

Utility of laboratory testing for the diagnosis of Hymenoptera venom allergy

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ABSTRACT

Background: A diagnosis of Hymenoptera venom allergy is based on clinical history and the results of skin tests and/or laboratory methods.

Objective: To analyze the utility of available laboratory tests in diagnosing Hymenoptera venom allergy.

Methods: Ninety-five patients with Hymenoptera venom allergy with a history of bee (35) or wasp (60) anaphylactic sting reaction and positive skin test with bee or wasp venom were included in this analysis. Specific immunoglobulin E (to bee venom extract, wasp venom extract, available recombinant molecules, and a basophil activation test with venom extracts were assessed in all the patients. Test sensitivity and specificity were calculated by using standard threshold values; then, receiver operating characteristic curve analysis was performed to compute optimal threshold values. Also, statistical analysis of the utility of different combinations of laboratory tests was performed.

Results: The optimal threshold values were revealed to be the following: 1.0 kIU/L for bee venom extract (sensitivity, 97.14%; specificity, 100%), 0.35 kIU/L for rApi m 1 (sensitivity, 68.57%; specificity, 100%), 1.22 kIU/L for wasp venom extract (sensitivity, 88.33%; specificity, 95.45%), 0.7 kIU/L for rVes v 5 (sensitivity, 86.67%; specificity, 95.45%), 1.0 kIU/L for rVes v 1 (sensitivity, 56.67%; specificity, 95.45%), 6.5% for basophil activation test with bee venom extract (sensitivity, 80%; specificity, 95.45%), and 4.5% for basophil activation test with wasp venom extract (sensitivity, 91.53%; specificity, 95.45%). The best test combinations were found to be the following: bee venom extract plus rApi m 1 (sensitivity, 97.14%; specificity, 95.45%) in bee and either wasp venom extract plus rVes v 5, or rVes v 5 plus rVes v 1 (both sensitivity, 98.33%; specificity, 95.45%) in patients with wasp venom allergy.

Conclusion: Our analysis confirmed that currently used laboratory tests represent effective tools in diagnosing Hymenoptera venom allergy. Moreover, our probabilistic approach offered another way to interpret concrete values of laboratory test results and opened possible direction on how to optimize the laboratory diagnostic procedure.

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Insect sting is one of the most frequent causes of anaphylaxis, which, because of its unpredictable, acute, and potentially life-threatening course, represents a serious medical problem. Proper diagnosis and treatment of Hymenoptera venom allergy, therefore, are critical and belong in the hands of experienced allergists. Regarding diagnosis and treatment of Hymenoptera venom allergy, the European Academy of Allergy and Clinical Immunology position paper recommendations from 2005 state^{1,2}: precise personal history, step skin testing, and the determination of specific immunoglobulin E (IgE) antibodies to bee and wasp venom extracts remain the criterion standard of examination. Making a definitive differentiation between bee and wasp venom allergy by means of commonly

used tests is often complicated because the frequently observed double positivity to bee and wasp venoms, in up to 59% of patients,³ usually reflects cross-reactivity rather than clinically relevant sensitization to both venoms, which also makes it difficult to choose the appropriate venom for immunotherapy.

True double sensitization to species-specific bee and wasp venom allergen components represents 20–40% of double-positive results, with the rest being due to cross-reactivity between venoms.^{1,3} Cross-reactivity can sometimes be caused by high sequence homology of protein allergens in both venoms; hyaluronidase (Api m 2/Ves v 2), dipeptidylpeptidase IV (Api m 5/Ves v 3), and vitellogenin (Api m 12/Ves v 6)⁴ are allergens that have high sequence homology. A more frequent cause of cross-reactivity is sensitization to cross-reacting carbohydrate determinants. Sensitization to carbohydrate determinants generates multiple positive test results with different allergens.^{1,5–8} The clinical relevance of carbohydrate determinant-reactive antibodies seems to be low or nonexistent in the case of insect venom allergy.⁹

The high prevalence of misleading double positivity to bee and wasp reported by standard test results has

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led to the incorporation of the basophil activation test (BAT) and molecular diagnosis into the standard diagnostic procedure. The BAT has been shown to be a useful tool in achieving correct diagnosis,^{10–15} but, in comparison with molecular diagnosis, the BAT is time-consuming, technically demanding, and, moreover, requires fresh blood. The BAT, however, should be used in patients with a convincing history of insect sting allergy but with negative skin test and venom IgE test results; in such cases, it can improve the overall sensitivity of a diagnostic evaluation.^{16,17} Thus, this method is a suitable “next step” in distinguishing patients who are missed by standard methods and/or molecular diagnosis.¹⁸ Moreover, a depression of allergen-specific basophil response seems to be associated with the induction of tolerance after completing a course of venom immunotherapy (VIT), which probably makes the BAT a promising tool for monitoring of VIT.^{10,19,20}

In the past decade, molecular diagnosis has developed and become available for Hymenoptera venom allergy diagnostic procedure. The bee venom allergen component rApi m 1 and the two wasp venom allergen components rVes v 5 and rVes v 1 have been commonly available for routine use since 2009. The benefit of these available allergens has been evaluated in several centers in Europe.^{3,5,21–26} It recently was stated by several investigators that the use of rVes v 5 combined with rVes v 1 is sufficient for a correct diagnosis of wasp venom allergy.^{21,22} For a precise diagnosis of bee venom allergy, however, the use of rApi m 1 unfortunately seems to be insufficient because of low sensitivity of rApi m 1. Sensitivity of rApi m 1 ranges from 56 to 82%.^{21–25} Patients with bee venom allergy are often sensitized to other bee venom allergens, such as Api m 2, 3, 4, 5, and 10. The inclusion of these additional bee venom allergens might be of clinical relevance and may increase the sensitivity of a molecular diagnosis for bee venom allergy.²⁴

Another concern that can hamper the diagnostic procedure of Hymenoptera venom allergy, apart from double sensitization, is a high prevalence of asymptomatic sensitization to insect venoms, which is estimated at between 9.3 and 28.7% in the general population,²⁷ which is why, to avoid misdiagnosis, only patients with a history of systemic sting reaction are indicated for diagnostic testing.¹ Our analysis, therefore, only involved patients with a clinical history of systemic sting reaction. The aim of our study was to assess the utility of commonly available laboratory tests in diagnosing Hymenoptera venom allergy.

METHODS

Patients

Ninety-five patients with Hymenoptera venom allergy were included in this retrospective analysis. The

inclusion criterion for the bee and wasp venom allergic group of patients was the conclusive indication for bee or wasp VIT. The indication for immunotherapy was made based on a clinical history of definite systemic bee or wasp sting reaction within the past 3 years and IgE sensitization to the respective venom confirmed by at least one of the available methods (skin test; IgE to venom extract, rApi m 1, rVes v 5, or rVes v 1; BAT). A skin test with the respective venom was positive in all the patients. None of our patients reported having had a systemic reaction after both bee and wasp stings. Patients with double sensitization were not excluded from our analysis.

The group of patients with bee venom allergy consisted of 35 patients: 17 boys and men and 18 girls and women, with an average age of 47 years (range, 14–73 years). In terms of the Müller grades of severity, 8 patients had a grade II, 16 had a grade III, and 11 had a grade IV systemic sting reaction. The group of patients with wasp venom allergy consisted of 60 patients: 23 men and 37 women, average age of 50.4 years (range, 19–74 years). Ten patients with wasp venom allergy had grade I, 13 had grade II, 21 had grade III, and 16 had grade IV systemic reactions. Nearly one-third of the patients with insect venom allergy were not able to reliably identify whether the stinging insect had been a bee or a wasp (Table 1), but sensitization to either bee or wasp was clearly confirmed by skin tests and/or by laboratory methods in these patients. The control group consisted of 22 subjects with no history of insect sting reaction.

Laboratory Tests

Specific IgE to bee venom extract (I1); to wasp venom extract (I3); to rApi m 1, rVes v 5, and rVes v 1; and the BAT were carried out in all of our study participants. Skin tests, however, although performed as part of the standard examination protocol in all the patients with a history of systemic allergic bee or wasp sting reaction, were not performed in the control group. Results of laboratory tests, therefore, were used for statistical analysis only. The study was approved by the ethics committee of the University Hospital and Medical Faculty in Pilsen March 6, 2014, and written informed consent was obtained from the study participants.

Specific IgE Determination. Specific IgE to bee venom extract (I1) and wasp venom extract (I3) as well as to available recombinant allergens rApi m 1, rVes v 1, and rVes v 5 were assessed. All specific IgE antibodies were measured by using the ImmunoCAP system (ThermoFisher Scientific, Uppsala, Sweden). The value of 0.35 kIU/L was considered the standard threshold for positivity.

Table 1 Demographic characteristics of patients with bee venom allergy and patients with wasp venom allergy

	No. Patients	Average age, y	Sex, no. (%)	Grade of Reaction, no. (%)	Stinging Insect, no. (%)
Patients with bee venom allergy	35	47.0	Male, 17 (49) Female, 18 (51)	I. 0 (0) II. 8 (23) III. 16 (46) IV. 11 (31)	Bee 25 (71) Unknown 10 (29)
Patients with wasp venom allergy	60	50.4	Male, 23 (38) Female, 37 (62)	I. 10 (16) II. 13 (22) III. 21 (35) IV. 16 (27)	Wasp 44 (73) Unknown 16 (27)

BAT. The BAT was performed as previously described.^{16,28} Briefly, whole blood was incubated with a stimulant buffer that contained interleukin 3 and bee or wasp venom (Pharmalgen Venom; ALK-Abelló, Hørsholm, Denmark) in a final concentration of 0.1 µg/mL at 37°C for 20 minutes. Incubation was stopped by chilling on ice. Monoclonal antibodies anti-CD63, anti-CD123, and anti-human leukocyte antigens corresponding to MHC class II, type DR (HLA-DR) (Becton Dickinson, Franklin Lakes, NJ) were then added. Finally, samples were analyzed on an FC500 flow cytometer (Beckman Coulter, Brea, CA). A value of 15% of activated basophils (CD123⁺, HLA DR⁺, CD63⁺) was considered as the standard threshold for positivity.

Statistical Analysis

Statistical analysis was performed by using SW SAS (Cary, NC). Descriptive statistics, such as mean, standard deviation, median, interquartile range, minimum, and maximum value, were computed within the bee- and wasp-venom allergic groups. Selected statistical data also were processed graphically in box and whisker plot diagrams. The nonparametric Wilcoxon two-sample test was used to compare differences between the groups for the parameters being investigated. Relationships among the variables being studied were analyzed by using Spearman correlation coefficients; statistical significance was determined at a *p* value of <5% level. The parameters being examined were first analyzed by using univariate tests.

Sensitivity and specificity for each method were calculated by using standard threshold values, and the receiver operating characteristic curve (ROC) analysis to search for optimal thresholds for each method was then performed. Optimal threshold values were defined as those values that had at least 95% specificity and the highest sensitivity. Also, the parameters being investigated were analyzed in two separate ways: by using “or” combinations and by means of multivariate analysis. The “or” combinations analyzed every possi-

ble combination of the two to four available diagnostic tests for bee and wasp allergy. Positivity of a given “or” combination of the selected parameters meant that at least one of the combination parameters was above the specified threshold value; parameters were computed by using both standard thresholds as well as the “optimal” ones revealed in our ROC analysis. Multivariate analysis was performed by using multiple regression; this method involves the analysis of more than one statistical variable at a time, and it also takes the effects of all variables on the outcome into account.

RESULTS

Standard and Optimal Threshold Values in Patients with Bee Venom Allergy

Sensitivity and specificity for the available laboratory tests were first calculated for standard threshold values (Table 2), and, then, to identify optimal thresholds for each method, an ROC analysis was performed. ROC curves were created for bee venom extract IgE, for rApi m1 IgE as well as for BAT (Fig. 1 A). Our optimal thresholds for bee venom extract IgE and BAT differed from standard thresholds in patients with bee venom allergy; however, we did not find a better threshold for rApi m 1 IgE than the standard one (Table 2).

Standard and Optimal Thresholds in Patients with Wasp Venom Allergy

Sensitivity and specificity of the laboratory tests were calculated for standard threshold values in patients with wasp venom allergy (Table 3). ROC curves were created for wasp venom extract IgE, for rVes v 5 IgE, and for rVes v 1 IgE as well as for BAT (Fig. 1 B). Our optimal thresholds differed from standard thresholds in patients with wasp venom allergy for all diagnostic tests (Table 3).

Table 2 Sensitivity and specificity of laboratory tests in patients with bee venom allergy according to different threshold values

Method	Threshold Value	Sensitivity, %	Specificity, %
Univariate analysis			
IgE I1	Standard, 0.35 kIU/L	97.14	81.82
	Optimal, 1.00 kIU/L	97.14	100.00
IgE rApi m 1	Standard, 0.35 kIU/L	68.57	100.00
	Optimal, 0.35 kIU/L	68.57	100.00
BAT I1	Standard, 15.0%	74.29	100.00
	Optimal, 6.5%	80.00	95.45
"Or" combinations			
I1 + rApi m 1	Standard	97.14	81.82
	Optimal	97.14	95.45
I1 + BAT I1	Standard	97.14	81.82
	Optimal	97.14	90.91
I1 + rApi m 1 + BAT I1	Standard	97.14	81.82
	Optimal	97.14	90.91
rApi m 1 + BAT	Standard	85.71	100.00
	Optimal	88.57	95.45
Multivariate analysis			
I1 + rApi m 1		97.14	95.45
I1 + BAT I1		91.42	95.45
I1 + rApi m 1 + BAT I1		88.57	95.45
rApi m 1 + BAT I1		88.57	95.45

IgE = immunoglobulin E; BAT = basophil activation test.

Analysis of "or" Combinations of Selected Parameters and Multivariate Analysis in Patients with Bee Venom Allergy

We performed statistical analysis of all possible combinations of laboratory tests in patients with bee venom allergy. The analysis of the "or" combinations showed that the best diagnostic performance among laboratory test combinations was represented by bee venom extract plus rApi m 1, when using optimal thresholds for both (Table 2). Multivariate analysis was performed by using multiple regression and showed the optimal combination of laboratory tests to be a combination of bee venom extract plus rApi m 1 (Table 2). The final regression curve was defined as the following: $0.4562188751 + 0.0102144018 \times \text{IgE bee venom extract} + 0.0241973532 \times \text{IgE rApi m 1}$, with an optimal threshold value of 0.47 (Fig. 1 A).

Analysis of "or" Combinations of Selected Parameters and Multivariate Analysis in Patients with Wasp Venom Allergy

A statistical analysis of all possible combinations of laboratory tests in patients with wasp venom allergy was also performed. First, an analysis of "or" combinations was computed; the best performance among laboratory test combinations was represented by wasp venom extract plus rVes v 5, wasp venom extract plus

rVes v 5 plus rVes v 1, or rVes v 5 plus rVes v 1, all when using optimal thresholds (Table 3). Multivariate analysis was performed by using multiple regression. The optimal combinations of laboratory methods were shown to be wasp venom extract plus rVes v 5 or rVes v 5 plus rVes v 1 (Table 3). The final regression curve for wasp venom extract plus rVes v 5 was defined as the following: $0.6433955587 + 0.0048772249 \times \text{IgE wasp venom extract} + 0.0075168973 \times \text{IgE Ves v 5}$, with an optimal threshold of 0.65 (Fig. 1 B). The final regression curve for rVes v 5 plus rVes v 1 was defined as the following: $0.6515693510 + 0.0084280548 \times \text{IgE Ves v 5} + 0.0038978292 \times \text{IgE Ves v 1}$, with an optimal threshold value of 0.66 (Fig. 1 B).

DISCUSSION

The standard diagnostic tools used for the detection of IgE sensitization to bee or wasp venom in patients with a history of systemic sting reaction are skin tests and venom extract specific IgE measurement.¹ Other available *in vitro* tests are the BAT and molecular diagnosis, which improve diagnostic accuracy, especially in unclear cases. The aim of our study was to reevaluate, in detail, the utility of all available laboratory diagnostic tests, both used separately or in combination, and to identify the optimal laboratory test or combination of tests suitable for confirmation of Hy-

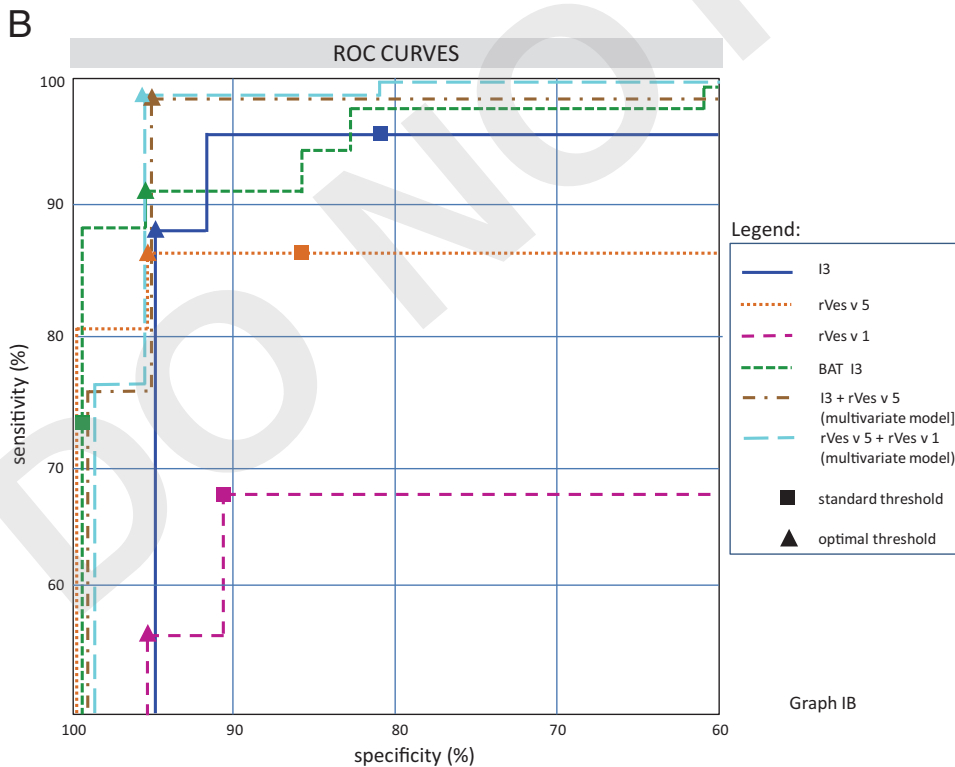
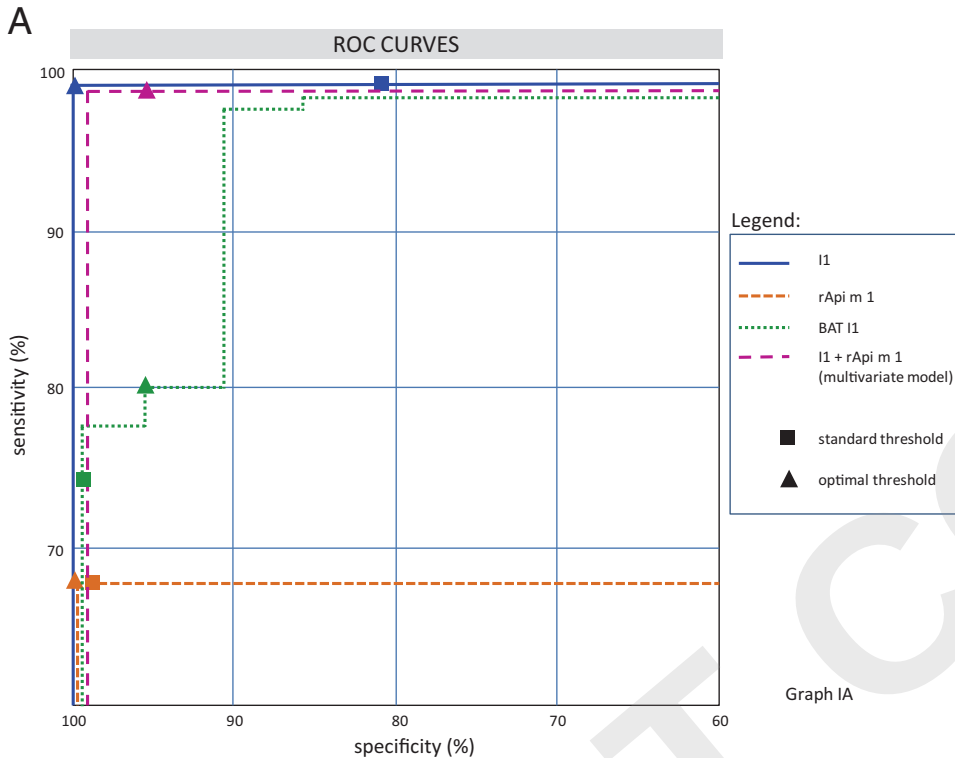


Figure 1 (A) Receiver operating characteristic (ROC) curves for laboratory tests in patients with bee venom allergy. (B) ROC curves for laboratory tests in patients with wasp venom allergy.

menoptera venom allergy. The sensitivity and specificity for each laboratory method were initially calculated by using standard threshold values; in contrast to the majority of previous studies, however, we then also performed an ROC analysis to search for the optimal threshold value for each of these tests.²⁹

Recently, ROC analyses that searched for optimal threshold values were published only for BAT (but not other tests) and reported differing optimal threshold values, which ranged from 15%^{15,16,30} to 25%¹³ of activated basophils. The problem in interpreting these findings is that the BAT protocol differed among stud-

Table 3 Sensitivity and specificity of laboratory tests in patients with wasp venom allergy according to different threshold values

Method	Threshold Value	Sensitivity, %	Specificity, %
Univariate analysis			
IgE I3	Standard, 0.35 kIU/L	96.67	81.82
	Optimal, 1.22 kIU/L	88.33	95.54
IgE rVes v 5	Standard, 0.35 kIU/L	86.67	86.36
	Optimal, 0.70 kIU/L	86.67	95.45
IgE rVes v 1	Standard, 0.35 kIU/L	68.33	90.91
	Optimal, 1.00 kIU/L	56.67	95.45
BAT I3	Standard, 15.0%	72.88	100.00
	Optimal, 4.5%	91.53	95.45
"Or" Combinations			
I3 + rVes v 1	Standard	98.33	81.82
	Optimal	90.00	95.45
I3 + rVes v 5	Standard	98.33	77.27
	Optimal	98.33	95.45
I3 + rVes v 5 + rVes v 1	Standard	100.00	77.27
	Optimal	98.33	95.45
rVes v 5 + rVes v 1	Standard	100.00	81.82
	Optimal	98.33	95.45
I3 + BAT I3	Standard	98.33	81.82
	Optimal	98.31	90.91
I3 + rVes v 5 + rVes v 1 + BAT I3	Standard	100.00	77.27
	Optimal	100.00	90.91
rVes v 5 + rVes v 1 + BAT I3	Standard	100.00	81.82
	Optimal	100.00	90.91
Multivariate analysis			
I3 + rVes v 5		98.33	95.45
I3 + rVes v 5 + rVes v 1		88.40	95.45
rVes v 5 + rVes v 1		98.33	95.45
I3 + rVes v 5 + rVes v 1 + BAT I3		94.90	95.45
rVes v 5 + BAT I3		96.60	95.45

IgE = immunoglobulin E; BAT = basophil activation test.

ies, especially in the way of identifying activated basophils and in insect venom concentration used for activation of these cells.^{10,13–16,28,30,31} In our study, we used a combination of anti-HLA-DR, anti-CD123, and anti-CD63 antibodies for detection of activated basophils, and only results obtained for the 0.1 µg/mL concentration of insect venoms were used for statistical analysis. According to our data, much lower threshold values than the standard ones (15%) seemed to be optimal for BAT both in patients with bee and wasp venom allergy. When using standard threshold values, 6% of patients with bee venom allergy and 19% of patients with wasp venom allergy were missed.

A detailed ROC analysis for specific IgE has not yet been published; 0.35 kIU/L is a standard threshold value used for extract venom IgE as well as for available recombinant allergen IgE molecules. The sensitivity of available recombinant molecules when using

standard threshold values, however, has been evaluated by several groups in Europe. The rApi m 1 sensitivity was first reported by Müller³ when using the Advia system (Siemens Medical Solutions Diagnostics, Tarrytown, NY) in 2009 as being 97%; several groups later registered lower sensitivity when using ImmunoCAP that ranged from 56 to 82%.^{5,21,23–25} Analysis of our data showed rApi m 1 sensitivity of 68.6% when using standard threshold values. Differences in rApi m 1 sensitivity among centers might be the result of different inclusion criteria used for selection of patients with bee and wasp venom allergy or may probably reflect regional differences in the sensitization pattern as well.²²

Sensitivity of rVes v 5 was reported to be 87% by Müller³ in 2009 when using the Advia system. Several other studies that used ImmunoCAP reported sensitivity of rVes v5, from 84 to 90%, and sensitivity of com-

bined rVes v 5 plus rVes v 1 from 92 to 98%.^{21,25,26,32,33} Analysis of our data showed sensitivity of 86.67% for rVes v 5 and of 100% for rVes v 5 plus rVes v 1.

Our analysis thus confirmed recently published data regarding specific IgE tests sensitivity for standard threshold values, but, moreover, it revealed that the same or similar sensitivity and significant higher specificity can be reached when threshold values are optimized. We found more suitable threshold values for extract venom IgE as well as for all available recombinant allergen IgE components except for rApi m 1. All assessed optimal threshold values were higher than standard ones. We originally supposed that the threshold values increase would be accompanied with a sensitivity decrease and a specificity increase. However, we found that sensitivity remained either unchanged or only insignificantly decreased and that specificity increased significantly for most of the IgE tests, which meant that the use of optimal threshold values significantly reduced the number of false-positive results. For example, optimal threshold reduced the number of false-positive results by 18% in the case of bee venom extract and by 9% in the case of rVes v 5.

Statistical analysis of all possible laboratory test combinations yielded interesting data. For that reason two kinds of statistical analyses were used. According to the “or” combination analysis, bee venom extract plus rApi m 1 (both used with optimal threshold values) represented the best combination of diagnostic laboratory tools in patients with bee venom allergy. Nevertheless, the sensitivity of the bee venom extract itself, when used with optimal threshold for positivity, was the same, and specificity was even 5% higher. The optimal combinations of tests in patients with wasp venom allergy according to the “or” combination analysis were represented by wasp venom extract plus rVes v 5, wasp venom extract plus rVes v 5 plus rVes v 1, or rVes v 5 plus rVes v 1; all these methods used optimal threshold values. Standard thresholds used for single tests in these combinations led to significantly lower specificity of all combinations, which meant that >18% of results were false positive. When performed individually, none of these tests had better sensitivity and specificity than these combinations. The second kind of statistical analysis, a multivariate analysis, yielded very similar results and that is why we did not present it in a detailed way here. From a practical point of view, then, performing multivariate analysis was not necessary.

CONCLUSION

Our analysis confirmed that currently used laboratory methods were able to determine a correct diagnosis in most of the patients with bee and wasp venom allergy. Moreover, our analysis provided another in-

sight into the laboratory diagnostic Hymenoptera venom allergy procedure. The probabilistic approach offers another way to interpret concrete values of laboratory test results and opens possible direction to optimize the laboratory diagnostic procedure. It is suitable to reconsider the meaning of the currently used threshold values for the respective methods and their probabilistic value. We showed that higher than currently used threshold values may yield better results in some methods that concern sensitivity and specificity of these methods in terms of reflecting the clinically relevant allergy.

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