

Clinical Routine Utility of Basophil Activation Testing for Diagnosis of Hymenoptera-Allergic Patients with Emphasis on Individuals with Negative Venom-Specific IgE Antibodies

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Key Words

Hymenoptera venom allergy · Diagnostic tests · Basophil activation test

Abstract

Background: Previous reports suggest the usefulness of basophil activation testing (BAT) in Hymenoptera-allergic patients with negative venom-specific IgE antibodies. We sought to evaluate the diagnostic utility of this testing in a routine clinical laboratory setting. **Materials and Methods:** Twenty-one patients with anaphylactic reactions to Hymenoptera sting (median grade III) and negative venom-specific IgE were routinely and prospectively tested with BAT. **Results:** We were able to diagnose 81% (17 of 21) of patients with BAT and 57% (12 of 21) with intradermal skin testing. Three wasp venom-allergic patients showed IgE positivity to rVes v 5. Four patients (19%) were negative for all tests. In the case of double-positive BAT, the culprit insect correlated with the venom that induced a significantly higher basophil response. **Conclusions:** BAT allows the identification of severe Hymenoptera-allergic patients with negative specific IgE and skin tests. The routine use of this cellular test should facilitate prescription of venom immunotherapy in complex cases with inconclusive diagnostic results.

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Introduction

Previous reports have clearly described patients with a history of systemic reactions after Hymenoptera sting, but with negative venom-specific IgE and skin test results [1–3]. Moreover, Golden et al. [1] showed that those patients could subsequently experience another severe reaction to sting because they demonstrated that after the sting challenge, the frequency of systemic reaction is comparable between patients with a positive (21%) and a negative (22%) skin test result. Furthermore, in cases of fatal sting anaphylaxis, venom-specific IgEs are very low or even undetectable in more than 30% of patients [3].

It was previously demonstrated that in patients with a positive history of anaphylactic sting reaction and negative venom-specific IgE and skin testing, the CD63 basophil activation test (BAT) could be a very useful tool that can improve overall sensitivity of the diagnostic evaluation [3, 4]. For this reason, a few years ago, we started using the BAT in routine diagnostic testing of patients with negative venom-specific IgE antibodies, preferably in those with a severe clinical reaction. In this report, we analyze the clinical utility of this cellular testing in our practice during the last 2.5 years, with special emphasis on comparison with other possible testing approaches for

double-negative patients and with the diagnostic and culprit consideration in the case of double-positive honeybee and wasp venom basophil response. We also analyzed whether the result of basophil testing in these complex cases could make it possible to accurately decide on and apply venom immunotherapy (VIT).

Materials and Methods

Study Patients

Twenty-one patients were recruited at the University Clinic of Respiratory and Allergic Diseases in Golnik from December 2009 to June 2012 (table 1). They all had a convincing history of at least one severe anaphylactic reaction to Hymenoptera sting (median Mueller grade III) and negative venom-specific IgE antibodies (table 1, 2). All investigations, except IgE reactivity to recombinant venom allergens, were prospectively performed in a routine clinical and laboratory setting.

Specific IgE, Total IgE and Tryptase Measurement

The serum concentration of specific IgE to wasp, honeybee, optional European hornet venom, rApi m 1, rVes v 5, and rVes v 1,

as well as of total IgE and tryptase was measured with ImmunoCAP-FEIA (Phadia, Uppsala, Sweden). In all patients, the measurements of specific IgE to wasp and honeybee venom were repeated within 1–2 months.

Intradermal Skin Testing

An intradermal skin test was done with up to 1 µg/ml of wasp and honeybee venom allergen as previously described [3].

Basophil Activation Test

A BAT was performed as previously described [3, 5–8]. Briefly, whole blood was incubated with 0.1 or 1 µg/ml of honeybee or wasp venom (Hal Allergie, Leiden, The Netherlands) or with 0.55 µg/ml of anti-FcεRI monoclonal antibody (mAb; Bühlmann Laboratories, Schönenbuch, Switzerland), or with 2 µM fMLP (Sigma, USA) as a control at 37°C for 15 min. Degranulation was stopped by chilling on ice, after which anti-CD63, anti-CD123 mAb and anti-HLA-DR mAbs (BD Biosciences, USA) were added and incubated for 20 min. Finally, whole-blood probes were lysed, washed, fixed and analyzed within 2 h on a FACSCalibur flow cytometer (BD Biosciences). The threshold value for diagnostically positive results was defined as 15% of CD63-positive basophils. This threshold was previously described in detail [3, 6, 8].

Table 1. Demographic data and the results of serum-specific IgE, total IgE and tryptase measurement

Patient No.	Sex	Age years	sIgE bee/wasp	Recombinant sIgE			Tryptase µg/l	Total IgE
				Api m 1	Ves v 5	Ves v 1		
1	F	63	<0.35/<0.35	<0.35	<0.35	<0.35	5.1	7.4
2	M	54	<0.35/<0.35	<0.35	<0.35	<0.35	3.0	16.6
3	F	33	<0.35/<0.35	<0.35	<0.35	<0.35	3.6	22.3
4	M	20	<0.35/<0.35	<0.35	<0.35	<0.35	4.1	741
5	M	55	<0.35/<0.35	<0.35	0.47	<0.35	4.6	11.4
6	F	74	<0.35/<0.35	<0.35	<0.35	<0.35	4.8	12
7	F	19	<0.35/<0.35	<0.35	<0.35	<0.35	6.7	45.7
8	F	40	<0.35/<0.35	<0.35	<0.35	<0.35	4.5	2.9
9	M	55	<0.35/<0.35	<0.35	<0.35	<0.35	7.4	19.1
10	F	52	<0.35/<0.35	<0.35	<0.35	<0.35	3.3	256
11	F	45	<0.35/<0.35	<0.35	<0.35	<0.35	6.6	8.1
12	F	40	<0.35/<0.35	<0.35	<0.35	<0.35	2.5	62.1
13	M	65	<0.35/<0.35	<0.35	<0.35	<0.35	5.0	13.5
14	M	50	<0.35/<0.35	<0.35	0.43	<0.35	7.5	39.5
15	M	43	<0.35/<0.35	<0.35	<0.35	<0.35	6.7	19.4
16	F	44	<0.35/<0.35	<0.35	<0.35	<0.35	3.1	266
17	F	49	<0.35/<0.35	<0.35	<0.35	<0.35	9.2	13.1
18	M	26	<0.35/<0.35	<0.35	<0.35	<0.35	5.4	16.3
19	M	16	<0.35/<0.35	<0.35	0.83	<0.35	1.4	251
20	F	33	<0.35/<0.35	<0.35	<0.35	<0.35	3.0	5.56
21	M	27	<0.35/<0.35	<0.35	<0.35	<0.35	41.9	3.25

Patients No. 11, 19 and 21 also showed negative specific IgE to hornet venom. In all patients, measurement of specific IgE to wasp and honeybee venom was repeated within 1–2 months. Data given in bold indicate positive sIgE results.

Results

Intradermal Skin Test

Twelve patients showed positive and 9 patients negative results for the intradermal skin test; 2 patients were positive for bee venom, 6 for wasp venom and 4 for both venoms (table 2).

Basophil Activation Test

The median percentages of basophil response to stimulation buffer (negative control), anti-FcεRI mAb and fMLP-positive control were 3% (range 2–4), 97% (28–100) and 60% (19–86), respectively. The median response to stimulation with allergens was 4% (range 2–72) and 17% (5–87) at 0.1 and 1 µg of honeybee venom, and 6% (range 1–68) and 46% (3–95) at 0.1 and 1 µg of wasp venom, respectively.

The cutoff value for positive results of allergen stimulation was set at 15% of CD63-positive basophils. Thus, 17 patients showed positive and 4 patients negative BAT results; 4 patients were positive for bee venom, 3 for wasp venom, and 10 patients for both venoms (table 2).

IgE Reactivity to Recombinant Venom Allergens

Three patients showed positive IgE reactivity to rVes v 5 and 18 patients were negative for rVes v 5. None of the patients demonstrated IgE reactivity for rVes v 1 and rApi m 1 (table 1).

Sensitivity in Patients with Negative sIgE

The diagnostic sensitivity of the intradermal skin test and BAT in patients with negative sIgE was 57% (12 of 21) and 81% (17 of 21), consequently. All 12 patients that tested positive for the intradermal test also showed posi-

Table 2. Clinical data and the results of basophil activation and intradermal skin tests

Patient No.	BAT, %		Intradermal skin tests		Culprit insect/time interval ¹	Clinical reaction	Planned VIT
	bee 0.1/1	wasp 0.1/1	bee 0.1/1	wasp 0.1/1			
1	4/23	16/57	neg./neg.	neg./neg.	?/3 years, 7 years	II	wasp
2	4/9	25/71	neg./neg.	pos./n.d.	wasp, hornet/18 years, 37 years	III	wasp
3	27/65	11/49	neg./ pos.	neg./ pos.	bee/15 years	III	bee
4	2/17	4/6	neg./ pos.	neg./neg.	?/3 months	IV	bee
5	4/7	23/86	neg./neg.	neg./ pos.	wasp/4 years	III	wasp
6	2/16	2/5	neg./neg.	neg./neg.	?/2 months, 6 years	III	bee
7	3/5	5/6	neg./neg.	neg./neg.	bee/4 months	III	no
8	9/19	16/30	pos./n.d.	pos./n.d.	?/2 months, 5 years	IV	bee and wasp
9	4/33	5/77	neg./neg.	neg./ pos.	wasp/2 months	IV	wasp
10	4/7	6/10	neg./neg.	neg./neg.	bee, wasp/2 months, 4 years	III	no
11 ²	7/17	2/4	neg./neg.	neg./neg.	hornet/12 years	IV	no
12	19/25	15/46	neg./neg.	pos./n.d.	wasp/10 years	II	wasp
13	72/87	4/36	pos./n.d.	pos./n.d.	bee/1 months, 1 per 2–3 years, 20 years	III	bee
14	6/16	6/53	neg./neg.	neg./neg.	wasp/2 years	IV	wasp
15	8/9	5/9	neg./neg.	neg./neg.	wasp/4 months	IV	no
16 ³	6/19	1/3	neg./ pos.	neg./neg.	wasp/2 months	IV	no
17	4/13	5/9	neg./neg.	neg./neg.	wasp/4 months	IV	no
18	4/16	24/84	neg./neg.	pos./n.d.	wasp/5 months	II	no
19	4/12	26/65	neg./ pos.	neg./ pos.	hornet/1.5 years	IV	wasp
20	5/22	68/95	neg./neg.	neg./ pos.	wasp/1 years	IV	wasp
21	3/25	10/70	neg./neg.	neg./neg.	hornet/8 years	III	wasp

Data given in bold indicate positive BAT or intradermal skin test results. n.d. = Not defined.

¹ Time interval between last sting reaction and the start of diagnostic evaluation.

² In patient No. 11, VIT was not planned due to conflicting diagnostic and culprit insect data.

³ In patient No. 16, VIT was not planned yet due to further testing for conformation of culprit venom.

tive results for BAT. All 4 patients that tested negative for BAT also showed negative results for the intradermal test. The sensitivity of specific IgE to rVes v 5 was 14% (3 of 21), and 23% (3 of 13) for wasp BAT-positive and/or intradermal-positive subjects.

Sensitivity in Patients with Negative sIgE and Intradermal Skin Test

Out of 9 patients that tested negative for the sIgE and intradermal skin test, 5 of them had a positive BAT response. Thus, the diagnostic sensitivity of the BAT in this subgroup of patients was 56%.

Specificity

The specificity was analyzed according to the culprit data, which were apparent for 10 intradermal and 13 BAT-positive patients (table 2). In 6 subjects with a single positive intradermal skin test, the culprit data were matching, and in 1 subject, they were not. Three subjects showed double-positive intradermal test results, but a single-positive culprit insect. In 3 subjects with a single-positive BAT, the culprit data were matching, and in 2 subjects, they were not. Eight subjects showed a double-positive BAT, but a single-positive culprit insect. However, in all those double-positive patients, the basophil response to culprit insect venom was markedly higher than the response to the clinically irrelevant venom: median 20% (range 5–72) and 74% (range 46–95) at 0.1 and 1 µg of culprit venom, and 5% (range 3–19) and 25% (range 16–49) at 0.1 and 1 µg of irrelevant venom, respectively ($p \leq 0.01$; fig. 1). Consequently, the diagnostic specificity of the intradermal test was 60% (6 of 10), and of BAT, with regard to higher response, 85% (11 of 13).

Adherence to VIT

The VIT was planned in 14 subjects; in 4 patients, it was indicated with bee venom, in 9 patients with wasp venom, and in 1 patient with both venoms. It was also planned in 4 patients (in 3 with wasp venom and in 1 with bee venom) that showed only positive BAT results. It was not indicated in 4 subjects negative for all tests, in 2 subjects with conflicting culprit and testing data (No. 11 and 16; table 2), and in 1 subject with a grade II reaction without risk factors.

Discussion

In this study, we showed that the routine use of BAT allows the prompt identification of severe Hymenoptera venom-allergic patients with inconclusive specific IgE

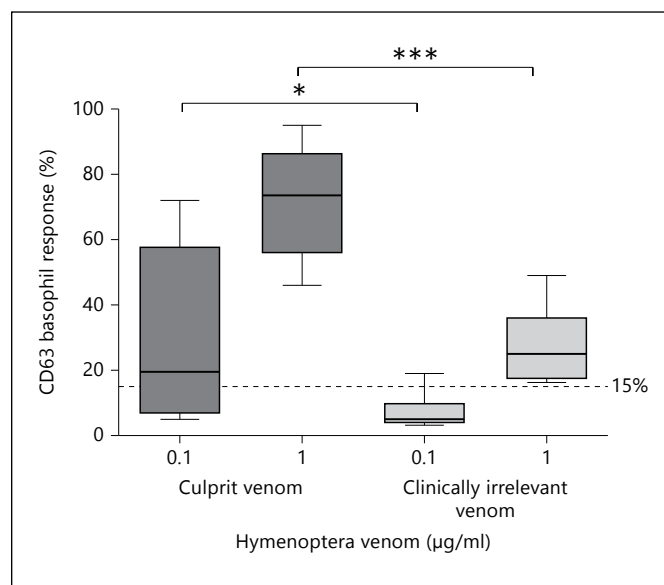


Fig. 1. Basophil response to culprit and clinically irrelevant venom in double-positive patients (n = 8). * $p < 0.05$; *** $p < 0.001$.

and skin test results. Thus, clinical utilization of BAT in practice should facilitate the prescription of VIT in complex cases.

Because VIT decisions rely on confirmation of allergic sensitization, it is clear that the management of those patients requires very sensitive diagnostic evaluation. According to current guidelines, the standard tests include skin testing and analysis of the serum for specific IgE to whole venom extract [9–11]. Nevertheless, a significant number of patients, and also those that may subsequently experience another severe or even fatal reaction to a sting, demonstrate negative sIgE and skin tests [1–3]. For such patients, the need for additional diagnostic approaches is obvious. In the field, a Hymenoptera venom allergy BAT was largely tested for the last 10 years either as a diagnostic or prognostic tool, or for monitoring VIT [3–8, 12–15]. However, those studies were carried out in an advance research setting on selected patients, and thus, there is a lack of data about the practical utility of BAT in a routine clinical setting and how those results could actually affect immunotherapy decision making. For this reason, in our study, all testing in all patients, except sIgE to recombinant venom allergens, was initiated prospectively by a practitioner allergist as a standard diagnostic evaluation of severe complex cases at our clinic.

Similar to a previous study [3], we clearly demonstrated that in patients with negative venom-specific IgE,

the diagnostic sensitivity of BAT is about 80% and the sensitivity of the intradermal skin test is about 50%. In other words, with BAT, we can successfully identify more than half of severe Hymenoptera-allergic patients with both negative sIgE and intradermal skin tests. Recently, it was suggested that more than half of wasp-allergic patients with negative sIgE to wasp venom might have positive IgE reactivity to rVes v 5 [15]. We also recently tested 25 patients with an anaphylactic reaction after wasp sting, but with negative wasp venom-specific IgE, and found that 32% of them showed positive rVes v 5 results [16]. However, all 25 of these wasp venom-allergic subjects showed clearly positive wasp-venom basophil responses. Furthermore, in the current report, only 14–23% of patients showed positive IgE reactivity to rVes v 5 and none of the patients were positive for other recombinants. Therefore, the addition of rVes v 5 or other recombinant IgE testing might be useful for diagnosing only a few patients.

Diagnosis and selection of the disease-causing insect venom is very important because VIT is the only effective prophylaxis for life-threatening reactions in patients allergic to Hymenoptera venom. Furthermore, in Hymenoptera-allergic patients, double IgE positivity to bee and wasp venom is often evident, and thus, the identification of the culprit venom could be difficult [17, 18]. Moreover, in the case of Hymenoptera-allergic patients with negative venom-specific IgE antibodies, both BAT and/or intradermal tests can show double-positive results [3], and

this double positivity can hamper the identification of the relevant venom for immunotherapy, especially if the patient has an anaphylactic reaction to only one insect. Importantly, when we analyzed BAT double-positive patients in whom a single insect causing an allergic reaction has been identified, we showed a markedly higher basophil response to the culprit venom than to the clinically irrelevant venom. Similar observations were recently demonstrated by Eberlein et al. [19] which showed a much stronger basophil reaction to the clinically relevant insect venom than to the irrelevant one in double IgE-positive patients. On the other hand, in cases with double-positive intradermal tests, there was no correlation with a single culprit insect, and similar observations were also demonstrated by Eberlein et al. [19]. These results clearly suggest the clinical relevance of basophil response, not only for conformation of sensitization or for monitoring VIT [5, 6], but also as a tool for dissecting bee and wasp allergy as a basis for accurately prescribing the culprit venom for immunotherapy.

In summary, we demonstrated that in complex cases with inconclusive diagnostic results, BAT is more clinically sensitive and relevant than any other type of testing. Therefore, Hymenoptera-venom BAT should become more broadly available in clinical practice and should be routinely performed in patients that have a history of a reaction suggestive of severe insect sting anaphylaxis, but in whom testing for specific IgE antibodies and skin tests yield negative results.

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