The relative value of skin tests and radioallergosorbent test in the diagnosis of bee sting hypersensitivity

Shlomo Bar-Sela, M.D., Meir Shalit, M.D., John H. Kalbfleisch, Ph.D., and Jordan N. Fink, M.D. Jerusalem, Israel, and Milwaukee, Wis.

The relative diagnostic value of ST and RAST was evaluated in 97 patients with BSH. Eighteen patients had LRs, 79 showed SRs including 18 with urticaria, 26 had bronchospasm, and 35 had anaphylactic shock. ST but not the RAST reactivity was strongly related to the severity of the clinical reaction (p ≤ 0.001) and found superior to the RAST in identifying patients with SRs in whom venom therapy was indicated. (J ALLERGY CLIN IMMUNOL 72:690-694, 1983.)

The diagnosis of BSH is based on the symptoms and physical signs manifested by the patient after a sting and identification of the stinging insect. For confirmation of BSH, additional testing such as STs or the RAST is used. Each test has its merits and disadvantages. STs measure the biologic reaction leading to the wheal-and-flare response and can be influenced by disease; RAST measures specific serum IgE antibodies and is not influenced by drugs or patients' responsiveness to mediators. The relative diagnostic efficacy of ST and RAST in ISH remains controversial. Some investigators claim equal diagnostic value, whereas others claim ST to be superior to RAST. Some of the controversy may lie in the fact that the populations studied have been vespid rather than bee sensitive in which case identification of the insect and, accordingly, the interpretation of the results are difficult. This study was carried out in patients with established BSH in order to compare the value of ST and RAST as diagnostic tools in the evaluation of BSH and perhaps hypersensitivity to other stinging insects.

MATERIAL AND METHODS

Subject selection

One hundred forty-two patients (103 male patients and 39 female patients), ages 4 to 64 years, were seen in the Allergy Unit of the Hadassah Medical Center for evaluation of BSH. Of these, the clinical history revealed bee sting sensitivity in 104 and vespid sensitivity in eight, and the stinging insect was not identified in 30 of these patients.

The population chosen for study consisted of the 97 patients with BSH who underwent both ST and RAST. All had a history of an immediate type of reaction (less than 1 hr) after a bee sting; identification of the insect was relatively certain, since the stinger remained in the puncture site. Eighty-six of the patients were evaluated not less than 6 wk and no more than 5 yr after the most recent sting. Seven were tested between 5 and 10 yr and four were tested more than 10 yr after the most recent sting. Reactions seen were grouped according to severity into LR or SR. SRs included urticarial lesions appearing distant to the stinging site, respiratory signs and symptoms (i.e., chest tightness, wheezing, dyspnea), and anaphylactic shock (syncpe or faintness with an objective drop in blood pressure). LRs included those limited to the sting site, appearing at variable periods after the sting, and not associated with other symptoms.

STs were performed with bee venom (Pharmacia GB Ltd., Uppsala, Sweden) by the intradermal end point dilution titration. Briefly, the volar aspect of the forearm was cleansed, and a venom dilution (0.02 ml) or control was injected intradermally by means of a 1 ml tuberculin syringe with a 26-gauge needle. Concentrations ranged from 10^{-6} to 10^{-2} mg/ml. A wheal of 5 mm or more with a surrounding flare of 10 mm or more was considered positive.

Specific serum IgE antibody levels were measured by RAST (Pharmacia). Briefly, 50 μl serum samples were incubated with venom disks for 3 hr at room temperature. Disks were washed three times with polysorbated 20 solu-
Skin tests and RAST in bee sting hypersensitivity

Abbreviations used
- ST: Skin tests
- RAST: Radioallergosorbent test
- BSH: Bee sting hypersensitivity
- LR: Local reaction
- SR: Systemic reaction
- ISH: Insect sting hypersensitivity

tion (Tween 20; Pharmacia, Uppsala, Sweden) and then incubated overnight at room temperature with 50 μl of 125I-anti-IgE. The disks were rewashelf three times, and the radioactivity of the anti-IgE bound was determined in a Hewlett-Packard gamma counter; results were compared to standard sera provided by the manufacturer. All tests were carried out in duplicate. Results were expressed as 0 to 4+ by comparison with the standards provided.

Statistical analysis
A frequency count table was constructed by classification of each subject according to the severity of the sting reaction and the ST and RAST results. ST results were graded into five classes as 0 (no reaction) or 1+ (reactivity at $10^{-3}$ mg/ml) to 4+ (reactivity at $10^{-6}$ mg/ml). The RAST results were also graded into five categories from 0 (no binding as compared to controls) to 4 (high degree of binding as compared to controls). Clinical reactions were graded according to severity as local or by varying degrees of SR, ranging from urticaria to anaphylaxis. To test for an association between the severity of the clinical reaction and the grade of positivity of each diagnostic test, the frequency table data were analyzed with a three-dimensional log-linear contingency table analysis. This procedure first fits the observed data with a model that allows all three study factors to interrelate with each other and then proceeds to test pairs of factors for a statistically significant association. Two factors are judged to be significantly related if both tests, that of partial association (measuring the relationship between two factors in the presence of other factors) and that of marginal association (which ignores a possible relationship with other study factors), produce probability levels of 0.05 or less.

In addition, ST and RAST were evaluated for their efficacy in differentiating between patients with LR (no indication for venom therapy) and those with SR (venom therapy indicated) by computing measures of sensitivity, specificity, predictive, and exclusion values for both tests and their combinations. Statistical analysis showed that optimum discrimination was observed when the five RAST categories were grouped into negative (RAST classes 0 to 2) and positive (classes 3 and 4) subsets. Similarly, ST results were grouped into negative (reaction to $10^{-3}$ mg/ml or more) and positive (reaction to $10^{-6}$ mg/ml or less) subsets.

RESULTS
Of the 97 patients with BSH, 18 had LR and 79 had SR. Of those with SR, 18 had urticaria, 26 experienced bronchospasm, and 35 had anaphylactic shock. The distribution of the ST and RAST results in those patients (Figs. 1 and 2) indicates that the severest allergic reactions were associated with the greatest in vivo or in vitro reactivity. These results are further statistically analyzed in Table 1.
TABLE I. The relationship between the severity of sting reaction, STs, and RASTs

<table>
<thead>
<tr>
<th>Factors correlated</th>
<th>Test of partial association</th>
<th>Test of marginal association</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi square</td>
<td>p Level</td>
</tr>
<tr>
<td>Sting reaction and ST</td>
<td>63.66</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Sting reaction and RAST</td>
<td>5.78</td>
<td>p = 0.9267</td>
</tr>
<tr>
<td>ST and RAST</td>
<td>24.74</td>
<td>p = 0.3096</td>
</tr>
</tbody>
</table>

Note partial association evaluates the relationship between two factors in the presence of effects of the remaining factor, whereas marginal association evaluates the relationship between two factors ignoring effects of the remaining factor.

TABLE II. Sensitivity, specificity, predictive, and exclusion values for STs and RASTs

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity*</th>
<th>Specificity†</th>
<th>Predictive value‡</th>
<th>Exclusion value§</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>0.887</td>
<td>0.944</td>
<td>0.986</td>
<td>0.654</td>
</tr>
<tr>
<td>RAST</td>
<td>0.582</td>
<td>0.722</td>
<td>0.902</td>
<td>0.283</td>
</tr>
<tr>
<td>ST or RAST</td>
<td>0.886</td>
<td>0.666</td>
<td>0.921</td>
<td>0.571</td>
</tr>
</tbody>
</table>

*Sensitivity = no. of patients with SR and a positive test

†Specificity = no. of patients with LR and a negative test

‡Predictive value = no. of patients with SR and a positive test

§Exclusion value = no. of patients with LR and a negative test

†Positive ST = immediate wheal-and-flare reaction to a concentration of $10^{-4}$ mg/ml or less.
§Positive RAST = score of 3 or more when compared with standards.

STs were not affected by the patient's age. When compared to younger age groups, however, lower specific IgE antibody levels were found in patients older than 46 yr who suffered from bronchospasm or anaphylactic shock after a bee sting. The time elapsed between most recent allergic reactions to bee sting and the current evaluation did not affect either ST or RAST.

The results of the simultaneous analysis of the sting reaction severity, skin reactivity, and RAST classes demonstrated that the only significant relationship ($p < 0.001$) was between ST and the severity of sting reaction. The associations between RAST results and the severity of the clinical reaction and between ST and RAST were not statistically significant.

When comparing the efficacy of ST and RAST (each subgrouped into its negative and positive subsets) in differentiating between the LR and SR groups, ST were superior to RAST in sensitivity and specificity, as well as in predictive and exclusion values (Table II). Thus in this study, STs have been shown to be superior to RAST in differentiating between LR and SR. Combining both tests did not improve any of the parameters measured.

DISCUSSION

The widespread use of insect venom for the diagnosis and treatment of ISH has been facilitated by the recent availability of pure venoms for clinical use. Venoms have been found superior to whole body extracts for the diagnosis and treatment of ISH. Therapy with venom is expensive and also may be accompanied by a higher percentage of reactions. Therefore it is important to confirm ISH before the institution of therapy.

Although a deliberate challenge with the suspected insect may be the only definitive test for the diagnosis of ISH, other laboratory tests such as ST and RAST are preferred by clinicians. Skin testing is inexpensive, simple to perform, and provides the answer without delay. It is, however, influenced by drugs and by the patient's responsiveness to mediators. It also causes discomfort to the patient, and SRs after STs have been reported. The search for specific IgE
antibodies in the serum by RAST overcomes the disadvantages of ST but requires special equipment, radioactive reagents and trained personnel and is more expensive than ST.

The relative diagnostic value of ST and RAST in the diagnosis of BSH has been disputed by several authors. Reisman claims that RAST is equal to ST, but this has not been confirmed by additional studies. Our study differs from other published comparisons of ST and RAST in several ways. The study population included only patients with BSH in whom identification of the stinging insect was definite. Furthermore, the patients studied were not beekeepers or family members in whom levels of venom-specific serum IgE and IgG antibodies are different from those in the general population. Finally, the statistical analysis compared the association between ST and RAST and the severity of clinical manifestations as well as the efficiency of ST and RAST in differentiating between patients with and without indications for venom therapy.

This study demonstrates that ST correlated with the severity of the clinical reaction better than RAST, a statistically significant association was found between ST and the clinical response to the bee sting. The study also showed that ST were superior to RAST in differentiating between patients with SRs and those with LRs. Patrizzi et al. studied 33 patients with BSH, and although RAST results were found to be less favorable than the results of ST, they recommended both ST and RAST in the clinical evaluation of patients with BSH. We do not agree with this recommendation since, in our study, neither sensitivity, specificity, predictive, nor exclusion values were improved by the addition of RAST results to those of ST.

The superiority of ST over RAST for evaluation of BSH, as demonstrated in the present study, is of interest. The immediate wheal-and-flare skin reaction depends on the presence of specific IgE antibody, its fixation to mast cells, the release of mediators, and the reactivity of skin, and STs likely represent the entire chain of events in the reaction. The RAST, on the other hand, measures only specific IgE antibody, and reliable results are influenced by technique and the presence of specific IgG antibodies, among other influencing factors.

In conclusion, our results indicate that STs are more specific and more sensitive than RASTs for evaluation of BSH. RAST does not add to the diagnosis of BSH nor to the identification of those patients with an indication for venom therapy when ST are used. It seems appropriate therefore to use only ST in the diagnostic workup of patients with BSH and perhaps in patients with other Hymenoptera hypersensitivity. RASTs should be reserved for patients in whom STs cannot be performed because of lack of cooperation, when there is a contraindication to the technique for investigational purposes, or for monitoring venom-specific antibody levels during and after immunotherapy.

We thank Shula Wollner, Lillian Antebi, and Zippora Cohen of the Allergy Unit of the Hadassah Medical Center for their technical assistance and Mavis E. Baurle and Catherine A. Walther for their typing and editorial assistance.

REFERENCES

3. Hoffman DR: Comparison of the radioallergosorbent test to intradermal skin testing in the diagnosis of the stinging insect venom allergy. Ann Allergy 43:211, 1979