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Diagnostic value of the basophil activation test in evaluating Hymenoptera venom sensitization

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Summary. *Background:* Diagnosis of allergy to Hymenoptera venom is usually confirmed with skin testing and measurement of specific serum IgE antibody, tests which are sometimes inconclusive. In these cases, additional in vitro tests are necessary. The aim of this study was to show the applicability of the basophil activation test in detecting sensitization to Hymenoptera venom and to compare the test sensitivity and clinical positive-predictive value with skin prick tests and measurement of allergen-specific serum IgE.

Methods: This prospective study was conducted between June 2004 and December 2007 and included a large group of 204 patients. All patients had a history of at least one systemic allergic reaction of Müller grades II–IV after a Hymenoptera sting. We compared results of the basophil activation test, specific serum IgE and skin prick tests with patients' clinical history and data on culprit insects.

Results: The overall clinical sensitivities of the basophil activation test, specific serum IgE and skin prick tests were 90%, 76% and 64%, respectively; the clinical positive-predictive values of the three tests were 79%, 73% and 78% for bee venom, 86%, 59% and 43% for wasp venom; and 84%, 77% and 22% for both venoms.

Conclusions: Our results revealed a higher clinical sensitivity and comparable or better clinical positive-predictive value of basophil activation tests than skin prick tests and allergen-specific serum IgE in the detection of allergy to Hymenoptera venom.

Key words: Basophil activation test, CD63, Hymenoptera venom hypersensitivity.

Introduction

Hypersensitivity to Hymenoptera venom is a common and potentially life-threatening condition that can be effectively treated with immunotherapy [1]. For that reason, it is important that allergologists diagnose all patients who are at a high risk of serious systemic reactions. Skin tests and measurement of specific IgE antibodies in the serum can confirm the diagnosis after a Hymenoptera sting [2, 3]. However, clinical histories and results of confirmatory tests are sometimes contradictory. In these cases, additional tests are necessary, including cellular in vitro tests and, in some rare cases, potentially harmful venom challenge tests.

Basophils are often used as target cells for in vitro detection of IgE-mediated sensitization, as they play an important role in the pathogenesis of allergic anaphylactic reactions [4–6]. Stimulation of basophils with an allergen induces the release of a number of mediators (histamine, leukotriene C_4) and increased expression of a number of surface proteins (for example: CD45, CD63, CD69, CD203c) [2, 7, 8]. Flow cytometric quantification of basophil activation by measurement of CD63 expression [2, 3, 9–14] has been used in investigation of IgE-mediated hypersensitivity caused by classic inhalant allergens, latex, food, drugs and Hymenoptera venom [11]. The technique has also been demonstrated in prediction of side-effects during venom immunotherapy (VIT) [15] and evaluation of allergen-specific basophil sensitivity in patients not responding to VIT [16]. It is also valuable in the diagnosis of non-IgE-mediated reactions, such as drug hypersensitivity and detection of autoantibodies in certain forms of chronic urticaria [11].

The aim of this study was to show the applicability of the basophil activation test (BAT) in detection of sensitization to Hymenoptera venom and to compare its clinical sensitivity and clinical positive-predictive values with those of routine tests such as skin prick tests and measurement of allergen-specific serum IgE. Clinical history was used as the gold standard. In this prospective study, a large cohort of 204 patients were investigated between June 2004 and December 2007.

Materials and methods

Patients

In total, 204 patients (16–74 years old, mean 43 years; 90 women) with a history of systemic allergic reaction Müller grades II–IV after a bee, wasp (*Vespula* spp.) or hornet sting were included in this study. Bee and wasp venoms (Venomenhal®, HAL, Haarlem, The Netherlands) were used in skin prick tests at concentrations of 1, 10 and 100 µg/ml. In accordance with national guidelines, end-point titration is used in standardized skin-prick venom testing in our clinical practice. The concentration of specific IgE in serum was measured using CAP FEIA (Pharmacia, Uppsala, Sweden).

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Basophil activation assay

BD FastImmune (BD Biosciences, San Jose, CA, USA) was used for basophil activation tests, as described previously [15]. Briefly, whole blood with heparin anticoagulant was preincubated with basophil stimulation buffer containing IL-3, a final concentration of 0.1 µg/ml or 1 µg/ml of honey bee or wasp venom (Hal Allergie), and 0.55 µg/ml anti-FcεRI mAb (Buehlmann Laboratories, Basel, Switzerland) or 2 µM fMLP (Sigma, St. Louis, MO, USA) at 37°C for 15 min. Chilling on ice was used to stop degranulation and then FITC-conjugated anti-CD63 mAb, PE-conjugated anti-CD123 mAb, and PerCP-conjugated anti-HLA-DR mAb (BD Biosciences) were added and the sample was incubated for 20 min on ice. Finally, the samples were lysed, washed, fixed and analyzed within 2 h on a FACSCalibur flow cytometer (BD Biosciences). Data were acquired with a threshold on FL2 set to eliminate most CD123-negative cells, and at least 600 CD123-positive cells per sample were acquired. Basophils were identified as low side-scatter, CD123-positive and HLA-DR-negative cells and the percentage of activated basophils was measured on FL1 (CD63). The cut-off for positive results was 15% of CD63-positive basophils [14].

Analysis of data

The clinical validity of a diagnostic test defines its ability to detect or predict the associated disorder. Clinical sensitivity measures the proportion of individuals who have a well defined clinical disorder and whose test values are positive; clinical positive-predictive values more meaningfully define test performance by prediction of associated clinical phenotype.

The clinical sensitivity and the clinical positive-predictive values according to the culprit data were calculated as follows: all (single and double) positive results for an individual method were considered *Positive*; all measured results for an individual method were considered *All*; positive results for an individual method for the respective insect in each group of patients (allergy to bee venom, wasp venom, both venoms) were defined as *TruePositive*; all measured results for the individual method for each group of patients according to the culprit data (allergy to bee venom, wasp venom, both venoms) were defined as *AllGroup*. Thus,

$$ClinicalSensitivity = \frac{Positive}{All} \text{ and}$$

$$ClinicalPositive\ \Pr\ edictiveValue = \frac{TruePositive}{AllGroup}$$

Results

Between June 2004 and December of 2007, 204 patients with a clinical history of anaphylactic reactions Müller grades II–IV after a bee, wasp (*Vespula* spp.) or hornet sting underwent BATs. (During this period, two individuals were non-releasers and were not included in the study group.) We compared the results of BAT, specific IgE antibodies and skin prick tests with clinical history and culprit data (Table 1).

Patients allergic to bee stings

Among 86 patients with systemic allergic reactions after a bee sting, 68 (79%) exhibited positive BAT responses to bee venom (52 positive for both venoms), nine (10.5%) tested positive for wasp venom and nine (10.5%) were negative for both venoms in BATs. Sixty-three (73%) of the 86 patients had elevated specific IgE for bee venom (33 for both venoms), five (5%) had elevated specific IgE only for wasp venom and 19 (22 %) patients had no detectable levels of specific IgE antibodies. In skin prick tests, 49 (63%) of 78 patients tested positive for bee ven-

Table 1. Results of basophil activation tests, specific IgE determination and skin prick tests in relation to culprit data. All patients had systemic allergic reactions (Mueller grades II-IV) after a Hymenoptera sting

Culprit insect	Number of patients	Basophil activation tests (b/w:no. of patients)	Specific IgE (b/w:no. of patients)	Skin prick tests (b/w:no. of patients)
Honey bee	86	P/P:34	P/P:25, P/N:7, N/N:2	P/P:5, P/N:12, N/P:1, N/N:7, P/ND:4, N/ND:1 ND:4
		P/N:34	P/P:4, P/N:21, N/N:9	P/P:1, P/N:17, N/N:8, P/ND:3, N/ND:2, ND:3
		N/P:9	P/P:2, N/P:4, N/N:3	N/P:3, N/N:3, P/ND:2, ND:1
		N/N:9	P/P:2, P/N:1, N/P:1, N/N:5	P/N:3, N/N:3, P/ND:2, ND/P:1
Wasp/hornet	64	P/P:29	P/P:18, P/N:5, N/P:1, N/N:5	P/P:11, P/N:7, N/P:3, N/N:6, ND/P:1, ND/N:1
		P/N:3	P/N:2, N/N:1	P/P:1, P/N:1, N/N:1
		N/P:26	P/P:6, N/P:11, ND/P:1, N/N:8	P/P:3, P/N:2, N/P:5, N/N:11, ND/N:2, ND:3
		N/N:6	P/P:1, P/N:1, N/N:4	P/N:1, N/P:1, N/N:3, ND/P:1,
Honey bee and wasp/hornet	13	P/P:11	P/P:10, N/N:1	P/P:2, P/N:4, N/N:1, Not assessable:1, ND:3
		N/P:1	N/P:1	N/P:1
		N/N:1	N/N:1	P/N:1
Unrecognized	41	P/P:20	P/P:16, N/P:1, N/N:3	P/P:5, P/N:3, N/P:3, N/N:9
		P/N:6	P/P:1, P/N:2, N/N:3	P/P:2, N/N:4
		N/P:11	P/P:2, N/P:7, N/N:2	P/N:4, N/P:2, N/N:5
		N/N:4	P/P:1, N/P:1, N/N:2	P/P:1, N/N:3

b bee; *w* wasp; *P* positive; *N* negative; *ND* not determined.

om (6 positive for both venoms), five (6%) tested positive only for wasp venom and 24 (31%) tested negative for both venoms (3 patients not tested for wasp venom). Eight of the 86 patients did not undergo skin prick tests. Seventy-one of the 86 patients received VIT (69 with bee venom, one with wasp venom and one with both venoms).

Patients allergic to wasp stings

Among 64 patients with systemic allergic reactions after a wasp sting, 55 (86%) had positive BAT results for wasp venom (29 positive for both venoms), three (5%) had positive results only for bee venom and six (9%) had negative results for both venoms. In detection of specific IgE, 38 (59%) of the 64 patients had elevated levels of antibody to wasp venom (25 positive for both venoms), eight (13%) had specific IgE only for bee venom and 18 (28%) had no detectable levels of specific antibodies. In skin prick tests in 61 of this group of patients, only 26 (43%) showed positive responses to wasp venom (15 positive to both venoms), 11 (18%) had positive reactions only for bee venom and 24 (39%) patients (3 patients not tested) had no reaction. Three of the 64 patients did not undergo skin prick tests. Forty-nine out of the 64 patients received VIT (46 with wasp venom, 3 with both venoms).

Patients allergic to both bee and wasp stings

Among 13 patients who had reactions after both bee and wasp/hornet stings, 11(84%) tested positive for both venoms in BATs, one (8%) patient tested positive only for wasp venom and one (8%) exhibited negative results for both venoms. In this group of patients, specific IgE for both bee and wasp venom was detected in 10 (77%) and specific IgE for wasp venom in one (8 %); two (15%) patients had no detectable levels of specific IgE antibodies. In skin prick testing, two (22%) of nine patients tested positive for both venoms, five (56%) for bee venom, one (11%) for wasp venom, and one (11%) tested negative for both venoms. Three of the 13 patients did not undergo skin prick tests and, because of dermographism, one patient's results were not assessable. Eleven of this group received VIT (4 with bee venom, 2 with wasp venom, 5 with both venoms).

Patients allergic to unknown insect

The culprit insect was unknown in the remaining 41 of the 204 patients. In BATs, 20 (49%) of these patients tested positive for both venoms, 17 (41%) only for a single venom (6 bee venom, 11 wasp venom), and four (10%) patients tested negative for both venoms. In tests for specific IgE, 20 (49%) of the 41 patients had elevated levels for both venoms, 11 (27%) had elevated levels only for a single venom (2 bee venom, 9 wasp venom), and 10 (24%) had no detectable levels of specific IgE. In skin prick testing, eight (20%) of the 41 patients showed positive reactions for both venoms, 12 (29%) only for a single venom (7 bee venom, 5 wasp venom), and 21 (51%) patients showed no reaction. Thirty-one of these 41 patients received VIT (11 with bee venom, 14 with wasp venom, 6 with both venoms).

Comparative performance of the basophil activation test

In relation to the clinical history stated by the patient, the overall clinical sensitivity of the BAT was 90%, higher than the sensitivities of specific IgE (76%) and skin prick tests (64%) (Fig. 1). In relation to culprit data, the clinical positive-predictive value of BATs was 79% for bee venom, 86% for wasp venom and 84% for both venoms (Fig. 2). The respective clinical positive-predictive values of specific IgE and skin prick tests were 73% and 78% for bee venom, 59% and 43% for wasp venom, and 77% and 22% for both venoms (Fig. 2).

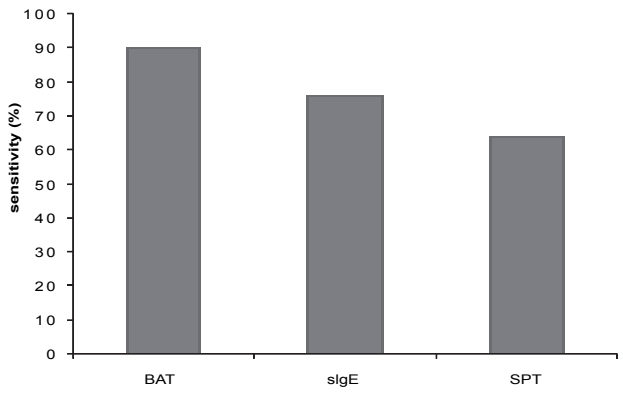

Fig. 1. Clinical sensitivity of BAT, specific IgE determination and skin prick tests in accordance with clinical data; *sIgE* specific antibody class E; *BAT* basophil activation test; *SPT* skin prick tests

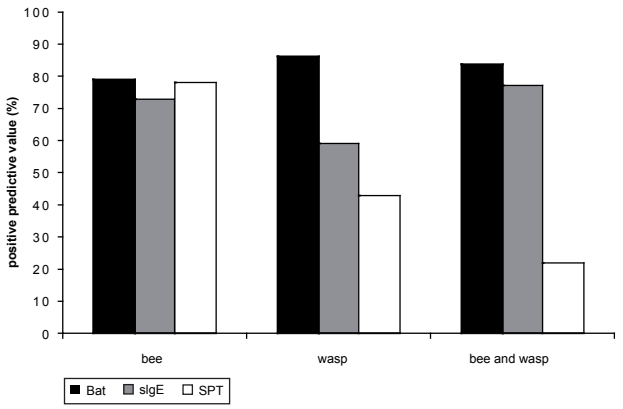

Fig. 2. Clinical positive-predictive values of BAT, specific IgE determination and skin prick tests for bee venom, wasp venom and for the two venoms in accordance with data on culprit insects; *sIgE* specific antibody class E; *BAT* basophil activation test; *SPT* skin prick tests

Discussion

Our results show that the BAT is a useful tool for diagnosing hypersensitivity to Hymenoptera venom, with a higher clinical sensitivity and comparable or even better clinical positive-predictive value than standard diagnostic procedures such as skin prick testing and measurement of allergen-specific serum IgE antibodies. These findings are consistent with those in other recent studies. For example, Erdmann et al. reported that BAT was comparable to intradermal skin tests and specific IgE in patients allergic to wasp venom, and was a useful tool for supplementation of routine diagnostic tests, as it increased the sensitivity of detection of venom sensitization [14]. Similar findings were obtained by Sturm et al. [13] and Eberlein-König et al. [12] for patients allergic to bee and wasp venom. However, we have shown the superiority of BAT over specific IgE measurement in a large group of patients in a prospective manner. Furthermore, 85% of patients in our group experienced severe systemic anaphylactic reactions of Müller grades III or IV after a bee, wasp (*Vespula* spp.) or hornet sting, stressing the importance of BAT in the diagnosis of venom allergy.

Double sensitization to bee and wasp venom typically raises the problem of choosing the correct venom for immunotherapy [17]. Our results suggest that the BAT could be a useful additional in vitro method in those cases. Of 88 patients with specific IgE antibody for both bee and wasp venoms, 15 patients (17%) were BAT-positive only for a single venom (10 for wasp venom, 5 for bee venom) and 69 for both venoms. Of 10 patients with positive BAT results for wasp venom, six (60%) claimed they experienced a systemic allergic reaction after a wasp sting, two after a bee sting and two patients did not recognize the insect. It is difficult to explain the wasp-positive BAT in two patients with a history of reaction to bee stings; however, patients quite frequently misidentify the culprit insect. We have to stress that we compared the diagnostic tests with the patient's history and not with the results of a sting challenge, which is also an unreliable option [18]. In addition, psychogenic reactions mimicking allergy are not rare and clinically irrelevant sensitization is found in at least 5% of the healthy population [19]. Of five patients with positive BAT results for bee venom, four (80%) had a history of systemic allergic reaction after a bee sting and one after the sting of an unrecognized insect.

The BAT is a very useful method for detecting venom sensitization, particularly in cases with inconclusive confirmatory tests that pose difficulties in the selection of venom for treatment [20, 21]. This was confirmed by our results, since the BAT was positive in 19 (73%) of 26 patients who experienced a systemic allergic reaction after an insect sting but had negative skin prick tests and no detectable specific IgE. Thirteen of these patients had a positive BAT for a single venom (6 for bee venom, 7 for wasp venom) and six for both venoms. Of all our patients, only four with negative skin prick tests underwent testing less than a month after the last sting. Measurement of specific IgE was repeated for all four patients, and thus the period between the systemic reaction and patient inclusion in the study could not affect the test results.

Nonreleasing basophils occur in 15–20% of the general population [22]. However, incubation with IL-3 restores expression of tyrosine kinase (Syk) and IgE-mediated activation of basophils [23]. As a consequence, the rate of nonreleasers decreases to approximately 5% and for those patients BAT is not a useful diagnostic tool [10, 11]. Among our patients, only two (1%) were nonreleasers. An important issue is the gating of basophils as CD123+/HLA DR- instead of IgE+ (anti-IgE/anti-CD63 technique) [10, 24]. The anti-IgE/anti-CD63 assay may fail in patients with low levels of IgE on their basophils, thus providing false-negative results and higher proportions of patients with nonreleasing basophils. Low levels of IgE, however, do not preclude functional responsiveness to a particular allergen [13, 24]. One of our two nonreleasers had an anaphylactic reaction of Mueller grade III after a bee sting and negative specific IgE and skin tests for both venoms. The second patient had an anaphylactic reaction of Mueller grade III after a wasp sting and had specific IgE antibodies and negative skin prick tests for both venoms and underwent wasp VIT.

In conclusion, the BAT is a useful diagnostic tool as it increases the sensitivity of in vitro tests for determining allergy to Hymenoptera venom. Furthermore, our results suggest that the BAT is particularly helpful in cases with negative or contradictory results of confirmatory tests and for the differentiation between clinically relevant and irrelevant sensitization.

Conflict of Interest
The authors declare that there is no conflict of interest.

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