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The Basophil Activation Test Is Not a Useful Screening Tool for Hymenoptera Venom-Related Anaphylaxis in Patients with Systemic Mastocytosis

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Key Words
Basophil activation · Hymenoptera · Systemic mastocytosis

Abstract
Background: Systemic mastocytosis (SM) patients are at a high risk for anaphylaxis, with Hymenoptera as the main culprit. A screening instrument to identify which patients are sensitized to Hymenoptera before they experience anaphylaxis would therefore be of great value. The basophil activation test (BAT) is proposed as a possible tool for diagnosing Hymenoptera venom-related allergy (HVA), especially in patients in whom conventional allergy tests yield contradictory results. ***Methods:*** We included outpatients with SM, according to WHO criteria, from September 2011 to January 2012. Next, to obtain various clinical data including intradermal test results, specific immunoglobulin E (sIgE) measurements and BAT were performed. ***Results:*** We included 29 patients, 9 of whom had a history of HVA and 4 of whom had experienced anaphylaxis due to other triggers. Sixteen patients had no history of anaphylaxis. sIgE was detected in 6 patients with HVA and in 2 patients with anaphylaxis due to other triggers. The BAT was positive in only 1 patient, in whom the skin test and sIgE were also positive. Compared to patients with skin lesions, those without skin lesions had significantly more anaphylaxis and sIgE to Hymenoptera. During a 3-year follow-up, no one experienced new anaphylactic episodes. ***Conclusion:*** The BAT is not a reliable tool for randomly screening SM patients for HVA.

Introduction

Systemic mastocytosis (SM) is a rare disease characterized by the proliferation of aberrant mast cells in which at least one extracutaneous organ is affected [1]. Since mast cells are the culprit cells of type I hypersensitivity reactions, SM patients are at a high risk for anaphylaxis, with a cumulative incidence ranging between 20 and 49% [2–4]. Another study showed that 12% of the patients of a cohort had life-threatening anaphylaxis, sometimes with serious cerebral hypoxic damage [5]. Patients with skin lesions have a lower lifetime risk of anaphylaxis than patients without skin lesions [6]. Many triggers can cause anaphylactic reactions, but most cases of SM are Hymenoptera venom related [5]. This can obviously lead to life-threatening situations. Therefore, it would be of great val-

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ue to identify those patients who are sensitized to Hymenoptera venom before they experience anaphylaxis, and maybe even preemptively treat them with immunotherapy. Conventional tests including intradermal tests and measurement of specific immunoglobulin E (sIgE) in serum are feasible to confirm sensitization after a patient has experienced an anaphylactic reaction, but they are not currently deemed useful for screening purposes. In particular, the presence of sIgE does not always predict Hymenoptera venom-related allergy (HVA). The basophil activation test (BAT) has been proposed as a useful adjunct in the diagnosis of allergic disease, especially in patients with negative or contradictory conventional tests [7–10]. In the BAT, basophils are used as an in vitro model for mast cells, despite their slightly different characteristics regarding appearance and function. Both, however, contain granules of preformed molecules that can cause an anaphylactic reaction after degranulation. Degranulation occurs after a wide range of stimuli, for instance activation by IgE, complement mediators, or bacteria-derived peptides [11]. Using the BAT, both IgE-mediated and IgE-independent type 1 hypersensitivity can be measured in vitro [12–14]. In previous studies, the BAT has been found to have varying diagnostic characteristics when compared with intradermal tests and sIgE measurement in populations with, as well as without, mastocytosis [15, 16].

Subjects and Methods

The objective of this study was to determine whether the BAT is also applicable for screening SM patients for Hymenoptera venom sensitization and thereby their risk for anaphylaxis due to a wasp sting. We used sIgE as a control measurement to determine who was sensitized to Hymenoptera venom.

Subjects

From September 2011 to January 2012, we prospectively included patients who presented to the outpatient clinic for a routine visit and who fulfilled the WHO criteria for SM [17]. We collected both demographic and disease related-data including personal characteristics, the subtype of SM, and a detailed history regarding anaphylaxis. All patients gave informed consent. We retrospectively checked all patient files until September 2015 (3 years) for new episodes of anaphylaxis.

Basophil Activation Test

Venom-activated basophils were identified by flow cytometry using the Flow2 CAST system (Bühlmann Laboratories AG, Schönenbuch, Switzerland) according to the manufacturer's instructions. Briefly, 50 µl EDTA-anticoagulated whole blood of the patient were incubated for 25 min at 37°C with 50 µl venom extract diluted to 100 ng/ml in the presence of 100 µl stimulation buffer containing calcium and IL-3. We used the Hymenoptera venom included in the Flow2 CAST kit. Subsequently, 20 µl fluorochrome-labeled monoclonal anti-CD63 antibodies and anti-CCR3 antibodies (staining reagent) were added (Bühlmann Laboratories). After washing the samples, flow cytometry was performed using a FACSCalibur device (BD BioSciences, USA) to detect degranulated CCR3-positive basophils based on the amount of CD63 expression. We used stimulation buffer as the negative control, and the positive control consisted of anti-FcεRI antibodies (all from Bühlmann Laboratories). An increase of <15% in CD63-positive

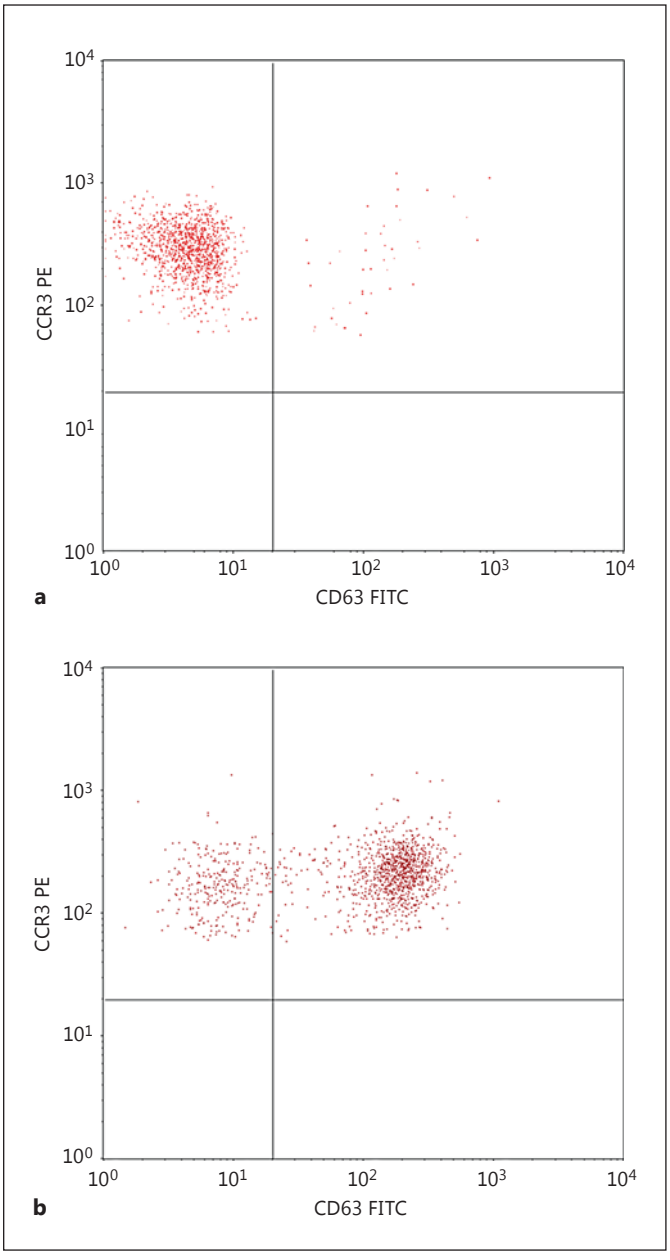

Fig. 1. Flow cytometry plots of a negative BAT (**a**) and a positive BAT (**b**), according to the surface CD63 expression on basophils.

Table 1. Patient characteristics (n = 29)

	No history of anaphylaxis (n = 16)	History of anaphylaxis not Hymenoptera-related (n = 4)	Hymenoptera-related anaphylaxis (n = 9)
Age at diagnosis, years	44 (25–56)	50 (38–63)	52.5 (36–72)
Females	9/16	4/4	4/9
Time anaphylaxis to BAT, years	N/A	2 (0.5–6)	3 (2–10)
Wasp sting after the diagnosis	0/14	0/4	0/8
Wasp immunotherapy before the BAT	N/A	0/4	1/9
Subtype of SM			
ISM	14/16	4/4	9/9
ISMs+	12/14	2/2	5/9
ISMs–	2/14	2/2	4/9
ASM	2/2	0/0	0/0
WHO criteria[a]			
Skin lesions	14/16	2/4	5/9
Bone marrow histology positive	5/6[b]	1/1	3/3
Bone marrow morphology positive	10/10	2/2	8/8
Tryptase at diagnosis, ng/l	38.7 (9.8–324.0)	31 (22.6–44.1)	23.7 (15.9–160)
D816V mutation detected	6/8	2/3	6/6
CD2 or CD25 detected	8/8	2/2	6/6
Conventional allergy tests			
Intradermal tests for wasp positivity	N/A	0/4	7/9
Specific IgE to Hymenoptera venom	0/16	1/4	6/9
Specific IgE levels, kU/l	–	0.29	0.60 (0.22–4.61)
Specific IgE to bee venom	0/16	0/16	1/9

Values are presented as numbers/total or medians (range) unless otherwise stated. ASM = Aggressive systemic mastocytosis; N/A = not applicable. [a] Some data are missing because the initial workup for SM was performed at other medical centers. [b] One patient had a negative bone marrow biopsy for SM but fulfilled all 3 minor WHO criteria for SM.

basophils, compared with the negative control, was considered negative. An increase >15% in CD63-positive basophils was considered a positive result. Results were only considered suitable for analysis when the negative and positive controls gave the expected results. Figure 1 shows a flow cytometry plot of patient-derived leukocytes indicating CCR3-positive basophils (selected), either <15% degranulated (fig. 1a) or >15% degranulated (fig. 1b), based on CD63 expression.

Specific IgE Measurement

sIgE antibodies against bee venom (i1) and wasp venom (i3) were determined using the Phadia 250 system (Thermo Fisher Scientific/Phadia B.V., Freiburg, Germany). Values of sIgE below 0.35 kU/l were considered negative. All analyses were performed according to the manufacturer's instructions.

Statistical Analysis

SPSS 21 (IBM SPSS Inc., USA) was used for all statistical analyses. Values are reported as medians with a range or medians with a standard deviation (SD). We used χ^2 and t tests to compare different subgroups. However, because of the small population, we only performed statistical analysis on relevant characteristics.

Results

We included 29 patients (table 1). The median age was 48 years (range 25–72), and 17 patients were female (59%). None of the patients were using systemic corticosteroids at the time of our study. Patients with a history of anaphylaxis had a lower median serum tryptase level (p = 0.438) than patients without anaphylaxis, although this did not reach statistical significance. Of the 13 patients who experienced at least 1 anaphylactic reaction, 9 patients reported a wasp sting as the trigger. Three patients had food-related anaphylaxis after ingestion of prawns, hazelnuts, or peanuts, respectively. In 1 patient, the trigger for anaphylaxis was unidentified.

Appropriate skin tests were performed in all patients with reported anaphylaxis after a wasp sting. In 2 of these 9 patients, skin tests were negative. These patients also had undetectable sIgE for either wasp or bee venom.

Of the 9 patients who reported Hymenoptera-related anaphylaxis, 5 had detectable sIgE levels for Hymenoptera venom. One of these patients had detectable sIgE for bee venom, without a clinical history of bee-venom related anaphylaxis. Skin testing in this patient was positive for wasp venom, but negative for bee venom, and thus the sIgE for bee venom probably represents irrelevant cross-reactivity with sIgE for wasp venom. Interestingly, 1 patient who reported anaphylaxis due to medication or prawns also had detectable sIgE for Hymenoptera venom.

All BAT were considered suitable for analysis. Among the 29 patients, the BAT was positive in only 1 patient. This patient had indolent SM (ISM) without skin lesions and had experienced wasp-related anaphylaxis 2 years before inclusion in our study. Of note, she also had the highest level of sIgE to Hymenoptera venom in our population (4.61 kU/l) and a positive intradermal test for wasp venom. sIgE for bee venom was not detectable in this patient. In the 3-year follow-up period, 1 patient was lost to follow-up. Of the other 28 patients, no one reported another wasp sting, and no one experienced a new episode of anaphylaxis.

Lastly, data were viewed from a different perspective for additional information. Irrespectively of their clinical history of anaphylaxis, patients with ISM were further divided into patients with or without skin lesions (ISMs+ vs. ISMs–, respectively). Anaphylaxis was significantly more frequent in ISMs– versus ISMs+ patients (6/7 vs. 7/20, $p = 0.021$). In accordance with this, sIgE to Hymenoptera venom also was significantly more often detectable in ISMs– versus ISMs+ patients (5/7 vs. 2/20, $p = 0.001$).

Discussion

We studied the feasibility of the BAT as a screening tool for Hymenoptera sensitization in patients with SM. Of all of the patients, only 1 had a positive BAT and this patient also tested positive for the conventional allergy tests (sIgE and skin test). However, 8 other patients with a clinical history of HVA, 5 of whom also had demonstrable sIgE to Hymenoptera and 6 of whom had positive intradermal tests, tested negative with the BAT. These tests thus did not correlate well in our population, nor did the BAT add useful information to the standard combination of clinical history, sIgE, and skin tests. In addition to new data about the BAT, our study provides additional evidence on the difference between ISMs+ and ISMs– patients. It has become increasingly clear in recent research that these two subtypes of ISM have entirely different clinical phenotypes [6, unpubl. data]. Our results confirm that anaphylaxis is more common in ISMs– patients versus ISMs+.

The role and usefulness of the BAT remains a topic of discussion in the current literature, with earlier studies reporting conflicting evidence. Bidad et al. [16] studied the role of the BAT in the diagnosis and monitoring of HVA in SM patients. They found a sensitivity of 87% and a specificity of 100% in their population. However, all patients with HVA also had a positive intradermal test, rendering the addition of the BAT redundant. Moreover, the sIgE level was significantly higher in this group compared to our population. We would therefore postulate that the BAT does not add useful information to the conventional diagnostic tests for HVA. This hypothesis is supported by the results of Bonadonna et al. [18]. They investigated the role of the BAT in SM patients with or without a reported reaction to Hymenoptera stings but all without demonstrable sIgE. In this population, BAT results were all negative and performing a BAT on top of sIgE and intradermal tests did not contribute to the diagnosis of HVA. Furthermore, Gonzalez-de-Olano et al. [19] investigated the BAT in patients with HVA and SM compared to patients with HVA but no SM. Specific IgE was detected in 15 of 22 SM patients, 9 of whom had a positive BAT result. Of the 7 SM patients without sIgE, 3 had a positive BAT. Using extracts of different wasp and bee species, they even found the culprit insect in 2 of these sIgE-negative patients. Another study by Eberlein-König et al. [8] also concluded that they were able to identify the culprit insect via the BAT in a few complicated cases in which intradermal tests and sIgE measurement could not lead to a diagnosis.

In addition to the different populations studied, an explanation for the strikingly different outcomes in all of these studies can be found in technical differences. The BAT has not been standardized completely yet, and different laboratories use different techniques. For instance, some laboratories use purified cells in their analyses, which can increase the sensitivity of the BAT [20]. Cut-off values and incubation substances also vary. Moreover, when working with basophils, one has to realize that basophils differ from (neoplastic) mast cells in certain ways, mainly in terms of non-IgE-mediated activation [21]. The main limitations of this specific study are the small number of patients investigated and the heterogeneity within our population. However, we chose this method on purpose to resemble daily practice. Moreover, the main aim was to investigate the role of the BAT as a screening method rather than to confirm the presence of an allergy that

had already been confirmed by either clinical anaphylaxis or conventional tests. Therefore, we also included patients without a history of anaphylaxis, which leads to a lower a priori incidence of HVA and thereby a lower sensitivity of the BAT in this group.

Lastly, 2 patients with HVA had received immunotherapy several years before this study, which could obviously have influenced the results of the BAT, since this is expected to become negative after immunotherapy. One of those patients, however, experienced an episode of wasp-related anaphylaxis after immunotherapy and therefore the effect of immunotherapy apparently was negligible.

Conclusions

Considering our results in relation to previous studies, we prudently conclude that the BAT is not a useful test to screen random SM patients for their risk of HVA. Furthermore, by combining the patient's clinical history, intradermal testing, and sIgE measurement, a diagnosis of HVA can be confirmed or declined in most patients. The BAT may be of additional use in very complicated patients with conflicting results of conventional tests, but we could not confirm this in our study. More systematic research in a carefully selected population might provide more insight in the role of the BAT in this niche.

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