



New directions in diagnostic evaluation of insect allergy

David B.K. Golden

Purpose of review

Diagnosis of insect sting allergy and prediction of risk of sting anaphylaxis are often difficult because tests for venom-specific IgE antibodies have a limited positive predictive value and do not reliably predict the severity of sting reactions.

Recent findings

Component-resolved diagnosis using recombinant venom allergens has shown promise in improving the specificity of diagnostic testing for insect sting allergy. Basophil activation tests have been explored as more sensitive assays for identification of patients with insect allergy and for prediction of clinical outcomes. Measurement of mast cell mediators reflects the underlying risk for more severe reactions and limited clinical response to treatment.

Summary

Measurement of IgE to recombinant venom allergens can distinguish cross-sensitization from dual sensitization to honeybee and vespid venoms, thus helping to limit venom immunotherapy to a single venom instead of multiple venoms in many patients. Basophil activation tests can detect venom allergy in patients who show no detectable venom-specific IgE in standard diagnostic tests and can predict increased risk of systemic reactions to venom immunotherapy, and to stings during and after stopping venom immunotherapy. The risk of severe or fatal anaphylaxis to stings can also be predicted by measurement of baseline serum tryptase or other mast cell mediators.

Keywords

insect sting allergy, predicting severity of anaphylaxis, venom allergy diagnostic tests

INTRODUCTION

The diagnosis of insect sting allergy is surprisingly complex, despite the simple recommendation that venom immunotherapy (VIT) is indicated in patients with a history of systemic reaction to a sting and positive venom-IgE by skin tests or serum test [1]. It is the indications for, and interpretation of, these tests that frustrates clinicians, leading to the search for new diagnostic materials and methods. This article will review the currently available diagnostic methods and their limitations, and will summarize the development of alternative approaches for diagnosis and prediction of risk in insect sting allergy.

CURRENT VENOM DIAGNOSTIC TESTS

Diagnostic testing is centered on the demonstration of venom-specific IgE antibodies by skin test or serum test [2,3]. As with all allergies, these tests are useful when they confirm a clear history of allergic reaction but have a poor overall positive

predictive value. To understand this, we must recall that venom-specific IgE can be demonstrated in about 20% of normal adults, and up to 40% of those who have been recently stung [4]. The presence of venom-specific IgE is not necessarily predictive of clinical reactivity or the severity of reaction to a sting [5]. The risk of anaphylaxis to a future sting is predicted by many factors, including the history of reaction, the presence of venom-IgE, the natural history of the disease, the baseline level of mast cell mediators, and age [6,7]. The demonstration of venom-IgE is associated with a modest chance of systemic reaction (10–15%) even in those with a negative history of sting reaction, and with a variable chance of systemic reaction in those with a

Johns Hopkins University, Baltimore, Maryland, USA

Correspondence to David B.K. Golden, MD, Associate Professor of Medicine, Johns Hopkins University, 7939 Honeygo Blvd, #219, Baltimore, MD 21236, USA. E-mail: dgolden1@jhmi.edu

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KEY POINTS

- Current venom skin tests and serum IgE tests are very good for confirming the diagnosis of insect-sting allergy, but have imperfect sensitivity and limited positive predictive value.
- Recombinant venom allergens can improve the specificity of serum IgE tests and differentiate cross-reactivity from specific sensitization, but optimal sensitivity may not be achieved without inclusion of multiple component allergens.
- BATs can identify some patients with insect allergy who are not detected by venom skin tests or serum IgE tests.
- The severity of sting anaphylaxis may be predicted by using BATs as well as by measurement of mast cell mediators or their metabolic pathways (e.g. tryptase, PAF acetylhydrolase).

positive history of allergic reaction, ranging from 5 to 75% depending on the severity of the prior reaction [1,8–10]. The clinician often orders a test with the expectation that it will provide a clear direction for treatment and guidance of the patient. The test is, however, often being done after the fact (after an allergic reaction to a sting), with up to 40% chance of a positive result because of the sting itself. In most cases, we cannot know what the result would have been before the sting.

In a prospective sting challenge study [6], we found that the severity of the past sting reaction was correlated with the frequency, but not the severity, of systemic reaction to a sting. In this study, we also noted that the chance of systemic reaction was also correlated with the species of yellow jacket used for the sting challenge. This demonstrates that a patient could have no reaction to one yellow jacket sting, but could still have a severe reaction to another yellow jacket sting. The venom skin test also showed a correlation with the frequency of systemic reaction to sting challenge, with the lowest risk being in patients with negative venom skin test but positive venom-IgE.

Negative venom skin and serum tests have been reported in about 15% of patients with a convincing history of systemic reaction to a sting, some of whom will have systemic reactions to future stings [11]. One effort to improve the sensitivity of skin tests was to dialyze the venom extract to eliminate small molecular weight components that cause non-specific irritant reactions in the skin. This resulted in skin tests that detected 79% of the patients who had negative skin tests to commercial venom, some of whom also had negative serum tests [12]. There remains a need to improve the sensitivity and

specificity of diagnostic tests to predict systemic reactions to stings.

RECOMBINANT ALLERGENS (COMPONENT-RESOLVED DIAGNOSIS)

The use of recombinant venom allergens for in-vitro diagnostic testing offers the possibility of increased sensitivity and/or increased specificity over existing methods. The former has been inconsistently demonstrated, but the latter appears quite promising. Although the sensitivity of serologic testing with native venoms is superior to that of recombinant allergens, some allergens may be underrepresented in commercial venoms. This may explain the identification of a small number of patients who have no detectable IgE to vespa venom in commercial serum tests, but are positive when tested with recombinant Ves v5 or Ves v1 [13]. This difference has also been related to geographic variation in responses to specific venom allergens because of either differences in the honeybees (and their venoms) in different areas or differences in genetic responsiveness to specific allergens in different populations (as has been observed for peanut) [13].

The most cited advantage of recombinant allergens is that they are expressed in cell cultures that do not add the cross-reactive carbohydrate determinants (CCDs) that are present on the native allergens. The area of greatest concern in this respect is the dual-sensitized patient with positive IgE tests for both honeybee and yellow jacket venoms. Muller *et al.* [14] reported that diagnostic sensitivity was 96% for rApi m 1 and 87% for rVes v 5. This was performed using an Advia-Centaur system for which Muller reports significantly greater specificity than in the ImmunoCAP system reported by Hofmann *et al.* [15] to have 79% sensitivity with rApi m 1. The source of the recombinant protein may account for some of the variability in other reports (e.g. 57% for rApi m 1) [16]. Sturm *et al.* [17] warns that 'using rApi m 1 and rVes v 5 is insufficient, and will miss genuine sensitization to other major allergens'. Greater accuracy accrues with the inclusion of additional allergens (e.g. rApi m 2, rVes v 1) [18–20,21[¶]]. Interestingly, Korosec notes that the sensitivity of the testing with native and recombinant allergens is not related to the severity of the reaction to stings, an observation made previously for both venom skin and serum tests for venom-IgE. The addition of specific allergens to the natural venom test for serum IgE also improves accuracy. The addition of rVes v 5 to the yellow jacket ImmunoCAP increased the sensitivity of the test from 83.4 to 96.8% [22]. More recently, Eberlein *et al.* [23^{¶¶}] confirmed the utility of component-resolved

diagnosis to distinguish dual from cross-reactive sensitivity to honeybee and yellow jacket venoms, but their results also raised questions regarding the role of CCDs. Jakob *et al.* [24] noted a similar lack of relation of honeybee or yellow jacket double positivity to CCDs, but found comparable reactivity to native and recombinant Api m 1 in their patients. The use of recombinant honeybee venom (HBV) allergens has also demonstrated the spectrum of sensitization to include all of the six allergens tested, and the use of all the six allergens achieved a sensitivity of 94% [25]. Perhaps more importantly, Kohler *et al.* also showed that Api m 3 and Api m 10 are underrepresented in commercial HBV extract leading not only to potential missed diagnosis but to incomplete protection on VIT, especially in the 5% of patients who were sensitized exclusively to these components. This could contribute to the long-recognized difference in degree of protection between HBV and vespid venoms. These data suggest the potential utility of adding these components to native HBV to improve diagnostic accuracy (similar to spiking yellow jacket with Ves v 5) and even to improve the therapeutic response to honeybee VIT.

A problem that is more common in the United States is double sensitivity to yellow jacket and wasp (*Polistes*) venoms, which occurs in about half of patients with yellow jacket allergy (which are the majority of all cases of insect sting allergy in the United States). In the past, radioallergosorbent test (RAST)-inhibition has been a useful tool for distinguishing dual sensitization (and therefore dual VIT) from cross-reactive sensitization (and single VIT) [26]. The use of recombinant venom allergens can also clarify the specific nature of the vespid sensitivities, although this may not be related to CCDs [27]. It has also been shown that both phospholipases and antigen 5s must be represented in the recombinant venoms to give optimal accuracy [20].

BASOPHIL ACTIVATION TESTS

A very different approach postulates that the level of basophil sensitivity is of clinical significance because it modulates the specific IgE in relation to the allergic reaction to a sting. There is a long history in the literature of allusion to such 'twitchy mast cells', 'basophil hyper-releasability', and 'leaky basophils' [28]. This heightened level of activation, which may stop short of actual mediator release, could be the result of many intracellular signaling processes, and can be monitored by the expression on the cell surface of basophil activation markers such as CD63 and CD203c [29].

One of the problems in translation of basophil activation tests (BATs) from a research tool to a routine clinical test is the need for a uniform and standard approach to the methodology, and the reporting and analysis of the results. Kosnik *et al.* [30] initially reported a ratio of 'positive' activation to 1.0 vs. 0.1 µg/ml of venom. This 'positive activation' is the proportion of basophils showing increased CD63 expression in response to allergen using a cutoff that varies in different studies, usually in the range of 10–15%. More recently, the 'CD-sens' is used to represent the inverse of the concentration for maximal CD63 response [23²²,31]. Other studies continue to report the absolute response to a single-concentration venom, and some studies still find the CD203c expression to correlate with some clinical outcomes.

Nevertheless, a growing literature attests to the potential utility of the test. The BAT was more sensitive than intradermal skin tests in patients suffering from clinical allergy with negative serum IgE and skin prick tests [32], especially in patients with mastocytosis [33²¹]. Although recombinant venom allergens have shown some utility in detecting clinical insect allergy in patients with negative tests for serum IgE to native venoms, the BAT was reported to be superior in such cases [34]. The BAT was highly correlated with the frequency of systemic reactions to VIT [30] and with successful protection during VIT [35], and predicted the relapse of patients after stopping VIT [36]. Eberlein *et al.* [23²²] has described a refinement of the BAT using recombinant venom allergens for improved diagnostic accuracy.

OTHER DIAGNOSTIC TESTS

Confirmation of sensitization to specific venoms or component allergens is essential for diagnosis and may have some value in predicting the frequency or severity of future sting reactions or the response to VIT or both, but there are other tests that are important in the prediction of risk. Most notably, baseline serum tryptase has been recognized for almost 20 years as an indicator of risk in patients with insect allergy [37,38]. Elevated baseline serum tryptase has been reported to indicate increased risk of severe anaphylaxis to stings, systemic reactions to VIT, incomplete protection with VIT, and relapse (including fatal reactions) after stopping VIT [7,39–41]. Measurement of baseline serum tryptase has become an essential part of the evaluation of patients who are sting allergic, and often leads to further evaluation for potential underlying systemic mastocytosis. It is noteworthy that insect stings are the most common cause of anaphylaxis in patients with indolent systemic mastocytosis [42].

Increased serum levels of PAF have also been identified as a risk factor for severe and fatal anaphylaxis, including insect stings [43,44]. This is because of abnormally low levels of PAF acetylhydrolase in these patients. When these measurements become available as commercial assays, they will undoubtedly become part of our repertoire for evaluation of risk in patients with insect sting allergy. One can imagine that future research will identify similar risk-associated and predictive measurements for other mast cell mediators or their metabolic pathways.

INDICATION FOR PERFORMING DIAGNOSTIC TESTS

The utility of each of the diagnostic assessments discussed here depends entirely on how it can answer the clinical question being asked (Table 1). For initial diagnosis, a detailed clinical history is essential. Diagnostic tests are not required for patients who are judged to be at a low risk for anaphylaxis, such as large local reactors, and children with cutaneous systemic reactions. For the majority of patients with sting anaphylaxis, standard venom skin tests or serum tests or both for venom-IgE will give sufficient information to guide clinical decision-making. When these tests are inconclusive, the BAT and baseline tryptase may be helpful. When there are multiple sensitivities, recombinant allergen tests (or RAST-inhibition tests) may clarify the primary clinical allergy for specific treatment. When there is a need to predict the severity (rather than the frequency) of reactions to stings, the most useful tests are the prior history, the baseline tryptase, and the BAT. There is often much uncertainty about what test gives the most guidance on when to discontinue VIT. This is because there is no test that actually predicts the

persistence of immune tolerance (vs. a waning immunity), although the BAT shows some promise in this respect, as does the expression of CD27 on allergen-specific T-cells [36,45].

INTERPRETATION OF DIAGNOSTIC TESTS

The presence or absence of venom-specific IgE does not by itself provide a clinical conclusion. This leads to great confusion when there is an expectation that the course of action will become clear when the test results become available. When the history is unclear or inconclusive, we cannot know from a positive result whether the person had venom-specific IgE present before the recent sting, or just as a result of the sting. There are many normal individuals (no history of sting allergy) who are positive for venom-IgE, but have a relatively low risk of sting anaphylaxis (<10%). The presence of venom-specific IgE is necessary, but not sufficient for sting anaphylaxis. The magnitude of the test result correlates with the frequency, but not always with the severity of reactions to stings. Therefore, identification of patients at greatest risk for sting anaphylaxis depends on recognition of high-risk factors [7,46].

Interpretation of negative tests for venom-IgE is also fraught with pitfalls. Venom skin tests have limited sensitivity because of the limited range of concentrations that can be used without causing nonspecific (false-positive) reactions. Serum IgE to venoms can be detected in about 10% of the patients who are clinically sting allergic and have negative venom skin tests, but there remain a small number of patients who have negative venom skin test and serum IgE tests, and yet have systemic reactions to stings [11]. It has been recommended that patients with a convincing history of moderate-to-severe sting anaphylaxis, who have negative venom skin

Table 1. Diagnostic evaluation of insect-sting allergy

	History	Skin test	Specific IgE	BAT	Recombinant allergen	RAST-inhibition	Tryptase baseline
Diagnosis							
No reaction	X						
LLR	X						
Mild systemic reaction	X	X	X				
Anaphylaxis	X	X	X	X	X	X	X
Predict severe reaction (to stings or VIT)	X			X			X
Cross-reactivity (honeybee/yellow jacket)					X	X	
Discontinue VIT	X			X			X

BAT, basophil activation test; LLR, large local reaction; RAST-inhibition, radioallergosorbent test-inhibition; VIT, venom immunotherapy. Reproduced with permission from [9].

tests, should have a serum test for venom-IgE, and if it is negative, the skin test should be repeated a few months later [47]. Both recombinant venom allergens and the BAT techniques have been shown to identify some patients who are suffering from sting allergy and have negative skin tests or serum IgE.

CONCLUSION

There is clearly a need for improved diagnostic and prognostic tests for insect sting allergy. Currently available tests have up to 95% accuracy, but when the diagnosis is difficult, there is an additional role for component-resolved diagnosis using recombinant allergens and for BATs. There is a need to standardize the materials and methods for these tests to avoid some of the discrepancies that are evident in the literature. Another diagnostic tool that would be most valuable is the ability to identify the patients who are at highest risk for the most severe reactions to stings. In this respect, the BAT holds some promise, but ideally it would be assessed in studies using live sting challenge for the most controlled observations of its correlation with clinical outcomes. Measurement of baseline serum tryptase or other mast cell mediators has shown significant correlation with severity of sting reactions.

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The author declares potential conflicts of interest as follows:

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Golden DBK, Moffitt J, Nicklas RA; AAAAI. Stinging insect hypersensitivity: a practice parameter update 2011. *J Allergy Clin Immunol* 2011; 127:852–854.
 2. Bernstein IL, Li JT, Bernstein DI, et al. Allergy diagnostic testing: an updated practice parameter. *Ann Allergy Asthma Immunol* 2008; 100:S1–S148.
 3. Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol* 2010; 125:S284–S296.
 4. Golden DBK, Marsh DG, Kagey-Sobotka A, et al. Epidemiology of insect venom sensitivity. *JAMA* 1989; 262:240–244.
 5. Sturm GJ, Kranzelbinder B, Schuster C, et al. Sensitization to Hymenoptera venoms is common, but systemic sting reactions are rare. *J Allergy Clin Immunol* 2013. pii: S0091-6749(13)01707-7. doi: 10.1016/j.jaci.2013.10.046.
 6. Golden DBK, Breisch NL, Hamilton RG, et al. Clinical and entomological factors influence the outcome of sting challenge studies. *J Allergy Clin Immunol* 2006; 117:670–675.
 7. Rueff F, Przybilla B, Bilo MB, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase – a study of the EAACI Interest Group on Insect Venom Hypersensitivity. *J Allergy Clin Immunol* 2009; 124:1047–1054.
 8. Golden DBK, Marsh DG, Freidhoff LR, et al. Natural history of Hymenoptera venom sensitivity in adults. *J Allergy Clin Immunol* 1997; 100:760–766.
 9. Golden DBK. Advances in diagnosis and management of insect sting allergy. *Ann Allergy Asthma Immunol* 2013; 111:84–89.
 10. Golden D. Insect allergy. In: Adkinson NFJ, Bochner BS, Busse WW, Holgate ST, Lemanske RF, Simons FER, editors. *Middleton's Allergy: Principles and Practice*, 8th ed. Philadelphia: Mosby Elsevier; 2013.
 11. Golden DBK, Kagey-Sobotka A, Hamilton RG, et al. Insect allergy with negative venom skin tests. *J Allergy Clin Immunol* 2001; 107:897–901.
 12. Golden DBK, Kelly D, Hamilton RG, et al. Dialyzed venom skin tests for identifying yellow jacket-allergic patients not detected using standard venom. *Ann Allergy Asthma Immunol* 2009; 102:47–50.
 13. Sturm GJ, Bilo MB, Bonadonna P, et al. Ves v 5 can establish the diagnosis in patients without detectable specific IgE to wasp venom and a possible north-south difference in Api m 1 sensitization in Europe. *J Allergy Clin Immunol* 2012; 130:817.
 14. Muller UR, Johansen N, Petersen AB, et al. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy* 2009; 64:543–548.
 15. Hofmann SC, Pfender N, Weskesser S, et al. Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. *J Allergy Clin Immunol* 2011; 127:265–267.
 16. Korosec P, Valenta R, Mitterman I, et al. Low sensitivity of commercially available rApi m 1 for diagnosis of honeybee venom allergy. *J Allergy Clin Immunol* 2011; 128:671–673.
 17. Sturm GJ, Hemmer W, Hawranek T, et al. Detection of IgE to recombinant Api m 1 and rVes v 5 is valuable but not sufficient to distinguish bee from wasp venom allergy. *J Allergy Clin Immunol* 2011; 128:247–248.
 18. Korosec P, Valenta R, Mitterman I, et al. High sensitivity of CAP-FEIA rVes v 5 and rVes v 1 for diagnosis of *Vespula* venom allergy. *J Allergy Clin Immunol* 2012; 129:1406–1408.
 19. Mitterman I, Zidarn M, Silar M, et al. Recombinant allergen-based IgE testing to distinguish bee and wasp allergy. *J Allergy Clin Immunol* 2010; 125:1300–1307.
 20. Monsalve RI, Vega A, Marques L, et al. Component-resolved diagnosis of vespine venom-allergic individuals: phospholipases and antigen 5s are necessary to identify *Vespula* or *Polistes* sensitization. *Allergy* 2012; 67:528–536.
 21. Muller UR, Schmid-Grendelmeier P, Hausmann O, Helbling A. IgE to recombinant allergens Api m 1 Ves v 1, and Ves v 5 distinguish double sensitization from crossreaction in venom allergy. *Allergy* 2012; 67:1069–1073.
- An excellent overview of the role of component-resolved diagnosis in assessing dual sensitization.
22. Vos B, Kohler J, Muller S, et al. Spiking venom with rVes v 5 improves sensitivity of IgE detection in patients with allergy to *Vespula* venom. *J Allergy Clin Immunol* 2013; 131:1225–1227.
 23. Eberlein B, Krischan L, Darsow U, Ollert M. Double positivity to bee and wasp venom: improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J Allergy Clin Immunol* 2012; 130:155–161.
- A provocative analysis of the role of component-resolved diagnosis, basophil activation tests, and the relevance of cross-reacting carbohydrate determinants in clinical evaluation of the patients with insect allergy.
24. Jakob T, Kohler J, Blank S, et al. Comparable IgE reactivity to natural and recombinant Api m 1 in cross-reactive carbohydrate determinant-negative patients with bee venom allergy. *J Allergy Clin Immunol* 2012; 130:276–278.
 25. Kohler J, Blank S, Muller S, et al. Component resolution reveals additional major allergens in patients with honeybee venom allergy. *J Allergy Clin Immunol* 2014; 133:1383–1389.
 26. Hamilton RH, Wisenauer JA, Golden DBK, Valentine MD Jr NFA. Selection of Hymenoptera venoms for immunotherapy based on patients' IgE antibody cross-reactivity. *J Allergy Clin Immunol* 1993; 92:651–659.
 27. Blank S, Neu C, Hasche D, et al. *Polistes* species venom is devoid of carbohydrate-based cross-reactivity and allows interference-free diagnostics. *J Allergy Clin Immunol* 2013; 131:1239–1242.
 28. MacGlashan DWJ. Releasability of human basophils: cellular sensitivity and maximal histamine release are independent variables. *J Allergy Clin Immunol* 1993; 91:605–615.
 29. MacGlashan DWJ. Basophil activation testing. *J Allergy Clin Immunol* 2013; 132:777–787.
- An excellent review of the methodology and pitfalls of basophil activation tests.
30. Kosnik M, Silar M, Bajrovic N, et al. High sensitivity of basophils predicts side effects in venom immunotherapy. *Allergy* 2005; 60:1401–1406.
 31. Zitnik SE, Vesel T, Avcin T, et al. Monitoring honeybee venom immunotherapy in children with the basophil activation test. *Pediatr Allergy Immunol* 2012; 23:166–172.
 32. Korosec P, Erzen R, Silar M, et al. Basophil responsiveness in patients with insect sting allergies and negative venom-specific immunoglobulin E and skin prick test results. *Clin Exp Allergy* 2009; 39:1730–1737.

33. Bonadonna P, Zanotti R, Melioli G, *et al.* The role of basophil activation test in special populations with mastocytosis and reactions to Hymenoptera sting. *Allergy* 2012; 67:962–965.

A recent advance in the application of basophil activation tests in patients with insect allergy, with useful references and review of the patients.

34. Korosec P, Silar M, Zidarn M, Kosnik M. Reply: Ves v 5 can establish the diagnosis in patients without detectable specific IgE to wasp venom and a possible north-south difference in Api m 1 sensitization in Europe. *J Allergy Clin Immunol* 2012; 130:818–819.
35. Petermelj A, Silar M, Erzen R, *et al.* Basophil sensitivity in patients not responding to venom immunotherapy. *Int Arch Allergy Immunol* 2008; 146:248–254.
36. Kucera P, Cvackova M, Hulikova K, *et al.* Basophil activation can predict clinical sensitivity in patients after venom immunotherapy. *J Investig Allergol Immunol* 2010; 20:110–116.
37. Haeberli G, Weber M, Muller UR. Sting challenge during venom immunotherapy for Hymenoptera venom allergy: the predictive value of basal serum tryptase level. *Allergy* 2000; 55:62–63.
38. Ludolph-Hauser D, Rueff F, Fries C, *et al.* Constitutively raised serum concentration of mast cell tryptase and severe anaphylactic reactions to Hymenoptera stings. *Lancet* 2001; 357:361.
39. Haeberli G, Bronnimann M, Hunziker T, Muller U. Elevated basal serum tryptase and Hymenoptera venom allergy: relation to severity of sting reactions and to safety and efficacy of venom immunotherapy. *Clin Exp Allergy* 2003; 33:1216–1220.

40. Muller UR. Elevated baseline serum tryptase, mastocytosis and anaphylaxis. *Clin Exp Allergy* 2009; 39:620–622.
41. Rueff F, Przybilla B, Bilo MB, *et al.* Predictors of side effects during the buildup phase of venom immunotherapy for Hymenoptera venom allergy: the importance of baseline serum tryptase. *J Allergy Clin Immunol* 2010; 126:105–111.
42. Niedoszytko M, deMonchy J, vanDoormaal JJ, *et al.* Mastocytosis and insect venom allergy: diagnosis, safety and efficacy of venom immunotherapy. *Allergy* 2009; 64:1237–1245.
43. Pravettoni V, Piantanida M, Primavesi L, *et al.* Basal platelet-activating factor acetylhydrolase: prognostic marker of severe Hymenoptera venom anaphylaxis. *J Allergy Clin Immunol* 2014; 133:1218–1220.

This study gives us a preview of the clinical application of new tests for mast cell mediators and their metabolic pathways.

44. Vadas P, Gold M, Perelman B, *et al.* Platelet-activating factor, PAF acetylhydrolase, and severe anaphylaxis. *N Engl J Med* 2008; 358:28–35.
45. Gilles S, Traidl-Hoffman C. CD27 expression on allergen-specific T cells: a new surrogate for successful allergen-specific immunotherapy? *J Allergy Clin Immunol* 2012; 129:552–554.
46. Stoevesandt J, Hain J, Kerstan A, Trautmann A. Over- and underestimated parameters in severe Hymenoptera venom-induced anaphylaxis: cardiovascular medication and absence of urticaria/angioedema. *J Allergy Clin Immunol* 2012; 130:698–704.
47. Reisman RE. Insect sting allergy: the dilemma of the negative skin test reactor. *J Allergy Clin Immunol* 2001; 107:781–782.