

ORIGINAL ARTICLE

Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results

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Summary

Background Current guidelines do not adequately address the question of how best to manage patients with a convincing history of insect allergy, but negative venom-specific IgE and skin test results.

Methods Forty-seven patients out of a total of 1219 (4%), with a positive history of sting allergy, were recruited over a period of 4.5 years. All recruited patients had a convincing history of a severe or a life-threatening anaphylactic reaction of Mueller grade II–IV (median grade III) after Hymenoptera sting, but negative venom-specific IgE and skin prick test results. Diagnostic work-up was prospectively followed by the CD63 basophil activation test and by intradermal skin testing. A control group of 25 subjects was also assessed.

Results Thirty-five out of 47 (75%) patients demonstrated a positive basophil CD63 response after stimulation with bee and/or wasp venom. Intradermal venom skin tests were performed for 37 patients, 17 (46%) of whom showed positive results. Out of 20 patients who demonstrated negative intradermal test results, 12 patients showed a positive CD63 response (60%). In contrast, out of 9 patients who showed a negative CD63 response, only one was detected by intradermal testing (11%). In the control group, only two out of 25 (4%) subjects displayed a positive basophil response and/or intradermal test.

Conclusion Here we show that, in complex cases with inconclusive diagnostic results, the CD63 activation test could be particularly useful and more sensitive than intradermal skin testing.

Keywords CD63 basophil response, hymenoptera allergy, insect venom, skin test

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Introduction

Recent advances in understanding basophil biology and the discovery of novel markers for measuring basophil activation have established new possibilities for the diagnosis and management of patients with insect sting allergies. Previous studies demonstrated that flow cytometry quantification of basophil activation by measurement of CD63 expression could be a valuable diagnostic tool for assessing Hymenoptera venom allergy [1–3]. The clinical utility of this cellular *in vitro* method was further challenged, as several reports suggested that differences in dose-dependent basophil responsiveness (sensitivity) to allergen-specific *in vitro* stimulation or changes in submaximal stimulation might be connected to adverse reactions [3] or a venom immunotherapy course [4, 5]. In contrast, some authors were not able to demonstrate such

correlations [1, 6]. Previous reports have described patients with a history of systemic reactions after Hymenoptera sting, but negative venom-specific IgE (<0.35 kU/L) and skin test results [7, 8]. Golden et al. [9] even suggest that approximately one-third of patients with a positive history might show negative intradermal skin test responses. Furthermore, those patients could subsequently experience another severe or even a fatal reaction to sting [8, 9]. In fact, in cases of fatal sting anaphylaxis, venom-specific IgE are very low or even undetectable in > 30% of the patients [8]. Ebo et al. [10] first suggested that in difficult cases, basophil testing constitutes a useful additional diagnostic tool. For this reason, we wanted to evaluate the diagnostic significance of the CD63 basophil activation test in a large group of patients showing a positive history of a systemic reaction to sting, but negative venom-specific IgE and skin prick test (SPT)

results, and compare these results with those obtained for intradermal skin testing.

Materials and methods

Study groups

The first study group included 47 patients (26 women and 21 men with a mean age of 43 years and an age range from 18 to 69 years) out of a total of 1219 with a history of Hymenoptera sting allergy. This group was recruited during a 4.5-year-long diagnostic testing at the University Clinic of Respiratory and Allergic Diseases. All recruited patients had a convincing history of a severe anaphylactic reaction of Mueller grades II–IV (median grade III 13 grade II, 15 grade III, and 19 grade IV) to Hymenoptera sting. All patients were subjected to an end-point titration SPT (Venomenhal, HAL, Haarlem, the Netherlands), CAP-FEIA (Phadia, Uppsala, Sweden) measurements of venom-specific IgE, total IgE, and basal serum tryptase (Table 1), followed by the basophil activation test and intradermal skin testing. CAP-FEIA serum tryptase measures the total tryptase level of all proforms of α -tryptase and β -tryptase as well as mature β -tryptase. For SPTs, venom concentrations were in the range from 0.1 up to 100 $\mu\text{g/mL}$. Intradermal testing was performed with up to 0.1 $\mu\text{g/mL}$ of allergen concentration in the majority of the first 34 (71%) patients and with up to 1 $\mu\text{g/mL}$ in the last 13 patients (numbers 35–47) (Table 1).

The second study group was a control group, that included 25 subjects with a negative history of reactions to Hymenoptera sting (16 women and six men with a mean age of 29 years and an age range from 21 to 50 years). Venom-specific IgE measurements and basophil activation and intradermal skin tests were performed for all control subjects. All subjects were volunteers and provided written consent after being fully informed of the purpose and nature of the study. The study was approved by the National Medical Ethics Committee.

Basophil activation assay

The basophil activation test was performed as described previously [3]. Briefly, whole blood (with a heparin anticoagulant) was pre-incubated in a basophil stimulation buffer with IL-3 (Buhlmann Laboratories, Basel, Switzerland) containing a final concentration of 0.1 or 1 $\mu\text{g/mL}$ of honey bee or wasp venom [1–3] (Hal Allergie), 0.55 $\mu\text{g/mL}$ of anti-Fc ϵ RI mAb (Buhlmann Laboratories) or 2 μM fMLP (Sigma, St Louis, MO, USA) at 37 °C for 15 min. Degranulation was stopped by chilling on ice, after which FITC-conjugated anti-CD63 mAb, PE-conjugated anti-CD123 mAb, and PerCP-conjugated anti-HLA-DR mAb (BD Biosciences San Jose, CA, USA) were added and incubated for 20 min on ice. Finally, whole-blood probes

were lysed, washed, fixed, and analysed within 2 h on a FACS Calibur flow cytometer (BD Biosciences). Data were acquired using an FL2 threshold that eliminated most CD123-negative cells, and at least 600 CD123-positive cells were acquired per sample. Basophils were identified as low side scatter, CD123-positive and HLA-DR-negative cells, and the quantitative percentage determination of degranulated basophils was carried out in FL1 (CD63).

Statistical analyses

Data distribution was recalculated using the Shapiro–Wilk test. Because the data were not normally distributed, we performed a Mann–Whitney test. Binominal results were analysed by Fisher's test. Probability values of $P < 0.05$ were accepted as significant. Analyses were performed using GraphPad Prism 5.

Results

The detailed characterization of the recruited patients is summarized in Table 1. The median time since the last systemic reaction to Hymenoptera sting and inclusion into the diagnostic work-up was 1 year, ranging from 1 month to 21 years. Twelve out of 47 (26%) patients experienced more than one anaphylactic reaction, with a period of 1 month to 48 years between reactions (median of 3 years). The culprit insect was identified in 32 out of 47 (68%) cases: bee in 17 cases, wasp in nine cases, bee and wasp in three cases, hornet in two cases, and wasp and hornet in one case. All patients had negative specific IgE and SPT results for bee and wasp venom, and rather low serum tryptase [median (range): 3.9 (1–19.4 $\mu\text{g/L}$)] and total IgE values [median (range): 24.4 (2.3–192 kU/L)].

The basophil CD63 venom response and intradermal skin test results are summarized in Table 2. The median percentages of basophil CD63 response to stimulation buffer (negative control), anti-Fc ϵ RI mAb, and fMLP-positive control were three (range 1.3–4.5%), 91 (range 11.4–99.5%), and 52.3 (range 15–91.9%), respectively. The basophil CD63 venom response was first evaluated by receiver operating characteristic (ROC) curve analyses (Fig. 1). The area under a ROC curve was 0.83 and the P -value was < 0.0001 . The optimal recalculated threshold was $> 14.2\%$, with a sensitivity of 75% and a specificity of 92%. Therefore, the cut-off value for positive results was set at 15% (Table 2), which corresponded with previous reports [1, 3, 5]. We found that 35 out of 47 patients showed a positive CD63 response after stimulation with bee and/or wasp venom. Twenty-five showed a positive response to one type of venom (16 bee and nine wasp venoms) and 10 to both venoms. Intradermal venom skin tests were performed on 37 patients, 17 (46%) of whom showed positive results; 11 were positive for bee venom, correlating with 10 bee venom-positive and one

Table 1. Demographic data and diagnostic results in 47 patients with history of insect sting anaphylaxis and negative venom sIgE and skin prick test results

Subject no.	Mueller grade	Past history of anaphylaxis	Culprit insect	Tryptase ($\mu\text{g/L}$)*	tIgE (kU/L)*
1	IV	21 yr	Bee	ND	ND
2	IV	10 yr	ND	3	15.2
3	IV, III	20 yr, 1 m	Bee	ND	ND
4	II	1 yr	Bee	< 1	54.6
5	III	20 yr, 5 yr, 3 yr, 1 yr	Bee	13.9	9.4
6	IV	8 yr	Bee	3.4	24.4
7	III	6 yr	Wasp	4.8	20.1
8	IV	2 yr	Bee	6.5	157.0
9	III	9 yr	Wasp	3.3	94.8
10	IV	1 m	Wasp	ND	79.7
11	II	1 per yr (beekeeper)	Bee	3.8	106.0
12	IV	18 yr, 3 m	ND	2.3	28.4
13	IV	3 yr, 2 m	ND	3	27.3
14	IV	2 m	Bee	10.3	9.7
15	IV	5 m	ND	13.2	68.2
16	IV, III	48 yr, 2 m	Hornet, Wasp	4.2	8.4
17	IV	14 yr	ND	4.3	39.6
18	IV	5 m	ND	5	20.1
19	IV	4 m	Hornet	19.4	49.4
20	IV	3 yr	Bee	ND	2.3
21	II	10 yr	Bee	2.6	190
22	III	1 yr	Wasp	4.4	13.5
23	IV	1 m	Bee	5.1	28.4
24	IV	1 yr	ND	6	21.9
25	II	16 yr, 7 yr	Wasp, Bee	2.1	63.9
26	III	1 yr	Wasp	3.1	40.4
27	II	1 m	Bee	3.2	192
28	IV	2 m	Hornet	2.9	10.9
29	II	2 m	ND	3.1	38.9
30	III	4 m	Bee	3.8	6.73
31	II	9 m	ND	11.7	55.5
32	III	6 m	Wasp	3.3	34.4
33	III	15 yr	Bee	3.3	6.36
34	III	6 m	Wasp	4.1	6.3
35	III	12 yr, 11 yr	Wasp	7.34	21.3
36	IV	5 yr	Bee	3.91	26.6
37	III	1 yr, every 2–3 yr	Wasp, Bee	6.04	103
38	III	1 yr	ND	4.49	177
39	II	1 yr	ND	5.5	10.4
40	II	4 m	Wasp	2.47	5.91
41	II	10 yr, 5 yr	ND	8.07	8.44
42	III, II	3 yr, 3 m, 4 m	Wasp, Bee	8.73	8.39
43	II	9 m	ND	2.89	39.7
44	III	2 m	Bee	3.07	16.5
45	III	10 yr	ND	3.79	21.1
46	III	7 yr, 3 yr, 1 yr	Bee	6.72	16.4
47	II	15 yr	ND	2.66	9.84

*Total IgE and baseline tryptase were measured by CAP-FEIA.

ND, not determined; yr, years; m, months.

double-positive basophil CD63 responders. Three patients were positive for wasp venom, correlating with three wasp CD63 responders. Two patients displayed a double-positive intradermal skin test result; one was double CD 63-positive and one showed a single-positive CD63 wasp

response. Only one patient demonstrated positive intradermal test results, but a negative CD63 response. Consequently, out of 9 patients who tested negative for CD63, one was detected by intradermal testing (11%). In contrast, 12 patients tested positive for CD63, but showed negative

Table 2. *In vitro* basophil response determined by the percentage of surface CD63-expressing CD123⁺ HLA-DR⁺ cells and results of intradermal skin testing in 47 patients with history of insect sting anaphylaxis and negative venom sIgE and skin prick test results.

Subject no.	Anti-FcεRI mAb (%)	Bee venom (1 µg/mL) (%)	Bee venom (0.1 µg/mL) (%)	Wasp venom (1 µg/mL) (%)	Wasp venom (0.1 µg/mL) (%)	Intradermal (bee/wasp)*
1 ^{†‡}	78.6	39.6	12.5	8.4	ND	Pos./Neg.
2 [†]	87	7.0	2	16	3.0	ND
3 [†]	54.1	31	2.9	8	4.6	ND
4	26	7.5	2.1	4.8	2.8	ND
5 [†]	87.9	16.7	5.5	2	1.3	ND
6 ^{†‡}	74.5	75.8	35	8.7	0.7	Pos./Neg.
7 [†]	83.7	70.9	3.5	95.4	69.2	Neg./Neg.
8 [‡]	70.5	4.6	2	2.1	1.8	Pos./Neg.
9	85.1	5	3	4.6	3.7	Neg./Neg.
10 [†]	97	89.7	19.6	100	35.4	ND
11	89.6	1.7	1.5	6.8	4.2	ND
12 [†]	90.6	16.2	5.9	91.4	33.3	Neg./Neg.
13 ^{†‡}	68.5	35.4	24.4	1.7	4.2	Pos./Neg.
14	93.2	8.6	3.6	12.1	7.6	Neg./Neg.
15	87.8	1.9	3.4	5.9	3	Neg./Neg.
16	92.8	6.8	3.2	1.2	0.9	ND
17 [†]	97.9	26.3	0.1	12.9	6.7	ND
18 [†]	95	74.2	28.5	66.4	11.1	ND
19 [†]	88	58.3	7.2	75.2	4.7	Neg./Neg.
20 [†]	77	47.3	5.2	41.2	20.7	Neg./Neg.
21	45.3	5.5	1.8	3.7	4.9	Neg./Neg.
22	74.3	4.4	1.6	3.9	4.2	Neg./Neg.
23 [†]	97	17.7	15.7	1.6	1	ND
24 ^{†‡}	91.9	36.8	8.1	10.9	2.8	Pos./Neg.
25 ^{†‡}	95.7	3	3	66.6	26.4	Pos./Pos.
26 [†]	99	3.6	3.4	16.3	8.3	Neg./Neg.
27 ^{†‡}	93.5	17.9	5.8	1.6	3.7	Pos./Neg.
28 [†]	93.5	90	9.3	88.9	21.8	Neg./Neg.
29 ^{†‡}	93.9	9.6	2.3	96.60	60.8	Neg./Pos.
30 ^{†‡}	90.8	82.1	81	4.6	3.8	Pos./Neg.
31	91.5	9.1	4	5.4	3.9	Neg./Neg.
32	96.5	6	5	0.8	3.4	Neg./Neg.
33 [†]	97.7	88	18.6	2.9	1.6	Neg./Neg.
34 [†]	83.6	86	83	6	1.3	Neg./Neg.
35 ^{†‡}	97	9.7	2.8	90.3	78.7	Neg./Pos.
36 [†]	92.4	17.8	1.2	6	1.1	Neg./Neg.
37 ^{†‡}	39.7	22	3.1	21.2	8.6	Pos./Pos.
38 ^{†‡}	91	2.8	2.3	41.5	3.3	Neg./Pos.
39	11.4	5.1	3.4	5	3.1	Neg./Neg.
40 [†]	94	4.8	3.6	47.7	1.5	Neg./Neg.
41 [†]	97.4	22.6	3.6	56.6	16.2	Neg./Neg.
42 ^{†‡}	98	98.5	88.3	82.1	42	Pos./Neg.
43 ^{†‡}	99.5	11.2	2.4	92.7	73.1	Neg./Pos.
44 ^{†‡}	96.9	19.9	9.2	9.9	7.1	Pos./Neg.
45 ^{†‡}	52.5	28.8	5.9	12.2	4.2	Pos./Neg.
46 ^{†‡}	92.7	75.7	31.6	5.2	1.3	Pos./Neg.
47 [†]	82.9	9.3	4.2	71.1	24.6	Neg./Neg.

*Intradermal skin test results indicate responses at allergen concentration up to 0.1 µg/mL in patients 1–34 and up to 1 µg/mL in patients 35–47.

[†]Thirty-five of 47 patients (75%) had positive basophile *in vitro* venom responses, with a cut-off of 15% CD63-positive basophiles.[‡]Seventeen of 37 patients (46%) had positive intradermal skin test results.

Pos., positive; Neg., negative.

results for the intradermal test. Consequently, out of 20 patients who tested negative for the intradermal test, 12 of them showed a positive response for CD63 (60%).

The sensitivities to basophil activation and the intradermal skin test are summarized in Fig. 2. This figure shows the comparison of 37 subjects on whom basophil activation and intradermal skin testing were performed.

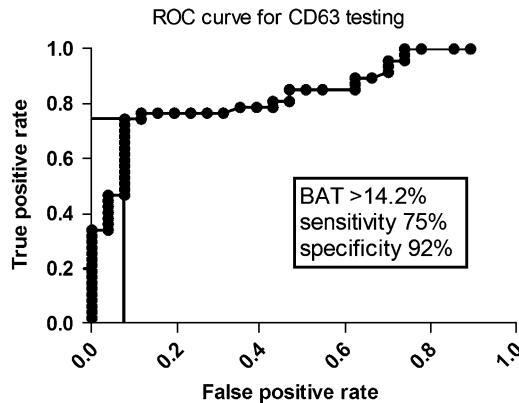


Fig. 1. Receiver operating characteristic (ROC) curve analysis of venom basophile CD63 testing. The area under a ROC curve was 0.83 and the P -value was <0.0001 . The optimal threshold was $>14.2\%$ of positive basophiles.

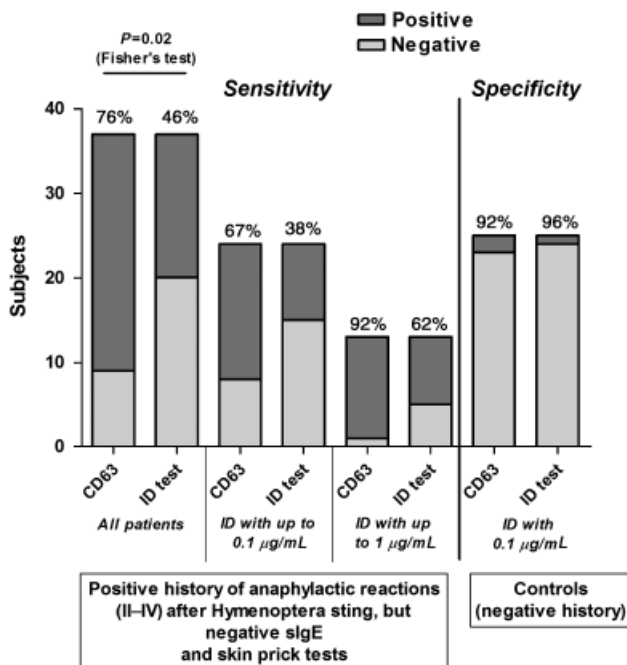


Fig. 2. Sensitivity of the basophile CD63 activation (CD63) and intradermal skin testing (ID test) in 37 patients with a history of convincing Hymenoptera sting anaphylaxis, but negative venom sIgE and skin prick test results; specificity in 25 control subjects without a history of systemic reaction to Hymenoptera sting. ID testing was prospectively performed with up to 0.1 µg/mL in the first 24 patients and with up to 1 µg/mL of allergen concentration in the last 13 patients. The cut-off value for positive CD63 results was set at 15%.

We found a significantly higher diagnostic sensitivity for the basophil CD63 response (28 out of 37 patients; 76%) as compared with the intradermal skin test (17 out of 37 patients; 46%) ($P=0.02$). Sensitivity was further compared according to the concentrations used for intradermal testing. The first subgroup, consisting of 24 patients (intradermal test performed with up to 0.1 µg/mL of allergen), showed a sensitivity response of 67% for CD63 as compared with an intradermal sensitivity of 38%, while in 13 other patients (intradermal test performed with a concentration up to 1 µg/mL), the sensitivity of CD63 response was 92% and the sensitivity of intradermal testing was 62% (Fig. 2).

Altogether, Hymenoptera venom sensitization was confirmed in 36 out of 47 (77%) patients. However, both subgroups were comparable with regard to time since the last systemic reaction to Hymenoptera sting [median (range): 12 vs. 12 months (1 month – 21 years vs. 1 month – 10 years), $P=0.86$] and had comparable levels of basal serum tryptase, total IgE [median (range): 3.9 vs. 4.2 (2.1–19.4 vs. 1–13.2 µg/L), $P=0.64$; 21.6 vs. 54.6 (2.3–192 vs. 8.4–190 kU/L), $P=0.16$], and severity of reactions [median Mueller grade (range): III (II–IV) vs. III (II–IV) ($P=0.17$)]. In addition, only three out of 44 patients showed a basal serum tryptase level higher than 12 µg/L, and six out of 45 patients showed total IgE levels higher than 100 kU/L.

The control group consisted of individuals with a negative history (stung but asymptomatic), and the diagnostic results for this group showed that four out of 25 (16%) control subjects showed a positive specific IgE response to bee and/or wasp venom (≥ 0.35 kU/L). Two of these showed a positive CD63 response after stimulation with wasp venom, and one showed a positive intradermal wasp venom skin test result (with concentration up to 0.1 µg/mL). The median percentage of basophil CD63 response to stimulation buffer (negative control), anti-FcεRI mAb, and fMLP positive control was 3.1 (range 0.7–4.9%), 92.1 (range 17.9–100%), and 54.1 (range 13.3–71.9%), respectively. The specificities of the venom IgE measurement, basophil activation test, and intradermal skin test in the control group were 84%, 92%, and 96%, respectively (Fig. 2).

Discussion

Negative venom skin test and specific IgE results in a patient with a history of systemic reaction to sting, as well as in patients with a history of very severe reactions or repeated systemic reactions after subsequent stings, might be more common than previously thought [8, 9]. As immunotherapy decisions rely on confirmation of allergic sensitization, it is clear that the management of these patients requires further diagnostic evaluation. One of the most promising methods currently available is the venom

basophil CD63 activation test. In this report, we tested the utility of the basophil activation test in the cases with inconclusive diagnostic skin test and sIgE results.

Because of potentially life-threatening reactions, the sensitivity of diagnostic tests for insect sting allergy is of great importance. However, it is desirable to avoid the inconvenience and the high costs of treating patients who would be reasonably safe without any treatment; therefore, a high diagnostic specificity is also required. According to EAACI guidelines, recommended diagnostic tests are skin tests with hymenoptera venoms performed by skin prick or intradermal testing and analysis of the serum for venom-specific IgE [11]. In the case of a negative prick test, they suggest the confirmation by the intradermal test. According to these guidelines, the first line of investigation of this study was a venom SPT, followed by sIgE measurement. Similarly, a recent publication of Diwakar et al. [12] showed that in the United Kingdom, the first-line investigation in 45% of practitioners is a venom SPT and measurement of venom-specific IgE in 55%. Only half of the UK practitioners proceeded with carrying out intradermal skin tests if both the SPT and sIgE were negative. On the other hand, in the United States, the venom intradermal test is the preferred testing modality for most patients [13]. However, regardless of the initial approaches, some patients show negative testing results. These numbers vary from study to study. In this report, which included a large cohort of > 1000 patients with a positive history of systemic reactions of at least Mueller grade II, we found that 4% of patients showed negative initial testing results. Golden et al. [9] reported that as many as one-third of the subjects (99 of 307) recruited with a comparable history of a systemic reaction to Hymenoptera sting showed negative venom intradermal skin test results, and 56 of these (18%) also displayed negative specific IgE. These two large studies clearly suggest the existence of a significant number of patients with negative standard diagnostic tests, although some previous investigators [14–16] found only a few cases. Furthermore, Golden et al. [9] demonstrated that 11 of the 51 patients (22%), who belonged to the diagnostically negative subgroup, experienced a systemic reaction after a sting challenge. These observations obviously suggest that additional diagnostic testing is needed in a valuable number of patients.

In this report, we showed that the basophil activation test is significantly more sensitive than intradermal skin testing in cases demonstrating inconclusive diagnostic results for SPT and sIgE measurement. Both tests showed a comparably high specificity in stung asymptomatic controls: CD63 testing with a concentration of up to 1 µg/mL and intradermal testing with a concentration up to 0.1 µg/mL. Sensitivity and specificity were compared with histories of previous sting reactions. The differences between the *in vivo* intradermal skin test and the *in vitro*

basophil activation test results may be due to a variety of factors. These factors may include different cellular targets (i.e. blood basophils in the CD63 activation test and cutaneous mast cells in the skin test), presentation of allergens, or high quantitative capabilities of flow cytometry. Moreover, the skin test is dependent on target organ mediator sensitivity, which is not necessarily correlated to basophil allergen sensitivity. It can be argued that the main reason might be the final concentration of allergen for intradermal testing because only 13 patients (35%) were tested with a concentration up to 1 µg/mL and another 24 with a concentration up to 0.1 µg/mL (65%). Nevertheless, both subgroups showed comparable advantages of CD63 testing where approximately one-third of the patients were identified solely by a CD63 basophil response. Moreover, this study showed that 20 patients were negative for skin testing (intradermal and prick testing) and sIgE; thus, more than half (12 patients, 60%) were identified by a positive CD63 basophil response. Of these 12 patients, eight experienced very severe or life-threatening reactions (four Mueller grade IV and four grade III), and one patient showed repetitive systemic reactions after subsequent stings. Notably, four of these 12 patients were from the subgroup tested with a concentration up to 1 µg/mL. In contrast, 9 patients were negative for a basophil CD63 response and only one of those 9 patients showed a positive intradermal test.

The non-allergenic components in Hymenoptera venoms, which can cause false-positive reactions at concentrations higher than 1 µg/mL, place a practical limit on the diagnostic range of both intradermal skin tests and the basophil activation test [2, 17]. It is also known that wasp sting, for example, releases only 1.7–3.1 µg of venom [18]. EAACI and AAAAI/ACAAI [13] guidelines stated that the 1 µg/mL is the maximum concentration for intradermal skin testing with an estimated sensitivity limit of about 90% up to 95% [11, 17]. Notably, we also found patients who did show negative intradermal skin tests at 1 µg/mL, but they evidently show a positive venom basophil CD63 response. Therefore, this study and a previous Ebo et al. [10] report clearly suggest that BAT is a reliable additional tool for diagnosing venom allergy in such difficult cases. Hence, ideally we would have all three tests (i.e. skin testing, specific IgE, and BAT) available to identify almost every affected patient. Currently, there is a major lack of basophil activation test utilization, as only specialized laboratories can validate standardized protocols for routine diagnostic practice [19].

Our diagnostic testing was initiated at a median time of 1 year after the sting reaction in patients with confirmed sensitization and those with negative results, and the period was comparable in both subgroups. Thus, the period between the systemic reaction and patient inclusion could not affect the test results. The earliest inclusion was at least 1 month after the reaction, decreasing the

possible influence of the refractory period. In fact, all three patients, who were included 1 month after a sting reaction, demonstrated a positive venom CD63 response. The basophil activation test also correlated with the single culprit insect recognized by the patient in 19 of 20 patients (95%, 12 bee, five wasp, and two hornet), although four demonstrated a double-positive CD63 response. In addition, three patients showed reactions to both bee and wasp sting; two of them showed a double-positive CD63 response and only showed a single positive CD63 response. Concerning the severity of reactions, we were unable to demonstrate that basal serum tryptase was a relevant prognostic marker for the severity of anaphylactic sting reactions, as it was suggested earlier [2, 20]. Nevertheless, we were unable to find this correlation in our previous report as well [3].

A short interval between stings might increase the risk of a systemic reaction compared with longer intervals and, with increasing interval durations between stings, the risk steadily declines [21]. The percentage of affected patients who have not been stung for 10 years or who discontinued venom immunotherapy prematurely (after 1–2 years) remains in the range of 20–30% [22]. Nevertheless, 12 of our patients showed more than one systemic reaction. Four experienced the second reaction after an interval of more than a decade (15, 18, 20, and 48 years), with a severity comparable to the first systemic reaction (three of them demonstrated a positive CD63 response). These observations suggest a clear possibility of a persistent risk of a systemic sting reaction of up to five decades after the previous reaction.

In summary, our results suggest that the current standard diagnostic tests are good for a majority of patients; however, in a significant number of patients with a clear history of insect allergies, this testing had a limited diagnostic sensitivity. We propose that in patients who are negative for skin prick and sIgE testing, the CD63 basophil activation test is a very useful tool that complements intradermal skin tests and can improve the overall sensitivity of our diagnostic evaluation.

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