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Component-resolved diagnosis in selecting patients for yellowjacket venom immunotherapy

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ABSTRACT

Background: Venom immunotherapy is effective in preventing systemic allergic reactions (SARs), but the diagnosis of venom allergy is problematic.
Objective: To compare the performance of component-resolved diagnosis and conventional tests in patients referred for venom immunotherapy.
Methods: We measured serum-specific immunoglobulin E to yellowjacket and honeybee venoms (Ves v 1 and Ves v 5 and Api m 1), cross-reactive carbohydrate determinants, serum basal tryptase (ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden), and skin prick test reactions in 84 patients referred to receive venom immunotherapy. History of SAR and its severity were evaluated.
Results: Of the 78 patients with suspected yellowjacket venom (YJV) allergy, a history of SAR was confirmed in 47 (60%) and 31 (40%) had a non-SAR reaction. The most accurate tests to confirm venom allergy after a SAR were serum-specific immunoglobulin E to yellowjacket whole-venom extract spiked with Ves v 5 (area under the curve 0.87, 95% confidence interval 0.77–0.97, $P < .001$) and Ves v 5 (area under the curve 0.86, 95% confidence interval 0.76–0.96, $P < .001$). Sensitization to Ves v 1 was infrequent and its area under the curve was low (0.62, 95% confidence interval 0.47–0.76, $P = .106$). Sensitivity of the YJV skin prick test was 86%, but its specificity was low at 54%. Double sensitization to yellowjacket and honeybee occurred frequently in skin prick tests. Of the patients without a SAR, 26% showed a positive reaction to YJV in any serum test and 46% showed a positive reaction in skin tests.
Conclusion: Specific immunoglobulin E to the YJV spiked with Ves v 5 confirmed the allergy after a SAR. A history of SAR should be confirmed before testing, because venom sensitization is frequent in other types of reactions.

Introduction

Hymenoptera venom allergy is a potentially life-threatening condition after an insect sting. Hymenoptera stings cause anaphylaxis more frequently in adults (3%) than in children (0.34%) and account for one fourth of fatalities caused by anaphylaxis.[1] Systemic mastocytosis increases the risk for severe anaphylaxis to Hymenoptera stings.[2–4] The risk of a systemic allergic reaction (SAR) to Hymenoptera re-stings is 25% to 75% in adults with a previous SAR. The risk depends on the severity of previous reactions and other known high-risk factors such as age and medication.[5] Children have lower risk of a SAR at re-stings and their SARs are mild in 60%.[6,7]

Venom immunotherapy (VIT) with extracted Hymenoptera venoms is effective in preventing a SAR to insect stings: fewer than 3% of patients treated with vespid VIT have a subsequent SAR. VIT can prevent fatal reactions, and it effectively improves quality of life.[8] The prerequisite for efficacious VIT is a history of a SAR to an insect sting and immunoglobulin E (IgE) sensitization to the culprit venom.

The assessment of IgE sensitization can be problematic because honeybee (*Apis mellifera*; Api m) and yellowjacket (*Vespula vulgaris*; Ves v) venom extracts are complex mixtures of proteins including venom-specific and cross-reactive components. The major allergens are hyaluronidases (Api m 2, Ves v 2), phospholipase A2 (Api m 1), phospholipase A1 (Ves v 1), acidic phosphatase (Api m 3), icarapin (Api m 10), and antigen 5 (Ves v 5).[6,9]

Double sensitization to yellowjacket and honeybee venoms as determined by skin prick tests and serum-specific IgE tests is common; up to 50% of patients show a positive reaction to the 2

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venoms. The major cross-reactive components in honeybee and yellowjacket venoms are hyaluronidases with 50% sequence identity. Furthermore, cross-reactive carbohydrate determinants (CCDs) cause double-positive test results.[6] In addition to clinically insignificant positive test results, venom extracts might lack important low-abundance allergens, resulting in false-negative test results.

The distinction between cross-reactivity and true sensitization is important for the choice of VIT, because treating patients who are not sensitized to the allergens in the VIT extract can cause de novo sensitizations, missing protection, and unnecessary costs.[9,10]

The current diagnostic framework in Hymenoptera venom allergy includes skin prick tests, stepwise intradermal testing, and serum-specific IgE to whole-venom extracts and to venom-specific allergen components.[11] The conventional yellowjacket venom (YJV) extract in ImmunoCAP alone has shown lower sensitivity than the combination of Ves v 5 and Ves v 1. Therefore, it is spiked (sYJV) with Ves v 5. When using the sYJV instead of the conventional YJV, the sensitivity increased from 83% to nearly 97%.[12] In the US guidelines, venom testing usually requires intradermal testing for optimal sensitivity, and prick tests are optional.[5] In addition, basophil activation tests and in-house immunoblot tests have been used. Component-resolved diagnostics has improved for the diagnosis of Hymenoptera venom allergy. Measuring specific IgE to allergen components instead of venom extracts helps to distinguish true sensitization from cross-reactivity. We studied the performance of diagnostic tests, including skin prick tests, serum-specific IgE, in-house immunoblot, and serum baseline tryptase, in patients with a strong suspicion of venom allergy referred for VIT.

Methods

Study Population and Classification of Allergic Symptoms

The study population of this case-control study consisted of children and adults referred for VIT for suspected Hymenoptera venom allergy. The cases had a SAR to a Hymenoptera sting and the controls had a large local reaction or other type of reaction. The study took place at a single center, a tertiary care hospital. We recruited 30 patients retrospectively from March 2010 through December 2013 and 54 prospectively in 2014. An allergist examined the patients and recorded the history of hymenoptera stings: date, symptoms, signs, emergency visits, and medication. The stinging insect was identified by the patient or by the caregiver. We defined a SAR as a sudden-onset reaction with rapid progression of signs and symptoms involving at least 2 organs including dermatologic (generalized urticaria or erythema, angioedema, or generalized pruritus and skin rash), cardiovascular (hypotension or clinical diagnosis of uncompensated shock), and respiratory (wheeze, stridor, upper airway swelling, or respiratory distress) symptoms. Generalized urticaria without any other symptoms was defined as a SAR in adults. Minor criteria included milder dermatologic, cardiovascular, respiratory, and gastrointestinal signs and symptoms. The definition was based on the Brighton Collaboration Anaphylaxis Working Group suggestion described in detail by Ruggeberg et al.[13] Severity of the reaction was graded as suggested by Mueller,[14] referenced in Bilo et al[6]: grade I was generalized urticaria, itching, malaise, and anxiety; grade II was any grade I reaction plus at least 2 of the following: angioedema, chest constriction, nausea, vomiting, diarrhea, abdominal pain, and dizziness; grade III was any grade II reaction plus at least 2 of the following: dyspnea, wheezing, stridor, dysarthria, hoarseness, weakness, confusion, and feeling of impending disaster; and grade IV was any grade III reaction plus at least 2 of the following: decrease in blood pressure, collapse, loss of consciousness, incontinence, and cyanosis. A large local reaction was defined as a swelling exceeding 10 cm lasting at least 24 hours.

Allergy Tests

Skin prick tests were carried out on the patient's inner forearm with a disposable single-use lancet. The yellowjacket and honeybee venoms were tested at 100 and 300 μg/mL, respectively (ALK-Abello, Hørsholm, Denmark). Histamine chloride (10 mg/mL) was the positive control, and the solvent was the negative control. A reaction wheal of at least 3 mm was considered positive. We measured serum basal tryptase and specific IgE to sYJV (i3), honeybee venom (i1), CCD (o214), Ves v 1 (i211), Ves v 5 (i209), and Api m 1 (i208; ImmunoCAP, ThermoFisher Scientific, Uppsala, Sweden). We defined sensitization conventionally as a specific IgE level of at least 0.35 kU/L. Since 2012, the conventional YJV ImmunoCAP has been spiked with Ves v 5.[12] All samples taken before September 2012 were reanalyzed using the sYJV ImmunoCAP. In addition, an in-house Immunospot method[15] was used to evaluate serum IgE antibodies to YJV and honeybee venom. For the test, 300 μg/mL of *Vespula* species (ALK-Abelló), 20,000 μg/mL of *Vespula* species European mix (Allergon, Ängelholm, Sweden), and 300 μg/mL of *A mellifera* (ALK-Abelló) venoms were used. A nonatopic serum served as a control.

Statistical Methods and Ethics

The main outcome measure was the accuracy of any diagnostic test in confirming venom allergy in a patient with SAR. We applied receiver-operating characteristics and area under the curve (AUC) to evaluate the performance of the tests in SAR and non-SAR categories. We calculated sensitivity, specificity, and likelihood ratios with 95% confidence interval (CI). We calculated positive and negative predictive values for specific IgE concentrations. Spearman rank correlation served to correlate severity with the specific IgE concentrations. Mann-Whitney *U* test was used to compare non-normally distributed data, and Pearson χ^2 or Fisher exact test was used for categorical data. SPSS 21 (SPSS, Inc, Chicago, Illinois) was used for the analyses. The local ethics committee approved the study protocol, according to which the patient (or 1 parent of the child) signed a written informed consent. The study followed the principles of the Declaration of Helsinki.

Results

Of the 84 patients, 78 were referred for VIT after a yellowjacket sting, 5 after a honeybee sting, and 1 after a sting by an unknown insect. The median age was 44 years (range 1–75); 24 (28%) were children or adolescents. All children with urticaria (13 of the 23) had other symptoms (dyspnea, vomiting, or angioedema). The performance of the allergy tests was evaluated for yellowjacket allergy in the 78 individuals. Of these patients, 47 (60%) had experienced a SAR and 31 (40%) had a non-SAR. The study included only 4 patients with a SAR triggered by honeybee venom. The demographic data and test results of the 78 subjects with a yellowjacket sting are presented in Table 1.

Patients with a SAR to yellowjacket sting were more frequently sensitized to sYJV ($P < .001$) and Ves v 5 ($P < .001$), and they had higher specific IgE to sYJV ($P < .001$) and Ves v 5 ($P < .001$) than those with a non-SAR. The best accuracy was obtained using specific IgE to sYJV (AUC 0.87, 95% CI 0.77–0.97, $P < .001$) and to Ves v 5 (AUC 0.86, 95% CI 0.76–0.96, $P < .001$). The AUC of specific IgE to Ves v 1 was lower (0.62, 95% CI 0.47–0.76, $P = .106$; Fig 1).

Specific IgE to sYJV had the best likelihood ratio (3.2), with 89% sensitivity and 74% specificity. Sensitivity of the skin prick test was 86%, but its specificity was lower at 54%. Accordingly, of the patients with a non-SAR to yellowjacket, 46% were sensitized to skin prick tests and 26% were sensitized to the serum sYJV test. Sensitivity of the immunoblot was the lowest at 62%. The performances