) were positive to CCD.

Specific IgE to sYJV correlated strongly to Ves v 5 ( $r = 0.98$ ,  $P < .001$ ), whereas the correlation to Ves v 1 was weaker ( $r = 0.60$ ,  $P < .001$ ). The correlation between Ves v 1 and Ves v 5 was 0.56

( $P < .001$ ). The YJV immunoblot correlated weakly to sYJV ( $r = 0.38$ ,  $P < .001$ ) and to Ves v 5 ( $r = 0.37$ ,  $P < .001$ ) but did not correlate to Ves v 1. Specific IgE to honeybee venom correlated strongly with Api m 1 ( $r = 0.98$ ,  $P < .001$ ). Honeybee venom immunoblot showed a moderate correlation to honeybee venom IgE ( $r = 0.58$ ,  $P < .001$ ) and to Api m 1 ( $r = 0.56$ ,  $P < .001$ ). None of the tests correlated with serum basal tryptase.

Severity of the reaction (grades I–IV) showed a moderate to weak correlation to sYJV ( $r = 0.40$ ,  $P < .001$ ) and to Ves v 5 ( $r = 0.38$ ,  $P < .001$ ). The severity did not correlate to Ves v 1 or the size of the skin prick test reaction wheal.

Serum basal tryptase level was increased (>13 μg/L) in 8 patients whose tryptase level ranged from 26 to 257 μg/L. Of these patients, 5 (62–75 years old) had mastocytosis and the KIT D816V mutation in bone marrow cells. Of these 5 patients, 4 were diagnosed after the allergic reaction to YJV and serum basal tryptase measurement.



**Figure 1.** Receiver-operating characteristics (ROC) curve showing the performance of the diagnostic tests for yellowjacket allergy. IgE, immunoglobulin E.

## Discussion

Specific IgE to the yellowjacket whole-venom extract that was spiked with Ves v 5 (sYJV) was superior to other in vitro tests or puncture skin tests in confirming venom sensitization after a SAR. In agreement with previous studies, specific IgE to Ves v 5 performed well, but Ves v 1 added no value. Despite the 86% sensitivity of skin prick tests, their usefulness was inferior because of the low specificity and large number of double-positive test results to YJV and honeybee venom. The in-house immunoblot test was inferior to serum-specific IgE or skin prick tests.

Measuring specific IgE to the venom allergen components instead of whole-venom extracts has improved the diagnosis of YJV allergy.<sup>16</sup> Spiking the yellowjacket whole-venom IgE test with Ves v 5 since 2012 has improved its sensitivity.<sup>12</sup> In our study, 47% of samples that were negative in the conventional non-sYJV-specific IgE test were positive in the test spiked with Ves v 5. Spiking explains the good correlation ( $r = 0.98$ ) between these 2 tests.<sup>12</sup> Sensitivity of the sYJV was higher (89%) than that of Ves v 5 alone (85%), but the combination of these 2 tests yielded 90% sensitivity. Measuring IgE

to the whole-venom extract seems to be useful, because the venom could contain other relevant allergen components.<sup>17</sup>

Unexpectedly, measuring IgE to Ves v 1 added no value in our patients with SAR. Previously, sensitization to Ves v 1 was found in 30% to 50% of patients with yellowjacket allergy,<sup>14,16,17</sup> but mono-sensitization has been rare.

The weakness of our study is the lack of intradermal testing. Intradermal tests have been highly sensitive and they are recommended as the first-line test in US guidelines.<sup>5</sup> The sensitivity of skin prick tests is lower than of intradermal tests (86%), and their usefulness is limited by the frequent double sensitization to yellowjacket and honeybee venoms. Skin prick tests also frequently indicate positivity in patients without a SAR.

Double positivity to the 2 venoms could indicate true double sensitization or could be caused by cross-sensitization. Previously, 50% to 60% double positivity has been reported in serum tests.<sup>18</sup> To our knowledge, little evidence exists on the performance and double positivity of skin prick tests compared with serum-specific IgE tests including recombinant allergen components. Previously, double positivity has been more common in conventional serum IgE tests than

**Table 2**  
Performance of Diagnostic Tests in Patients With a Systemic Allergic Reaction ( $n = 47$ ) and With a Large Local or Other Reaction ( $n = 31$ ) to a Yellowjacket Sting

Positive diagnostic test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Likelihood ratio
slgE > 0.35 kU/L in ImmunoCAP					
sYJV <sup>a</sup>	89 (76–96)	74 (55–88)	84 (74–90)	82 (66–92)	3.4 (1.9–6.3)
Ves v 5	85 (71–94)	68 (48–83)	80 (70–87)	75 (59–86)	2.6 (1.6–4.4)
Ves v 1	11 (4–24)	94 (78–99)	71 (34–92)	42 (39–45)	1.7 (0.4–8.3)
sYJV <sup>a</sup> or Ves v 5	90 (78–97)	61 (42–78)	79 (71–86)	79 (61–90)	2.3 (1.5–3.7)
Immunoblot	62 (46–76)	73 (54–88)	78 (61–90)	56 (40–72)	2.3 (1.2–4.4)
Skin prick test reaction wheal > 3 mm					
Titrated <sup>b</sup>	86 (70–95)	54 (33–74)	73 (63–81)	72 (51–86)	1.9 (1.2–2.9)
100 µg <sup>c</sup>	58 (41–75)	62 (41–81)	69 (55–80)	51 (39–64)	1.6 (0.9–2.8)
300 µg <sup>d</sup>	84 (66–94)	59 (36–79)	74 (63–83)	72 (52–86)	2.0 (1.2–3.5)

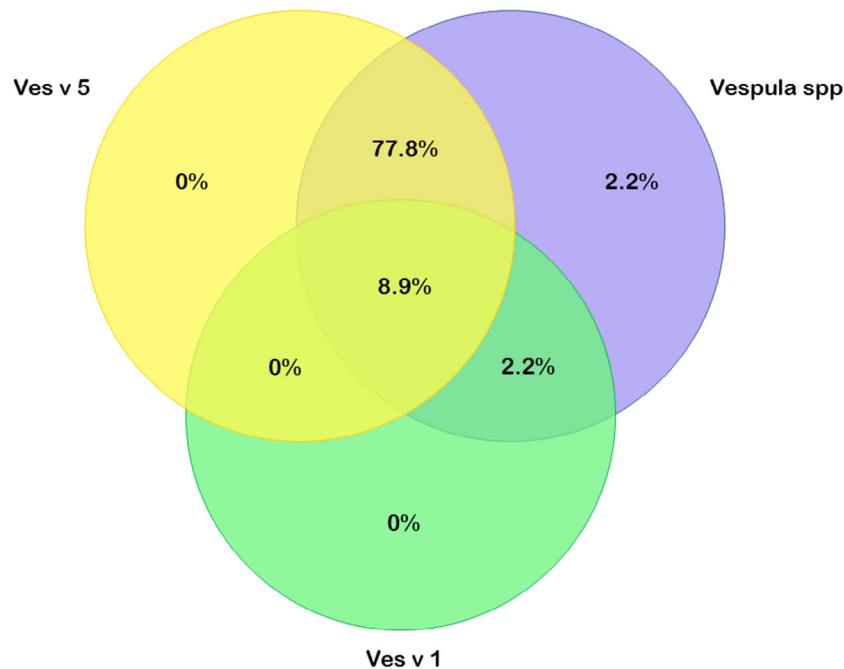
Abbreviations: slgE, specific immunoglobulin E; sYJV, spiked yellowjacket venom.

<sup>a</sup>Specific IgE to sYJV was measured using an ImmunoCAP test that was spiked with Ves v 5.

<sup>b</sup>Fifty-nine test patients.

<sup>c</sup>Fifty-eight test patients.

<sup>d</sup>Fifty-three test patients.



**Figure 2.** Venn diagram of diagnostic combinations of yellowjacket systemic allergic reactions. Percentages of patients with a positive test immunoglobulin E sensitization to *Vesputa* species extract and Ves v 5 and Ves v 1 allergen components are shown (n = 45).

in skin prick tests,<sup>19</sup> but regional differences can occur in the sensitization profiles of patients with insect venom allergy.<sup>20</sup> In our study using the sYJV, double positivity to YJV and honeybee venom was much more common in skin prick tests than in serum tests. In agreement with previous studies, double sensitization was mainly independent of CCD sensitization.<sup>21</sup>

In this population with suspected venom allergy, sensitization to sYJV was frequent in patients without a SAR but with a large local reaction, and the specificity of the tests was not optimal. This could be due to the fact that up to 80% of patients with a large local reaction are sensitized to venom IgE. Up to 46% of patients without a SAR were sensitized in the titrated skin prick tests, and 26% were sensitized in conventional serum tests. Although all patients were referred for suspected venom allergy, the thorough history did not always confirm a SAR. Therefore, before screening for venom sensitization, the history of SAR should be confirmed. The prevalence of sensitization to Hymenoptera venoms is estimated to be 10% to 40% in the general population.<sup>11</sup> Sensitized individuals have an increased risk of having a large local reaction to a sting, but the risk for a SAR in sensitized asymptomatic patients is lower than 6%.<sup>11</sup>

The 5% of our patients who had serum basal tryptase level higher than 20 µg/L were newly diagnosed with systemic mastocytosis and the KIT D816V mutation and fulfilled the World Health Organization minor diagnostic criterion. An anaphylactic reaction to Hymenoptera sting could be an indicator of indolent mastocytosis; therefore, it is useful to measure serum basal tryptase after a SAR to Hymenoptera stings.<sup>4,22,23</sup>

The small number of patients with a SAR to honeybee sting and the lack of commercial tests to Api m 10 and Api m 4 during the study did not allow us to evaluate the performance of the serum tests in honeybee allergy.

The wide interval from the latest sting to blood sampling could affect the results; after an initial decrease (hours), venom-specific IgE increases up to 5-fold from 1 to 4 weeks after the sting and then gradually decreases.<sup>11</sup> The results did not change after adjusting for the time interval.

In conclusion, use of a venom-specific IgE measurement should be reserved for patients with a verified SAR who would be considered for VIT. For this indication, in patients with suspected YJV allergy, when intradermal venom skin tests are not performed, the diagnostic tests of choice are serum-specific IgE to sYJV and Ves v 5. Using venom allergen component-specific IgE tests could improve the diagnosis of IgE-mediated YJV allergy, a prerequisite for VIT after a SAR. An in-house immunoblot added no value and should not be used in selecting patients for VIT, because even if the result is positive, the VIT extract might not contain the specific allergens. VIT in patients who are not sensitized to the culprit allergens carries risks and might not be efficient.<sup>10</sup>

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