

Letter to the Editor

Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy*To the Editor:*

Diagnosis of Hymenoptera venom allergy is based on a history of anaphylactic sting reactions, positive skin test responses, and/or detection of specific IgE to honeybee venom or *Vespa* species venom (VV).¹ Positive results in skin and serologic tests with conventional venom extracts do not always reflect genuine sensitizations but are frequently caused by antibodies cross-reactive to homologous peptide sequences in protein allergens (eg, hyaluronidases and dipeptidyl peptidase IV homologs)^{2,3} or to cross-reactive carbohydrate determinants (CCDs), which are present in the majority of Hymenoptera venom allergens.^{4,5} Double positivity (DP) to both bee venom (BV) and VV in patients who have not identified the culprit insect necessitates additional laboratory tests (eg, IgE inhibition assays) to distinguish genuine double sensitization from cross-reactivity.^{6,7} These tests are expensive, time-consuming, difficult to interpret, and therefore rarely used in the clinical routine. Here we assessed whether the routine use of recombinant species-specific major allergens (SSMA) of honeybee venom (rApi m 1) and VV (rVes v 5), both devoid of CCD reactivity in a novel ImmunoCAP solid-phase assay (Phadia, Uppsala, Sweden), improves the diagnosis of Hymenoptera venom allergy and facilitates the discrimination between genuine double sensitization and cross-reactivity.

Sera from 123 patients with anaphylactic reactions to either honeybee or *Vespa* species stings were analyzed. The classification in different groups was based on the patient's history of an anaphylactic reaction to an insect sting and the identification of the culprit insect by the patient. All patients had positive skin test responses to BV, VV, or both. Thirty-four (37%) patients reported a history of anaphylaxis to BV and 59 (63%) to VV, and 30 patients had not identified the responsible insect. Forty nonatopic patients with a negative history for Hymenoptera venom allergy served as control subjects. All patients and control subjects were analyzed for specific IgE to BV, VV, CCDs, rApi m 1, and

rVes v 5 by means of ImmunoCAP i1, i3, o214, i208 and i209, respectively (Phadia). A value of greater than 0.35 kU/L was considered positive.

Conventional venom extract-based IgE detection demonstrated monosensitization in 50 patients (BV, $n = 18$; VV, $n = 32$), DP in 64 patients, and double-negative results in 9 patients. When the responsible insect was known, IgE to rApi m 1 was detected in 27 (79%) of 34 patients with BV allergy, and IgE to rVes v 5 was detected in 53 (90%) of 59 patients with VV allergy (sensitivity of 79% and 90%, respectively; Table I). In the group with a positive history of BV allergy, 9 (27%) of 34 patients displayed IgE to rVes v 5, whereas only 4 (7%) of 59 patients with VV allergy reacted to rApi m 1, corresponding to a specificity of 73% and 93%, respectively. When the diagnosis of isolated BV or VV allergy was based not only on history but also on IgE detection to conventional venom extracts, IgE to rApi m 1 was detected in 0 (0%) of 32 patients with VV allergy and IgE to rVes v 5 was detected in 1 (6%) of 18 patients with BV allergy, corresponding to a specificity of 100% and 94%, respectively. None of the control sera displayed IgE reactivity to rApi m 1, whereas IgE reactivity to rVes v 5 was detected in 3 of 40 control sera (specificity of 100% and 93%, respectively). The negative predictive value (using control subjects and monosensitized patients as true negatives) was 91% for rApi m 1 and 90% for rVes v 5. The correlation (Spearman rank test) between IgE to BV or VV and the corresponding SSMA was significant ($P < .0001$; $r = 0.82$ for rApi m 1/BV and $r = 0.69$ for rVes v 5/VV).

Among patients with DP who had not identified the culprit insect, 17 (57%) of 30 displayed isolated IgE to either rApi m 1 ($n = 8$) or rVes v 5 ($n = 9$), whereas the remaining 13 patients had IgE to both allergens. Interestingly, 5 (56%) of 9 patients with a positive history of Hymenoptera venom-induced anaphylaxis but double-negative test results to venom extracts displayed IgE reactivity to rVes v 5. Analysis of IgE reactivity to CCDs revealed that 31 (48%) of 64 patients with DP had IgE to CCDs (Fig 1). Of these, 15 (48%) patients reacted with both SSMA, 10 reacted only with rApi m 1, and 6 reacted only with rVes v 5. Of the 33 patients with DP without specific IgE to CCDs, only 8 (24%)

TABLE I. Clinical and serologic data of patients with Hymenoptera venom allergy

	Api m 1 positive	Api m 1 negative	Ves v 5 positive	Ves v 5 negative	Api m 1 and Ves v 5 DP	Api m 1 and Ves v 5 DN
History of BV allergy						
BV allergy (total), $n = 34$	27/34 (79%)	7/34 (21%)	9/34 (27%)	25/34 (73%)	7/34 (21%)	5/34 (15%)
Monosensitized to BV extract, $n = 18$	17/18 (94%)	1/18 (6%)	1/18 (6%)	17/18 (94%)	1/18 (6%)	1/18 (6%)
DP to venom extracts, $n = 15$	10/15 (67%)	5/15 (33%)	8/15 (53%)	7/15 (47%)	6/15 (40%)	3/15 (20%)
Without IgE to venom extracts, $n = 1$	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
History of VV allergy						
VV allergy (total), $n = 59$	4/59 (7%)	55/59 (93%)	53/59 (90%)	6/59 (10%)	4/59 (7%)	6/59 (10%)
Monosensitized to VV extract, $n = 32$	0/32 (0%)	32/32 (100%)	30/32 (94%)	2/32 (6%)	0/32 (0%)	2/32 (6%)
DP to venom extracts, $n = 19$	4/19 (21%)	15/19 (79%)	18/19 (95%)	1/19 (5%)	4/19 (21%)	1/19 (5%)
Without IgE to venom extracts, $n = 8$	0/8 (0%)	8/8 (100%)	5/8 (63%)	3/8 (37%)	0/8 (0%)	3/8 (37%)
Unknown insect						
DP to venom extracts, $n = 30$	21/30 (70%)	9/30 (30%)	22/30 (73%)	8/30 (27%)	13/30 (43%)	0/30 (0%)
Control subjects						
Without history of BV or VV allergy, $n = 40$	0/40 (0%)	40/40 (100%)	3/40 (7%)	37/40 (93%)	0/40 (0%)	37/40 (93%)

DP, Double positive.

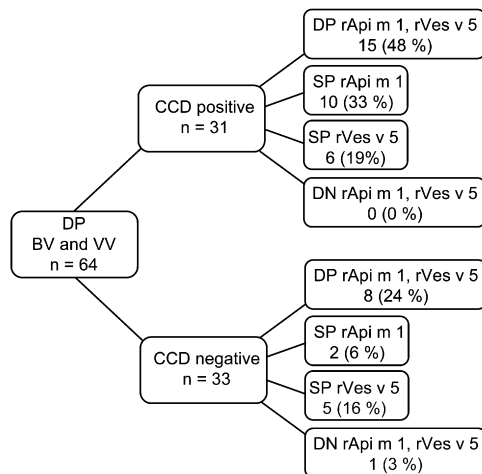


FIG 1. Presence of IgE to recombinant major allergens of honeybee venom (BV; Api m 1) and *Vespula* species venom (VV; Ves v 5) in the group of patients with DP to BV and VV. DN, Double negativity; SP, single positivity.

reacted with both SSMA, whereas 2 reacted only with rApi m 1 and 20 only with rVes v 5, and 3 were nonreactive with both SSMA.

Here we demonstrate, for the first time, that a widely available novel solid-phase assay (ImmunoCAP, Phadia) using the recombinant SSMA of bee venom, rApi m 1, and *Vespula* species venom, rVes v 5, detects IgE in patients with Hymenoptera venom allergy with high sensitivity and specificity. Similar results for IgE detection to rApi m 1 and rVes v 5 in patients with insect venom allergy (sensitivity of 97% and 96%) have been reported recently with a liquid-phase detection system (ADVIA Centaur; Siemens Medical Solution Diagnostics, Deerfield, Ill), which in the meantime has been discontinued by the manufacturer.⁸ The lower sensitivity in our study (79% and 90%, respectively) is likely because of different patient selection. Although the previous study⁸ analyzed selected patients with a documented sting reaction within the last year, our cohort consisted of unselected patients from our allergy clinic and included subjects with sting reactions up to 10 years ago, a setting likely to provide a more realistic picture of the test performance in daily routine. The sensitivity of 79% and 90% might simply reflect the fact that a certain percentage of patients are sensitized to other clinically relevant venom allergens. Expanding the spectrum of species-specific venom allergens for routine use (eg, Api m 2, Api m 3, Api m 4, Api m 10, or Ves v 1) will help to address this question.^{9,10}

Hymenoptera venom allergy diagnosis is often hampered by positive test results to both honeybee and VV extracts. DP to venom extracts might be explained by IgE cross-reactivity with homologous peptide sequences in protein allergens or with CCDs.²⁻⁵ Similar to previous studies, about 50% of patients with DP had IgE to CCDs, explaining DP at least in some patients.⁴ In current clinical practice immunotherapy with both BV and VV, is usually performed in patients who had not identified the culprit insect but display DP to venom extracts, especially in patients without IgE to CCDs.⁶ The present study, however, demonstrates that only a minority of CCD-negative patients with DP to venom extracts (8/33 [24%]) display a genuine sensitization to both SSMA, whereas the majority (22/33 [66%])

present a sensitization only to one of the SSMA (Fig 1). In the latter group immunotherapy only with venom from the primary sensitizing insect should be sufficient, and it is unlikely that immunotherapy with the second venom would be of additional benefit.

In conclusion, detection of specific IgE to rApi m 1 and rVes v 5 is a reliable diagnostic method that enables us discriminate between genuine double sensitization and cross-reactivity in patients with double-positive IgE results to conventional venom extracts. This way it allows us to optimize patient selection for immunotherapy and to avoid unnecessary treatment protocols with both insect venoms.

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