

Double positivity to bee and wasp venom: Improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants

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Background: Specific IgE (sIgE) antibodies to both bee and wasp venom can be due to a sensitivity to both insect venoms or due to cross-reactive carbohydrate determinants (CCDs).

Objective: Investigating whether a basophil activation test (BAT) with both venoms as well as with bromelain and horseradish peroxidase (HRP) or recombinant allergen-based IgE testing can improve the diagnostic procedure.

Methods: Twenty-two Hymenoptera-venom allergic patients with sIgE antibodies to both bee and wasp venom were studied. sIgE antibodies to MUXF3 CCD, bromelain, HRP, rApi m 1, and rVes v 5 were determined, and a BAT (Flow2 CAST) with venom extracts, bromelain, and HRP was performed. Further recombinant allergen-based IgE testing was done by using an ELISA, if required. The reactivity of basophils was calculated from the insect venom concentration at half-maximum stimulation.

Results: Double positivity/double negativity/single positivity to rApi m 1 and rVes v 5 was seen in 12/1/9 patients. Further recombinant allergen-based IgE testing in the last ones revealed positive results to the other venom in all cases except one. BAT was double positive/double negative/single positive in 6/2/14 patients. Four patients with negative results in sIgE antibodies to CCDs had positive results in BAT. BAT with bromelain/HRP showed a sensitivity of 50%/81% and a specificity of 91%/90%. **Conclusion:** Component-resolved IgE testing elucidates the pattern of double positivity, showing a majority of true double sensitizations independent of CCD sensitization. BAT seems to add more information about the culprit insect even if the true clinical relevance of BAT is not completely determined because of ethical limitations on diagnostic sting challenges. BAT with HRP is a good method to determine sensitivity to CCDs. (J Allergy Clin Immunol 2012;■■■:■■■-■■■.)

Key words: Insect venom allergy, basophil activation test, CD63, recombinant allergens, rApi m 1, rVes v 5, bromelain, horseradish peroxidase

The diagnosis of insect venom allergy is based on history, skin tests, and the determination of specific IgE (sIgE) antibodies to bee and wasp venom. It is estimated that up to 59% of the patients with Hymenoptera venom allergy reveal positive results to both bee and wasp venom in these tests.¹ This poses the problem of identifying the relevant venom for immunotherapy especially if the patient has an anaphylactic reaction to only 1 insect. The cause of this double positivity can be true double sensitization to both venoms, cross-reactions due to sequence homology among bee and wasp venom proteins, for example, hyaluronidases, or sIgE to cross-reactive carbohydrate determinants (CCDs). For a long time, only complex IgE inhibition assays were able to help in distinguishing genuine double sensitization from cross-reactivity.² Basophil activation tests (BATs) with venom extracts were also used to solve this problem, but they showed a positive result to only 1 venom in only about one quarter to one third of these cases.^{3,4} Another novel possibility is the determination of sIgE antibodies to recombinant species-specific major allergens devoid of CCD reactivity. sIgE antibodies to rApi m 1, a major allergen (phospholipase A2) of honeybee venom, and rVes v 5, a major allergen of *Vespula*-specific venom, are now commercially available. It could be shown in double-positive, CCD-positive patients having sIgE antibodies to bromelain that 49% have sIgE antibodies to either rApi m 1 or rVes v 5, thus avoiding immunotherapy against both venoms in about half of these cases.⁵ Another recent study showed that the use of CCD-depleted venom in BATs could detect the mono-sensitivity in 8 of the 18 cases with a double-positive intracutaneous test.⁶ As the production of CCD-depleted venoms is difficult, we were interested in the question whether a BAT with commercially available bee and wasp venom extracts, calculation of the concentration at half-maximum stimulation (C50), and a ratio of reactivity would be helpful in solving problems with double positivity. These data were compared with the results of sIgE antibodies to rApi m 1 and rVes v 5 and further recombinant allergen-based IgE testing, if required. Furthermore, BAT with 2 markers of CCD sensitization, bromelain and horseradish peroxidase (HRP) carrying several N-glycan chains, was compared with sIgE antibodies to MUXF3 CCD, bromelain (MUXF3) and HRP.

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Abbreviations used

BATs: Basophil activation tests
 CCDs: Cross-reactive carbohydrate determinants
 HRP: Horseradish peroxidase
 sIgE: Specific immunoglobulin E

METHODS**Patients**

At first, 28 patients with a history of insect venom allergy were included in the study. They had systemic immediate-type reactions of severity grade I to IV (according to the classification of Ring and Messmer⁷) due to a Hymenoptera sting. Inclusion criteria were the demonstration of sIgE antibodies to both bee and wasp venom. Out of these 28 patients, 4 patients were nonresponders to anti-IgE receptor positive control and 2 patients revealed high negative controls in BAT. Thus, 22 patients (17 men, 5 women) could be further analyzed. Eight patients did not remember the species at least once, 7 patients were convinced that they were stung by bees, 5 patients by wasps, and 2 patients by both insects. Fifteen patients had a positive skin test result to both insect venoms and 4 patients to 1 insect venom (2 wasp venom, 2 bee venom). In 3 patients, a skin test was not done. For details, see [Table 1](#).

Controls

Controls comprised 10 individuals with a negative history for an anaphylactic reaction to Hymenoptera stings and no sIgE antibodies to bee venom, wasp venom, and MUXF3 from bromelain and HRP. The study protocol was in accordance with the local ethical committee guidelines, and all subjects had given informed consent before being included.

Skin tests

Intradermal skin testing with honeybee or yellow jacket venom (Venomil; Bencard, Munich, Germany) was done on the ventral aspect of the forearm with incremental concentrations of 0.0001, 0.001, 0.01, and 0.1 $\mu\text{g/mL}$ with a positive (histamine dihydrochloride) and negative control (saline). Positive test results were defined according to the recommendations of the European Academy of Allergology and Clinical Immunology.⁸ Intradermal test results were considered to be positive when the difference to control was greater than 3 mm.

Determination of sIgE antibodies

sIgE antibodies against bee venom (i1), wasp venom (i3), rApi m 1 phospholipase A2 (i208), rVes v 5 (i209), MUXF3 CCD, bromelain (o214) and HRP (o400) were determined by using the system UniCAP 250 (Phadia GmbH, Freiburg, Germany). Values of sIgE below 0.35 kU/L were considered negative. For patients with sensitization to 1 or none of the above-mentioned recombinant allergens, sIgE immunoreactivity to other recombinant insect venom allergens was analyzed by using an ELISA according to previous studies.^{9,10}

Basophil activation tests

The Flow2 CAST (BÜHLMANN Laboratories AG, Schönenbuch, Switzerland) was used for the quantitative determination of *in vitro* basophil activation. Venous blood was collected in 10 mL EDTA tubes and stored at 4°C. The anticoagulated blood samples were gently homogenized by inverting several times.

For each patient and allergen, polystyrene tubes were prepared with 50 μL of allergen in various concentrations ([Table II](#)) diluted in stimulation buffer. As positive controls, both a monoclonal anti-Fc ϵ RI antibody and *N*-formyl-methionyl-leucyl-phenylalanine (2 μM) were used. In order to evaluate basal values without stimulation, 50 μL of stimulation buffer was applied to a separate tube. To each tube 100 μL of stimulation buffer (containing calcium,

heparin and IL-3 [2 ng/mL]), 50 μL of patients' blood, and 20 μL of staining reagent containing a mix of anti-CD63-fluorescein isothiocyanate and anti-CCR3-phycoerythrin mAbs was added.

The tubes were covered with an adhesive plastic sheet and incubated at 37°C for 25 minutes in an incubator. The stimulation was stopped by adding 2 mL of lysing buffer, and the tubes were left at room temperature for 5 minutes. After centrifugation for 5 minutes at 500g, the supernatant was decanted and 300 μL of washing buffer was added to each tube. The cells were resuspended by gently vortexing before flow cytometric analysis.

Flow cytometric analysis of the cells was performed on a FACScan flow cytometer (Becton-Dickinson Immunocytometry System, Heidelberg, Germany) equipped with a 488-mW argon ion laser, using CellQuest software. Basophilic cells were selected out of the lymphocyte population by using anti-CCR3. In each assay, at least 300 basophils were assessed. The upregulation of the activation marker CD63 was calculated by the percentage of the CD63⁺ cells compared with the total identified basophilic cells. A cutoff of 10% CD63⁺ cells was used for bee and wasp venom as recommended by the supplier and confirmed in earlier studies.¹¹

For bromelain and HRP, receiver operator curves were calculated to establish the best concentration and cutoff for BAT whereas sIgE results were used as reference because of the lack of a clinical manifestation. Results of both methods were tested for agreement with the kappa test. Comparison of CD63 activation between the 2 substances at each concentration was done with the Student *t* test for paired samples.

Determination of half-maximum stimulation

The data were transferred into diagrams. The x-axis showed 5 different concentrations of the allergen, beginning with the lowest (C5) up to the highest concentration (C1). On the y-axis, the activity marker CD63 was applied (in percent). In order to get the half-maximum stimulation (C50), a line for the maximum stimulation was drawn, either of the insect venom or of the positive control (anti-Fc ϵ RI mAb). The half of this value was taken and demonstrated by a further line. The point where this half-maximum line cuts the curve shows the half-maximum concentration on the x-axis. For determination of the predominant sensitivity, the following ratio was applied: C50 (ng/mL) bee venom/C50 (ng/mL) wasp venom.

A ratio of less than 0.1 indicated a predominant bee venom sensitivity, a ratio of more than 10 indicated a predominant wasp venom sensitivity, and a ratio between 0.1 and 10 indicated an equal predominant sensitivity to both venoms. An example is given in [Fig 1](#).

Statistical analysis

Sensitivity of these methods with regard to a clear history in patients (*n* = 14) and specificity were calculated as follows:

Sensitivity = true-positive results/(true-positive results + false-negative results)

Specificity = true-negative results/(true-negative results + false-positive results).

RESULTS**sIgE to rApi m 1, rVes v 5, and other recombinant allergens**

Fifteen patients showed sIgE antibodies to rApi m 1 and 18 patients to rVes v 5. Nine patients had sIgE antibodies to only 1 of these recombinant allergens, 12 patients to both recombinant allergens, and 1 patient neither to rApi m 1 nor to rVes v 5. With regard to a clear identification of the stinging insect in history, sensitivity of this method was 50%. Controls were all negative (specificity: 100%). Further recombinant allergen-based IgE testing of the 10 patients with at least 1 negative result to rApi m 1 or rVes v 5 showed positive results to other recombinant allergens in all cases except for one (patient no. 7). For details, see [Table I](#).

TABLE I. Clinical characteristics, skin tests, determination of sIgE antibodies, and BATs (Flow2 CAST) in the study group

Patient no.	Culprit insect/ time interval	Clinical reaction	Positive skin test (intradermal)	sIgE antibodies (class)	ELISA	Flow2 CAST for insect venoms (C50, ratio and positivity)	Flow2 CAST* for basal values and CCDs (% antigen activated basophils)
1	W/8 y B/1 y	II	B, W	B 5, W 4, rApi m1 2, rVes v5 2, MUXF3 3, HRP 3	ND	B: C50: neg; W: C50: neg Ratio B/W: NA Negativity to B and W	Basal value: 1.3% Bromelain: 27.9% at c1 HRP: 46.8% at c1
2	W/10 y ?/2 y	I	ND	B 3, W 3, rApi m1 0, rVes v5 0, MUXF3 0, HRP 0	Positive: nApi m1, rApi m2, rApi m3, rApi m5, rVes v1	B: C50: 1.7 ng/mL; W: C50: 41 ng/mL Ratio B/W: 0.041 Positivity to B	Basal value: 0.3% Bromelain: neg HRP: neg
3	B/25 y B/5 y	II	B	B 3, W 3, rApi m1 1, rVes v5 3, MUXF3 2, HRP 2	ND	B: C50: neg; W: C50: neg Ratio B/W: NA Negativity to B and W	Basal value: 3.5% Bromelain: neg HRP: 86.5% at c2
4	?/20 y B/1 y	II	B, W	B 4, W 4, rApi m1 2, rVes v5 3, MUXF3 0, HRP 0	ND	B: C50: 210 ng/mL; W: C50: 20 ng/mL Ratio B/W: 10.5 Positivity to W	Basal value: 0.9% Bromelain: neg HRP: neg
5	?/10 y	II	W	B 3, W 1, rApi m1 0, rVes v5 2, MUXF3 0, HRP 0	Positive: rApi m2, rApi m10, rVes v1	B: C50: 5 ng/mL; W: C50: 285 ng/mL Ratio B/W: 0.018 Positivity to B	Basal value: 7.6% Bromelain: 23.5% at c5 HRP: 32.4% at c4
6	B/7 y	II	B, W	B 3, W 2, rApi m1 2, rVes v5 2, MUXF3 0, HRP 1	ND	B: C50: 4 ng/mL; W: C50: 70 ng/mL Ratio B/W: 0.057 Positivity to B	Basal value: 1.4% Bromelain: neg HRP: 21.5% at c3
7	B/26 y B/6 y B/1.5 mo	III	B	B 4, W 3, rApi m1 2, rVes v5 0, MUXF3 2, HRP 3	Negative: rVes v1, rVes v2, rVes v3, rVes v5	B: C50: 0.45 ng/mL; W: C50: 120 ng/mL Ratio B/W: 0.004 Positivity to B	Basal value: 0.7% Bromelain: 78.2% at c1 HRP: 94.7% at c4
8	W/2 mo	II	B, W	B 3, W 2, rApi m1 2, rVes v5 1, MUXF3 1, HRP 2	ND	B: C50: 140 ng/mL; W: C50: 285 ng/mL Ratio B/W: 0.491 Positivity to B and W	Basal value: 2.9% Bromelain: neg HRP: neg
9	W/1 y	II	B, W	B 3, W 3, rApi m1 2, rVes v5 2, MUXF3 3, HRP 4	ND	B: C50: 0.45 ng/mL; W: C50: 285 ng/mL Ratio B/W: 0.002 Positivity to B	Basal value: 2.3% Bromelain: 89.5% at c5 HRP: 86.7% at c2
10	B/9 y	II	B, W	B 3, W 2, rApi m1 1, rVes v5 2, MUXF3 2, HRP 2	ND	B: C50: 180 ng/mL; W: C50: 285 ng/mL Ratio B/W: 0.632 Positivity to B and W	Basal value: 8.0% Bromelain: neg HRP: 20.3% at c2
11	?/4 y	I	B, W	B 2, W 2, rApi m1 0, rVes v5 2, MUXF3 0, HRP 0	Positive: rApi m2, rApi m3	B: C50: 285 ng/mL; W: C50: 22 ng/mL Ratio B/W: 12.95 Positivity to W	Basal value: 1.9% Bromelain: neg HRP: neg
12	B/4 y B/2 y	I	ND	B 5, W 3, rApi m1 4, rVes v5 3, MUXF3 3, HRP 3	ND	B: C50: 20 ng/mL; W: C50: 285 ng/mL Ratio B/W: 0.07 Positivity to B	Basal value: 0.6% Bromelain: neg HRP: 81.3% at c3
13	B/30 y B/8 y B/1 mo	IV	B, W	B 3, W 3, rApi m1 2, rVes v5 0, MUXF3 2, HRP 3	Positive: rVes v1	B: C50: 0.45 ng/mL; W: C50: 40 ng/mL Ratio B/W: 0.01 Positivity to B	Basal value: 4.1% Bromelain: neg HRP: 39.9% at c4
14	?/0.5 mo	I	B, W	B 1, W 3, rApi m1 0, rVes v5 4, MUXF3 0, HRP 0	Positive: rApi m3	B: C50: 200 ng/mL; W: C50: 4 ng/mL Ratio B/W: 50.0 Positivity to W	Basal value: 1.3% Bromelain: neg HRP: neg
15	?/30 y B/23 y ?/3 y	III I	B, W	B 3, W 1, rApi m1 1, rVes v5 0, MUXF3 0, HRP 0	Positive: rVes v1, rVes v2, rVes v3	B: C50: 1 ng/mL; W: C50: 285 ng/mL Ratio B/W: 0.004 Positivity to B	Basal value: 1.4% Bromelain: neg HRP: neg

(Continued)

TABLE I. (Continued)

Patient no.	Culprit insect/ time interval	Clinical reaction	Positive skin test (intradermal)	sIgE antibodies (class)	ELISA	Flow2 CAST for insect venoms (C50, ratio and positivity)	Flow2 CAST* for basal values and CCDs (% antigen activated basophils)
16	W/15 y B/1 mo	IV III	B, W	B 4, W 3, rApi m1 3, rVes v5 3, MUXF3 2, HRP 2	ND	B: C50: 1.4 ng/mL; W: C50: 8 ng/mL Ratio B/W: 0.18 Positivity to B and W	Basal value: 1.2% Bromelain: 34.3% at c1 HRP: 84.4 % at c3
17	?/3 y	I	B, W	B 3, W 3, rApi m1 2, rVes v5 3, MUXF3 0, HRP 0	ND	B: C50: 1 ng/mL; W: C50: 4 ng/mL Ratio B/W: 0.25 Positivity to B and W	Basal value: 2.4% Bromelain: neg HRP: neg
18	W/3 y	II	ND	B 1, W 1, rApi m1 0, rVes v5 1, MUXF3 0, HRP 0	Positive: rApi m10	B: C50: 285 ng/mL; W: C50: 9 ng/mL Ratio B/W: 31.7 Positivity to W	Basal value: 2.0% Bromelain: neg HRP: 74.6% at c3
19	W/0.5 mo	III	W	B 1, W 3, rApi m1 0, rVes v5 2, MUXF3 1, HRP 1	Positive: rApi m1, rApi m2, rApi m3	B: C50: 100 ng/mL; W: C50: 7 ng/mL Ratio B/W: 14.3 Positivity to W	Basal value: 1.8% Bromelain: 78.7% at c1 HRP: 85.4% at c4
20	W/5 y W/3 y	II	B, W	B 2, W 2, rApi m1 0, rVes v5 2, MUXF3 0, HRP 0	Positive: rApi m10	B: C50: 130 ng/mL; W: C50: 4 ng/mL Ratio B/W: 32.5 Positivity to W	Basal value: 3.6% Bromelain: 9.0% at c1 HRP: 40.4% at c1
21	B/10 y B/3 mo	I	B, W	B 5, W 3, rApi m1 1, rVes v5 3, MUXF3 0, HRP 0	ND	B: C50: 22 ng/mL; W: C50: 8 ng/mL Ratio B/W: 2.8 Positivity to B and W	Basal value: 0.3% Bromelain: 27.1% at c1 HRP: 41.2% at c1
22	H/7 y ?/0.5 mo	IV II	B, W	B 3, W 4, rApi m1 2, rVes v5 3, MUXF3 0, HRP 2	ND	B: C50: 7 ng/mL; W: C50: 5 ng/mL Ratio B/W: 1.4 Positivity to B and W	Basal value: 0.7% Bromelain: 52.2% at c1 HRP: 66.3% at c5

B, Bee (venom); c1, c2, c3, c4, c5, concentration 1, 2, 3, 4, 5; H, hornet; MUXF3, MUXF3 CCD, bromelain; NA, not applicable; ND, not done; neg, negative; W, wasp (venom).

*The highest percentage of activated basophils obtained in each patient with bromelain or HRP.

BAT to bee and wasp venom with determination of half-maximum stimulation

Calculation of the ratio C50 bee venom/C50 wasp venom revealed in 8 cases a sensitivity to only bee venom, in 6 cases to only wasp venom, and in 6 cases a double positivity to bee and wasp venom. Two cases were negative. With regard to a clear history, sensitivity of this method was 57.1%. Controls were positive in 1 case for wasp venom with 35% CD63 upregulation at the highest concentration (specificity: 90%).

Concordant and discordant results for insect venoms

The 6 cases with double positivity in BAT to bee and wasp venom had sIgE antibodies to both rApi m 1 and Ves v 5. Monopositivity to sIgE antibodies to either rApi m 1 or Ves v 5 was concordant with the corresponding venom in BAT in 8 cases (bee venom: n = 3, wasp venom: n = 5); 1 case was discordant (patient no. 5). In all 6 cases (out of these 9 monopositive cases) with the known stinging insects, the result in BAT was concordant with the culprit insect. In 12 cases with positive sIgE to both commercially available recombinant allergens, BAT revealed a positive result to only 1 insect in 4 cases (bee venom: n = 3; wasp venom: n = 1) or was negative (n = 2). In 2 cases, the positive insect defined by BAT was concordant with the culprit insect. BAT was also positive to

1 insect (bee) in a case (patient no. 2) with no sIgE antibodies to rApi m 1 and rVes v 5, but the first stinging insect in history was a wasp and the second one was unclear. Further component-resolved diagnosis showed positivity to other bee and wasp allergens.

sIgE to CCDs (MUXF3 and HRP)

Ten patients showed sIgE antibodies to MUXF3 and 12 patients to MUX F3 from HRP. Two patients had sIgE antibodies only to HRP, 10 patients to both MUXF3 and HRP, and 10 neither to MUXF3 nor to HRP. For details, see Table I.

BAT to bromelain and HRP

Receiver operator curves revealed a cutoff of 4% (sensitivity 50%, specificity 91%) for bromelain at a concentration of 568 ng/mL related to the CCD-overall results (sIgE antibodies to MUXF3 or HRP). For HRP at a concentration of 4.5 ng/mL, the calculated cutoff was 7%, showing a sensitivity of 82% and a specificity of 90%.

The BAT result was positive in 9 patients with bromelain and in 15 patients with HRP. Basophil activation was significantly ($P < .05$) higher with HRP than with bromelain at all concentrations (Fig 2). Controls were negative with all tested concentrations.

TABLE II. Tested allergens and their concentrations in stimulation*

Allergen	Concentration	Flow2 CAST concentration (ng/mL)
Honey bee venom	c1	284
	c2	56.8
	c3	11.4
	c4	2.27
	c5	0.45
Yellow jacket venom	c1	284
	c2	56.8
	c3	11.4
	c4	2.27
	c5	0.45
Bromelain	c1	2841
	c2	568
	c3	114
	c4	22.7
	c5	4.5
HRP	c1	2841
	c2	568
	c3	114
	c4	22.7
	c5	4.5

c1, c2, c3, c4, c5, Concentration 1, 2, 3, 4, 5.

*All allergens (honey bee venom and yellow jacket venom purchased from BÜHLMANN Laboratories AG, Schönenbuch, Switzerland; bromelain from pineapple stem and peroxidase inactivated from Sigma-Aldrich, St Louis, Mo) were in lyophilized form. They were reconstituted and diluted in stimulation buffer immediately before performing BAT.

Concordant and discordant results for CCDs

The Kappa test showed a good agreement (κ .64) for HRP and a moderate agreement (κ .45) for bromelain for both methods (BAT and sIgE antibodies). Sixteen patients were CCD positive having at least 1 positive result in the determination of sIgE antibodies to MUXF3 or to HRP and in BAT with bromelain or HRP. Six patients showed positive results neither for sIgE antibodies MUXF3 or HRP nor for BAT with bromelain or HRP. Four patients with negative results in the determination of sIgE antibodies to CCDs had positive results in BAT to bromelain or HRP. One patient with negative results in BAT to CCDs had a positive result in the determination of sIgE antibodies to MUXF3 and HRP. For details, see Table I.

DISCUSSION

This study compared results of sIgE antibodies to recombinant Hymenoptera allergens and of BAT in the difficult group of patients with insect venom allergy and double positivity to bee and wasp venom considering also the data of CCDs.

Up to 60% of patients with Hymenoptera venom allergy had positive results in the skin test or for sIgE antibodies to both bee and wasp venom despite the fact that the majority reacted systemically to only 1 insect. In order to distinguish between double sensitivity to both venoms and cross-reactivity due to CCD, until recently RAST inhibition assays were possible methods, but they were not routinely used. In order to find a sensitivity to CCD, sIgE antibodies to MUXF3 and HRP can be determined. Meanwhile sIgE antibodies to recombinant allergens of bee and wasp venom are

commercially available. It was thought that component-resolved diagnostics would solve the problem of double sensitivity in patients with a sensitivity to CCD, but still about 50% of these patients show sIgE antibodies to both rApi m 1 and rVes v 5.⁵ It could be shown that BAT with determination of CD-sens or using submaximal doses reflects clinical symptoms (eg, after sting challenge during immunotherapy) better than other biomarkers.^{12,13}

Furthermore, another study showed that basophil activation with the irrelevant insect venoms was lower than with the relevant one, which was thought to be due to the weaker potential of CCDs in insect venoms to activate basophils.⁶

In our study, BAT with HRP showed a much stronger reaction than with bromelain. This may be due to the different numbers of carbohydrate chains (HRP: 7; bromelain: 1). We also defined sensitivity and specificity for these CCDs, showing good results for HRP (sensitivity: 92%). Four additional patients had a sensitivity to CCDs with BAT compared to sIgE testing. Therefore, negative results in the CAP may not exclude CCD sensitivity, and BAT with HRP is a good method to determine CCD sensitivity. Furthermore, we confirmed observations that CCD sensitization does not preclude double positivity to bee and wasp venom allergens.

For defining a stronger reaction to the relevant insect, we calculated a ratio between C50 of bee venom and C50 of wasp venom. In 6 patients, results of the commercially available recombinant allergens (rApi m 1 and rVes v 5) and BAT were concordant in double sensitivity. This means immunotherapy to both venoms still should be considered despite a history of reaction to only 1 insect in 3 patients. The remaining 16 cases showed a double positivity in extended recombinant allergen-based IgE testing (CAP system and/or ELISA) in 15 cases, while BAT was single positive in 14 cases. BAT was in accordance to a clear history of the stinging insect in 7 cases (patient nos. 6, 7, 12, 13, 18, 19, and 20). In these cases, BAT was helpful in defining the relevant insect venom for immunotherapy. In patients with discordant results and unclear history, immunotherapy with 2 venoms still has to be considered.

Our results show that the determination of sIgE antibodies for commercially available recombinant allergens and extended assessment of IgE immunoreactivity to other recombinant allergens could solve the problem of double positivity in only 1 out of 22 cases. This kind of IgE testing elucidates the pattern of sensitization, but it does not resolve the problem of clinical relevance in most cases. It also means that routine detection of sIgE only to 2 major allergens is not sufficient for elucidating double positivity. This was also found by another group showing that in several of such cases sIgE antibodies to rApi m 2 and nApi m 4 were detectable.¹⁴ Furthermore, in our study, double positivity due to CCDs was most likely present in only 1 case showing negative results to all recombinant wasp venom allergens but a strong reactivity to CCDs in BAT (patient no. 7). The value of BAT in the diagnosis of insect venom allergy was shown in several studies even though BAT was done with different methods (using different activation markers [CD63 vs CD203c], methods of basophil identification, and allergen sources).^{3,4,15-18} Regarding patients with positivity, BAT (with the use of a simple cutoff) was positive to only 1 insect in about a quarter to one third of the cases.¹⁹ In our study group, we would have identified 1 insect venom with this method in only 1 case. As shown in our study, the calculation of the

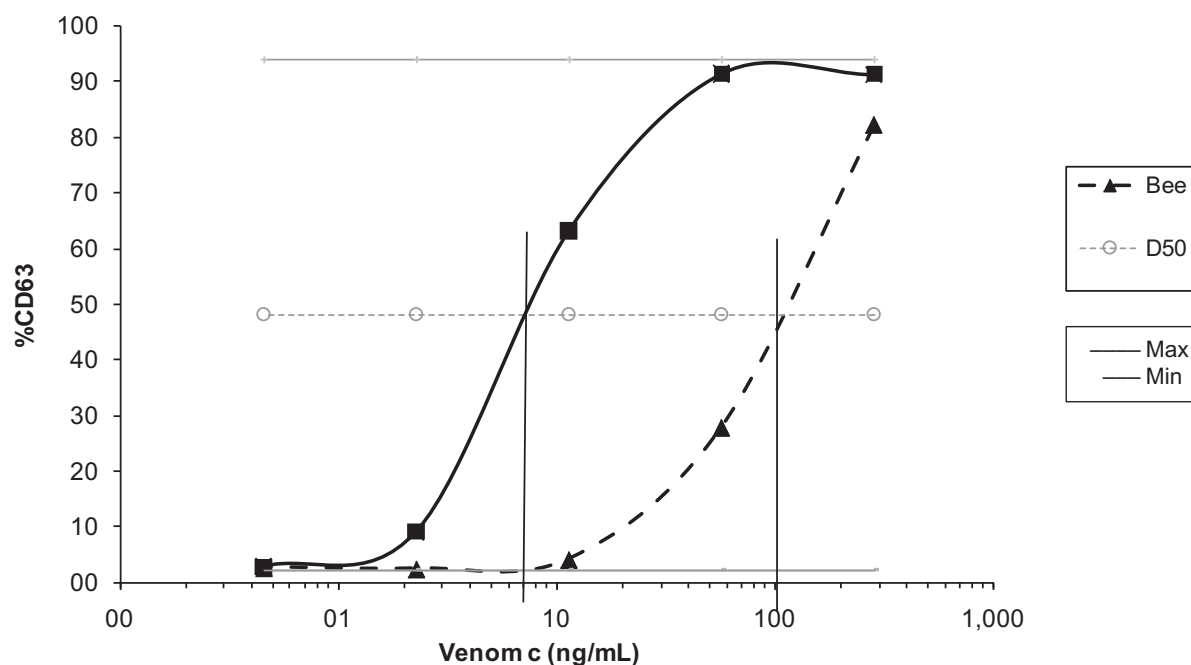


FIG 1. Determination of the half-maximum stimulation. *Max*, Maximum stimulation: 93.9% CD63 (stimulation control); *Min*, Minimal stimulation: 2.5% CD63 (honey bee c2); D50 (bee venom): 100 ng/mL; D50 (yellow jacket venom): 7 ng/mL; ratio D50 = 14.29. Conclusion: Patient shows a reactivity to wasp venom.

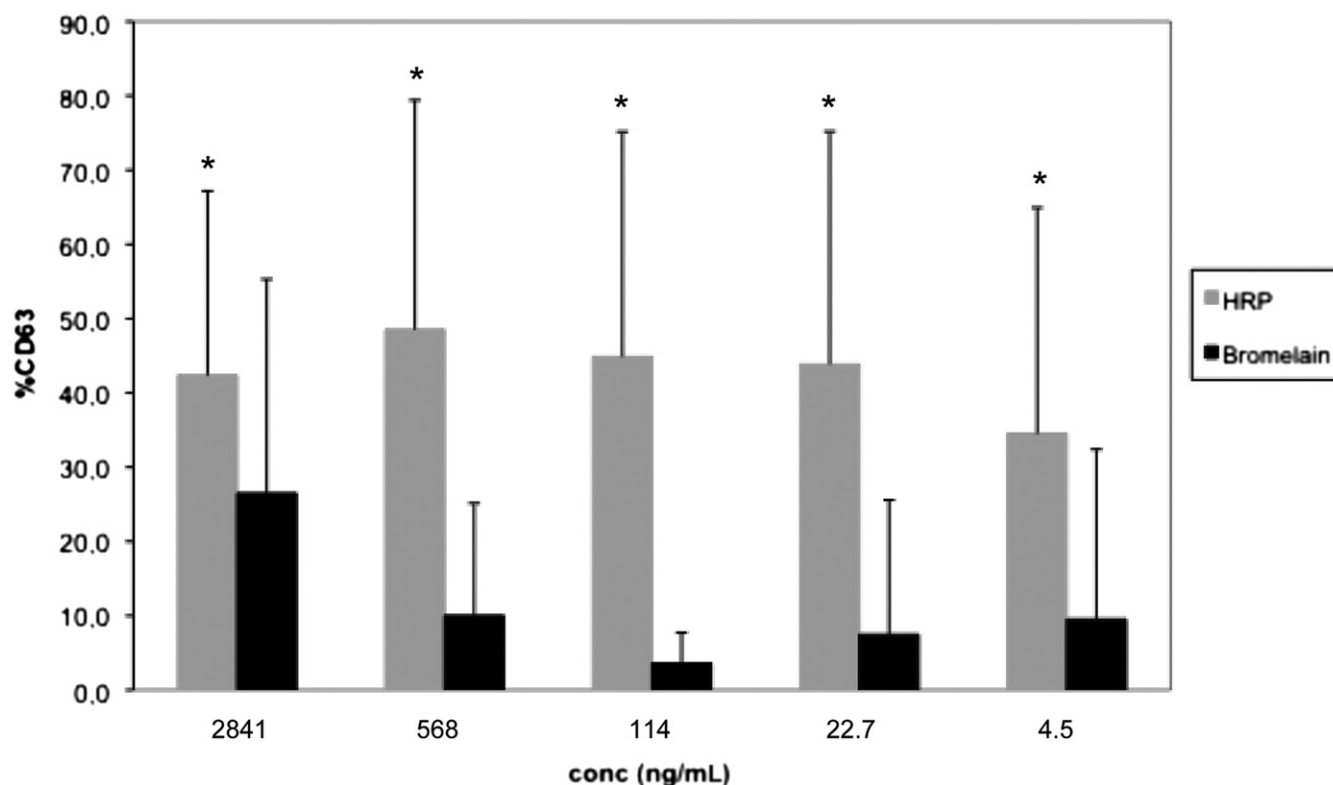


FIG 2. BAT (mean $\pm$ SEM) with different concentrations of bromelain and HRP in all patients. (* $P < .05$). *conc*, Concentration.

half-maximum stimulation seems to be superior to the other method. Taken together, this cellular *in vitro* test adds clinically relevant information in several patients, whereas component-

resolved diagnosis almost exclusively reflects the double-sensitization pattern to insect allergens independent of CCD sensitization.^{9,20,21}

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Clinical implications: Facilitation of the decision for the selection of insect venom species for allergen-specific immunotherapy in patients with double positivity and knowledge about the way of application of basophil activation tests.

REFERENCES

- Müller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespa* venom by estimation of IgE antibodies to species-specific major allergens Api m 1 and Ves v 5. *Allergy* 2009;64:543-8.
- Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hubsch-Müller C, Enk A. In vitro Hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy* 2006;61:1220-9.
- Peternelj A, Silar M, Bajrovic N, Adamic K, Music E, Kosnik M, et al. Diagnostic value of the basophil activation test in evaluating Hymenoptera venom sensitization. *Wien Klin Wochenschr* 2009;121:344-8.
- Sturm GJ, Böhm E, Trummer M, Weiglhofer I, Heinemann A, Aberer W. The CD63 basophil activation testing Hymenoptera venom allergy: a prospective study. *Allergy* 2004;59:1110-7.
- 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;127:265-7.
- Mertens M, Amler S, Moerschbacher BM, Brehler R. Cross-reactive carbohydrate determinant strongly affect the results of the basophil activation test in Hymenoptera-venom allergy. *Clin Exp Allergy* 2010;40:1333-45.
- Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitution. *Lancet* 1977;26:466-9.
- Bonifazi F, Jutel M, Bilo BM, Birnbaum J, Müller U. EAACI Interest Group on Insect Venom Hypersensitivity. Prevention and treatment of Hymenoptera venom allergy: guidelines for clinical practice. *Allergy* 2005;60:1459-70.
- Blank S, Seismann H, Bockisch B, Baren 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;184:5403-13.
- Blank S, Seismann H, Michel Y, McIntyre M, Cifuentes L, Braren I, et al. Api m 10, a genuine *A. mellifera* venom allergen, is clinically relevant but underrepresented in therapeutic extracts. *Allergy* 2011;66:1322-9.
- Scherer K, Weber JM, Jermann TM, Krautheim A, Tas E, Ueberschlager EV, et al. Cellular in vitro assays in the diagnosis of Hymenoptera venom allergy. *Int Arch Allergy Immunol* 2008;146:122-32.
- Nopp A, Cardell LO, Johansson SGO, Öman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy* 2009;64:811-4.
- Ebo DG, Hagendorens MM, Schuerwegh AJ, Bridts CH, De Clerck LS, Stevens WJ. Flow-assisted quantification of in vitro activated basophils in the diagnosis of wasp venom allergy and follow-up of wasp venom immunotherapy. *Cytometry B Clin Cytom* 2007;72:196-203.
- Sturm G, Hemmer W, Hawranek T, Lang R, Ollert M, Spillner E, et al. Detection of IgE to recombinant Api m 1 and rVes v 5 is valuable but not sufficient to distinguish bee from wasp venom allergy. *J Allergy Clin Immunol* 2011;128:247-8.
- Sturm G, Chunsheng J, Kranzelbinder B, Hemmer W, Sturm E, Griesbacher A, et al. Inconsistent results of diagnostic tools hamper the differentiation between bee and vespine venom allergy. *PLoS ONE* 2011;6:e20842.
- Erdmann SM, Sachs B, Kwiecien R, Moll-Slodowsky S, Sauer I, Merk HF. The basophil activation test in wasp venom allergy: sensitivity, specificity and monitoring specific immunotherapy. *Allergy* 2004;59:1102-9.
- Eberlein-König B, Schmidt-Leidescher C, Rakoski J, Behrendt H, Ring J. In vitro basophil activation using CD63 expression in patients with bee and wasp venom allergy. *J Invest Allergol Clin Immunol* 2006;16:5-10.
- Eberlein-König B, Varga R, Mempel M, Darsow U, Behrendt H, Ring J. Comparison of basophil activation tests using CD63 or CD203c expression in patients with insect venom allergy. *Allergy* 2006;61:1084-5.
- Kosnik M, Korosec P. Importance of basophil activation testing in insect venom allergy. *Allergy Asthma Clin Immunol* 2009;5:11.
- Seismann H, Blank S, Cifuentes L, Baren I, Bredehorst R, Grundwald 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.
- Mittermann I, Zidar 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;125:1300-7.