

Hymenoptera Venom Allergy: Taking the Sting Out of Difficult Cases

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■ Abstract

Background: Correct identification of the culprit venom is a prerequisite for specific venom immunotherapy.

Objective: To assess whether the basophil activation test (BAT) constitutes an additional diagnostic instrument in patients with equivocal or negative specific immunoglobulin (Ig) E or venom skin test (VST) results.

Methods: One hundred eighteen patients with a compelling history of IgE-mediated hymenoptera venom allergy were enrolled. Venom-specific IgE was quantified by ImmunoCAP and VST was performed in all patients. Basophil activation was analyzed by flow cytometry after labeling with anti-IgE and anti-CD63.

Results: In 64 out of 118 patients, diagnosis was considered as definite and the entomologic description was confirmed by unequivocal and concordant positive specific IgE and VST results. In 53 of those 64 patients, BAT confirmed diagnosis, whereas the remaining 11 patients were nonresponsive in the BAT analysis. Forty-seven patients (40%) had a tentative diagnosis of venom allergy, as they had divergent specific IgE or VST results. In 31 of those patients, BAT was positive only for the suspected venom and helped to establish diagnosis of wasp and honeybee venom allergy in 28 and 3 patients, respectively. BAT was diagnostic in 7 patients with complete negative results for specific IgE and VST, despite clear entomologic identification.

Conclusions: In about half the patients with diagnosis of venom allergy, unequivocal specific IgE and VST results are obtained and additional tests are not needed. In the remainder, diagnosis is less straightforward due to discrepant or negative specific IgE or VST results. In these patients, BAT constitutes a helpful additional instrument to identify the culprit venom and start venom immunotherapy accordingly.

Key words: Anaphylaxis. CD63. Basophil activation test. Flow cytometry. Immunoglobulin E. Immunotherapy. Skin test.

■ Resumen

Antecedentes: La identificación correcta del veneno responsable es un requisito previo a la administración de inmunoterapia específica con veneno.

Objetivo: Valorar si el test de activación de basófilos (TAB) constituye un instrumento diagnóstico adicional en los pacientes con resultados de inmunoglobulina (Ig) E específica negativos o equívocos o de la prueba cutánea con veneno.

Métodos: Se inscribieron al estudio 118 pacientes con antecedentes incuestionables de alergia al veneno de himenópteros mediada por la IgE. Se cuantificó la IgE específica al veneno con ImmunoCAP y se realizaron pruebas cutáneas con veneno a todos los pacientes. La activación de basófilos se analizó por citometría de flujo con anti-IgE y anti-CD63.

Resultados: En 64 de los 118 pacientes, el diagnóstico se consideró definitivo, y la descripción entomológica se confirmó mediante los resultados de la prueba cutánea y la IgE específica positiva concordantes e inequívocos. En 53 de los 64 pacientes, el TAB confirmó el diagnóstico, mientras que no se produjo una respuesta en el análisis TAB en los 11 pacientes restantes. Cuarenta y siete de los pacientes (40%) tuvieron un diagnóstico no definitivo de alergia al veneno, ya que los resultados de la prueba cutánea o de la IgE específica fueron divergentes. En 31 de estos pacientes, el TAB sólo fue positivo para el veneno sospechoso y ayudó a establecer el diagnóstico de alergia al veneno de avispa y de abeja en 28 y en 3 de los pacientes, respectivamente. El TAB sirvió como diagnóstico de 7 pacientes con resultados completamente negativos para la IgE específica y prueba cutánea, a pesar de la clara identificación entomológica.

Conclusiones: En aproximadamente la mitad de los pacientes con diagnóstico de alergia al veneno, se obtienen resultados inequívocos a la prueba cutánea e IgE específica y no es necesaria la realización de pruebas adicionales. En el resto de pacientes, el diagnóstico no es tan claro debido a la IgE específica negativa o discrepante o a los resultados de la prueba cutánea. En estos pacientes, el TAB constituye un instrumento adicional de utilidad para identificar el veneno responsable e iniciar la inmunoterapia específica correspondiente.

Palabras clave: Anafilaxia. CD63. Test de activación de basófilos. Citometría de flujo. Inmunoglobulina E. Inmunoterapia. Prueba cutánea.

Introduction

Immunoglobulin (Ig) E-mediated hymenoptera venom allergy is an important health problem and correct diagnosis is a prerequisite for effective management with specific immunotherapy. Therefore, diagnosis of venom allergy ideally should rest upon different confirmatory tests rather than on a single one. At present, physicians generally rely upon quantification of specific IgE and venom skin tests (VST) to confirm their clinical suspicion. Although both methods can provide useful information, neither of them has an absolute predictive value [1-8]. Recently, the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity stated that in cases where quantification of specific IgE and VST remain negative or yield contradictory or equivocal results, cellular tests may be used to demonstrate immunologic sensitization [9].

Upon encounter of specific allergen that crosslinks FcεRI-bound IgE, basophils not only synthesize and secrete bioactive mediators, but also upregulate the expression of certain activation markers that can be quantified flow cytometrically in the basophil activation test (BAT) [10,11]. On several occasions, BAT has proved to be reliable to diagnose hymenoptera venom anaphylaxis, with a sensitivity and specificity readily exceeding 80% to 85% [12-15]. In contrast, the potential of BAT in the diagnosis of more difficult cases has only been addressed in a small study of patients with uncertain histories regarding insect species and inconsistent test results [16].

The main objective of this study was to evaluate the use of BAT in venom-allergic patients for whom a clear entomologic identification of the culprit insect was available but who had equivocal or negative specific IgE or VST results.

Methods

Subjects

The study included 118 patients with a compelling history of hymenoptera venom allergy: 100 to wasp venom and 18 to honeybee. All had suffered from pronounced urticaria, severe angioedema, rhinoconjunctivitis, bronchospasm, or generalized anaphylaxis with hypotension or shock and provided an unequivocal entomologic identification of the culprit insect.

Serum IgE

Specific IgE for wasp (*Vespa vulgaris*) and honeybee (*Apis mellifera*) venom was quantified by Immuno-CAP (Phadia AB, Uppsala, Sweden). According to the manufacturer's instructions, results ≥ 0.35 kU_A/L were considered positive.

Venom Skin Tests

All patients had a VST using an intradermal endpoint titration method (10^{-4} up to 1 μ g/mL solutions of wasp and honeybee venom; Pharmalgen, ALK-Abelló, A/S, Denmark).

VST were considered positive when the wheal and flare reaction exceeded a diameter of 5 mm.

Basophil Activation Test

The details of the BAT technique are described elsewhere [15]. Briefly, within 3 hours of sampling, aliquots of whole blood were preincubated with an interleukin 3-containing stimulation buffer. Preactivated blood samples were stimulated with commercial wasp and honeybee venom (Pharmalgen, ALK-Abelló, A/S, Denmark), anti-IgE (Pharming, BD Biosciences, Erembodegem, Belgium) as a positive control, or washing solution to measure spontaneous CD63 expression (negative control). To quantify activated basophils, cells were stained with biotinylated anti-human IgE (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and phycoerythrin (PE)-conjugated anti-human CD63 (Pharming, BD Biosciences, Erembodegem, Belgium) or a PE-conjugated irrelevant (control) antibody of identical isotype (Pharming). After washing, 20 μ L of Streptavidin Alexa 488 (Molecular Probes, Leiden, The Netherlands) was added for 15 minutes at room temperature. Red blood cells were lysed and white blood cells fixed (FACS Lysing solution, BD Biosciences, Erembodegem, Belgium) for 10 minutes at room temperature. After centrifugation, cells were resuspended in washing solution and added to the cell pellets. Flow-cytometric analysis of basophil activation was performed on a FACScan flow cytometer (BD Immunocytometry Systems, San-José, USA). IgE-staining and side scatter were employed to gate on at least 500 basophils that expressed a high density of surface IgE. Within this gate the percentage of activated basophils (coexpressing CD63) was measured. Percentages of activated basophils were corrected by subtracting spontaneous CD63 expression from the value obtained with allergen stimulation. Thresholds for positivity of BAT were calculated by receiver operating characteristics analysis and were found to be 26% of CD63-positive basophils for wasp venom [15] and 15% for honeybee [17].

Results

Definitive Diagnosis of Venom Allergy

As summarized in the figure, in 64 of the 118 patients (54%) diagnosis of their venom allergy was considered as definitive. In those patients (57 with wasp and 7 with honeybee allergy), their history was confirmed by unequivocal and concordant specific IgE and VST results for the culprit venom, whereas specific IgE and VST were negative for the irrelevant venom. Eleven of the 64 patients (17%) were nonresponsive in the BAT and failed to upregulate expression of CD63 after stimulation with anti-IgE (positive control) and relevant venom.

Tentative Diagnosis of Venom Allergy

Forty-seven of the 118 patients (40%) had a tentative diagnosis of wasp (37) or honeybee (10) venom allergy on the basis of divergent specific IgE and VST results (figure). The

most frequently observed barrier to a definite diagnosis in these patients was the presence of double-positive specific IgE results for wasp and honeybee venom. Such results were present in 28 of these 47 patients (or 24% of the total population). Twenty-one patients with wasp venom allergy had specific IgE for honeybee venom and 7 patients with honeybee venom allergy had specific IgE antibodies for wasp venom. In 6 of the 28 patients (or 5% of the total population) with double-positive IgE results, the VST was of no additional diagnostic help, as it was either double negative or double positive. However, all had their diagnosis of wasp venom allergy confirmed by a positive BAT for wasp venom and negative BAT for honeybee venom, except 1 beekeeper with honeybee venom allergy (figure).

In 16 patients, diagnosis of wasp venom allergy was hindered by the fact that only 1 confirmatory test was obtained: either positive specific IgE ($n = 9$) or a positive VST ($n = 7$). In 14 of those patients, BAT was confirmative and contributed to the final diagnosis.

Three beekeepers with honeybee venom anaphylaxis had inconclusive double-positive ($n = 2$) or double-negative ($n = 1$) VST. In 1 of them, BAT confirmed diagnosis of honeybee venom allergy.

Taken together, BAT was confirmative in 24 patients and diagnostic in 5 out of the 47 patients (62%) with a tentative diagnosis of venom allergy (figure).

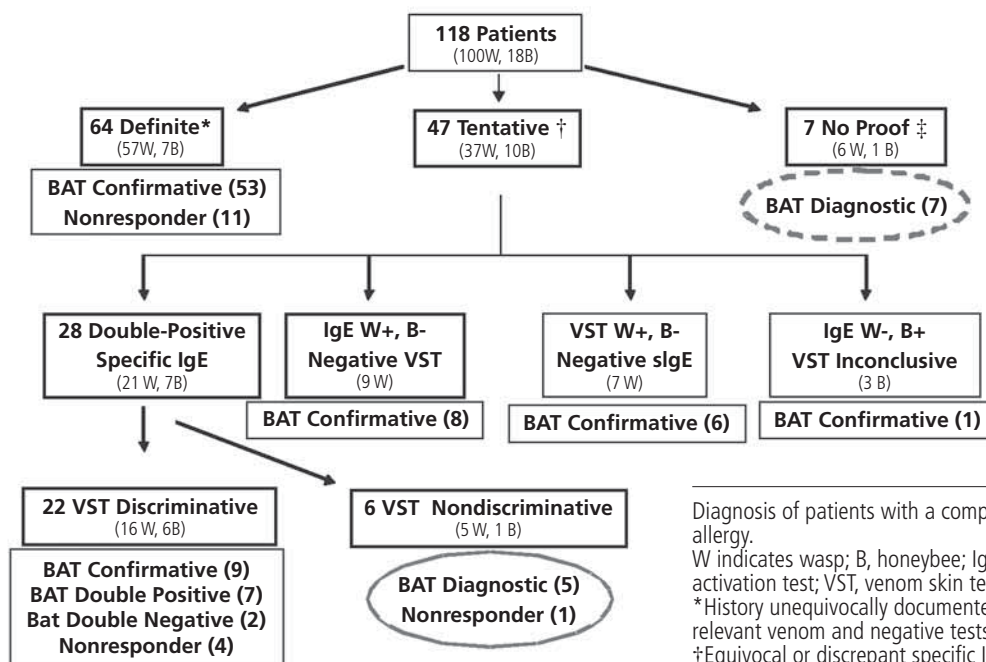
No Evidence of IgE-Mediated Venom Allergy

In 7 of the 118 patients (6%) with a compelling history of venom allergy, no specific IgE or VST responsiveness was demonstrable and BAT constituted the sole method to establish diagnosis (figure).

Discussion

IgE-mediated hymenoptera venom allergy is an important health problem and correct identification of the offending venom is a prerequisite for effective management with specific immunotherapy. According to the current guidelines, venom immunotherapy is limited to patients in whom an IgE-mediated response is demonstrable by quantification of specific IgE antibodies or VST [18]. However, diagnosis of hymenoptera venom allergy is not always straightforward. From our data it emerges that only half of the patients with a compelling history of hymenoptera venom allergy have their diagnosis robustly established by unequivocal specific IgE and VST results. BAT confirmed diagnosis in all these patients, provided the cells were responsive to stimulation. However, this study was not primarily designed to assess the diagnostic reliability of BAT in hymenoptera venom allergy [12-15], but rather to evaluate the potential of BAT to facilitate diagnosis of difficult cases where quantification of specific IgE and VST remain negative or yield contradictory or equivocal results.

In agreement with the results of other studies [2,3,8], double positivity for specific IgE was observed to be the most common barrier to correct diagnosis in patients with a single venom allergy. Double-positive specific IgE results for wasp and honeybee venom were present in almost a quarter of the total population. In addition, it emerged that in 6 of the 28 patients with double-positive specific IgE results, correct identification of the culprit venom was further hampered by negative or equivocal VST results. However, all these patients, except for 1, had their diagnosis finally established by a single positive BAT result for relevant venom and venom immunotherapy



Diagnosis of patients with a compelling history of hymenoptera venom allergy.

W indicates wasp; B, honeybee; IgE, immunoglobulin E; BAT, basophil activation test; VST, venom skin test.

*History unequivocally documented by positive specific IgE and VST for relevant venom and negative tests for irrelevant venom.

†Equivocal or discrepant specific IgE or VST results.

‡Entirely negative specific IgE and VST.

was started accordingly. In contrast, a confirmative BAT result was only obtained in 41% of the patients with double-positive specific IgE results who had their diagnosis established by a single positive VST for relevant venom. In the remainder of the patients with double-positive specific IgE results, the majority had a double-positive BAT result.

In 16% of our patients, a definite diagnosis was hindered by the fact that they demonstrated only 1 confirmatory test—ie, either positive specific IgE or a positive VST. In almost all of those patients, the BAT results were confirmative and contributed to the final diagnosis.

A major finding of our study was that about 6% of our patients with a compelling history of hymenoptera venom allergy had a complete negative result for specific IgE and VST. In all those patients, venom allergy was clearly documented by BAT. Moreover, as the results of BAT were unequivocal and pointed to a single culprit venom, the results were considered diagnostic and guided the selection of venom for immunotherapy.

Taken together, the results of this study indicate that a definite diagnosis of venom allergy is established in about 55% of patients. As these patients have unequivocal and concordant specific IgE and VST results, no additional diagnostic tests are mandatory. In the remaining patients, diagnosis is complicated by equivocal, discrepant, or entirely negative specific IgE and VST results. In the majority of these difficult cases, BAT constitutes a useful additional diagnostic instrument and contributes to the correct selection of potentially life-saving venom immunotherapy.

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