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The Diagnostic Importance of Recombinant Allergen IgE Testing in Patients with Hymenoptera Venom Allergy: Comparison of Two Methods

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ABSTRACT

Adults with systemic anaphylactic reactions (SAR) to insect sting show often multiple-positivity of serum-specific IgE (sIgE) to *Hymenoptera* venoms. Unnecessary long-lasting venom-specific immunotherapies (VIT) in false-positive patients increase the risk of recurrent SAR. This report aims to analyze the diagnostic importance of recombinant allergen IgE testing in patients with SAR to *Hymenoptera* sting.

In 82 patients we measured sIgE to honeybee venom (HBV), wasp venom (WV) and hornet venom (HV) extracts, recombinant phospholipase A2 from HBV (sIgE-rApi m1), recombinant antigen 5 from WV (sIgE-rVes v5), and cross-reactive carbohydrate determinants-CCD-bromelain by ImmunoCAP. We analyzed the correlation of ImmunoCAP and Immunoblot for HBV and WV extracts, rApi m1, and rVes v5 in 39/82 patients. According to the history of the culprit insect, we compared sensitivity and specificity between the two methods.

The severity of the SAR does not depend on the sIgE level to venom extracts and recombinant allergens. Fifty-one percent of the patients had a multiple-positivity to HBV/WV or HBV/WV/HV extracts. Severe SAR and CCD-sIgE were more frequent in multiple-positive than single-positive patients. CCD-sIgE were more frequent in HBV allergic patients than WV and HV allergic patients. There was a significant correlation between levels of sIgE to venom extracts and recombinant allergens measured by ImmunoCAP and Immunoblot. ImmunoCAP has higher sensitivity and specificity than Immunoblot for diagnosis of SAR to *Hymenoptera* venoms.

IgE testing to recombinant CCD-free allergens is necessary for the adequate selection of long-lasting VIT, especially in patients with multiple sensitivities to venom extracts.

Keywords: Anaphylaxis; Hymenoptera; Honeybee venoms; Immunoblotting; Wasp venoms

INTRODUCTION

Hymenoptera venoms are considered to be one of the most frequent triggers of systemic anaphylactic reactions (SAR) in adults.[1] They contain a complex mixture of glycosylated and non-glycosylated proteins,

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peptides, and biogenic amines. Specific IgE (sIgE) to more than one Hymenoptera venom could be found in up to 50% of adults with a history of SAR to an insect sting, although most of them report an allergic reaction to only one insect.[2] When positive antibodies to multiple venoms are detected they may be related to 1) genuine double IgE-sensitization to specific allergens of honeybee (*Apis mellifera*) and yellow jacket (*Vespula vulgaris*, in Europe also called wasp) or hornet venoms (*Vespa crabo*) or 2) presence of clinically irrelevant IgE to cross-reactive carbohydrate determinants (CCD) present in all three venoms.[3]

Concurrent presence of sIgE to specific and cross-reactive allergens of honeybee venoms (HBV), wasp *v*enoms (WV), and hornet venoms (HV) indicates further discrimination between genuine sensitization and cross-reactivity which is essential for the selection of appropriate allergens for venom-specific immunotherapies (VIT).[4] Nowadays, molecular diagnosis with non-glycosylated recombinant allergens has significantly improved diagnostic accuracy, particularly in patients showing positive sIgE for multiple *Hymenoptera* venoms.[5] The major allergen of WV and HV is antigen 5 (Ves v5), while phospholipase A2 (Api m1) is one of the most important allergens of HBV.[6] Serum sIgE to recombinant major antigen 5 (rVes v5), measured by fluoro-enzyme immunoassay (ImmunoCAP-FEIA) had a satisfactory sensitivity (84-93%) and specificity (94-100%) for the diagnosis of genuine sensitization to WV or HV and may help adequate selection of suitable allergens for VIT.[2,7-9] However, low sensitivity of recombinant Api m1 (rApi m1) ranging from 57% to 79%, has been reported in honeybee allergic patients.[8,10,11] A higher sensitivity of rApi m1 (88% to 97%) in honeybee allergic patients was reported by other groups.[2,12,13] On the other hand, the highest sensitivity, up to 100% was found for HBV and WV extracts.[2,7,8,12] So far it is not clear why there are such large variations in sensitivity and specificity of sIgE to venom extracts and recombinant major venom allergens between different European countries.

This study aimed to analyze the diagnostic importance of recombinant allergen IgE-testing in patients with a history of SAR to *Hymenoptera* stings. We determined the differences in groups found to be sIgE-positive for single (HBV or WV or HV) extract or multiple-positive (HBV/WV and HBV/WV/HV) extracts using severity of SAR and positivity for rApi m1, rVes v5, and CCD. We also examined whether there were correlations between concentrations of sIgE to venoms measured by ImmunoCAP and Immunoblot. According to the accurate history of insect sting, we compared the sensitivity and specificity of IgE-testing to venom extracts and recombinant allergens by ImmunoCAP and Immunoblot.

MATERIALS AND METHODS

Patients

This retrospective study included 82 patients: 62 male (75.6%) and 20 female (24.4%) (mean age 46.4±13.2 years, range 18-70 years) with a newly-diagnosed SAR to venom sting. The study was performed between March 2015 and November 2019 at the Clinical Centre of Serbia, Belgrade. The protocol for the research has been approved by the Ethics Committee of the Medical Faculty, University of Belgrade, and number 1550/V-8.

Patients with a documented history of SAR to more than one sting were excluded. 42 patients showed multiple-positivity, while 40 showed single-positivity: 12 to HBV extract, 18 to WV extract, and 10 to HV extract (Table 1). In all single-positive patients, a history of insect sting matched with positive sIgE to respective venom.

The severity of SAR was estimated based on Mueller's classification, including four degrees of severity of symptoms from a sting from mild to life-threatening: I-urticaria, II-angioedema, III-respiratory disorders, and IV-fall in blood pressure and loss of consciousness.[14]

None of our patients had mast cell disorder or elevated baseline serum tryptase (value≤11 mcg/L) measured by ImmunoCAP (Phadia, Uppsala, Sweden).

Fluoro-enzyme Immunoassay (FEIA)

Serum levels of sIgE to HBV, WV, HV extracts, rApi m1, rVes v5, and cross-reactive carbohydrate determinants-CCD-bromelain were measured by FEIA (ImmunoCAP Phadia, Uppsala, Sweden). Levels of sIgE were expressed in quantitative units (kUA/L). A value of sIgE ≥0.35 kUA/L was considered positive.

Immunoblot

In 39/82 patients, serum sIgE levels to HBV, WV extracts, rApi m1, rVes v5, and CCD were measured by Immunoblot (Euroimmun, Germany, Euroline DPA-Dx insect venoms). Levels of sIgE were expressed in

quantitative units (kUA/L). A value of sIgE ≥0.35 kUA/L was considered positive.

Statistical Analyses

Obtained data were analyzed using IBM SPSS Statistics software for Windows (version 17; IBM, Armonk, NY). Mean quantitative variables were used and the frequency of qualitative variables was also calculated. Nonparametric Chi-square and Fisher exact tests were used to evaluate the relationship between the qualitative variables. In 39 patients we analyzed Spearman's correlation between levels of sIgE to HBV, WV extracts, rApi m1, and rVes v5 by ImmunoCAP and Immunoblot.

According to a history of insect sting, we compared sensitivity and specificity between the two methods. The sensitivity and specificity of these methods were calculated using the formulas:

Sensitivity = true-positive / true positive + false negative

Specificity = true negative / true negative + false positive.

RESULTS

In our group of 82 patients, male patients more frequent had SAR (75.6%) comparing to female patients (24.4%) (p=0.029).

A comparison of demographic characteristics of multiple-positive and single-positive patients is shown in Table 1.

The majority of patients (55/82) had more severe SAR (III and IV grades of SAR) (67.1%) (p<0.001). The frequency of more severe SAR (85.7%) was higher in the group of patients with multiple-positivity compared to the group with single-positivity (47.5%) (p<0.001). The number of beekeepers did not differ significantly between groups of multiple-positive and single-positive patients.

94.4% of WV single-positive and 100% of HV single-positive patients had positivity of sIgE-rVes v5, while 83.3% of HBV single-positive patients had positivity of sIgE-rApi m1 (Table 2).

True double sensitizations to both venoms determined by positivity for venom-specific recombinant allergens were found in 26.2% of multiple-positive and none of the 40 single-positive patients(p<0.001) (Table 2). Five of 82 (6.1%) patients were negative to both recombinant allergens. CCD-sIgE were more often present in multiple-positive 17/42 (40.5%) than single-positive patients 5/40 (12.5%) (p<0.001).

The majority of multiple-positive patients (35/42) had an exact history of an insect sting. Based on the history (beekeeping, proximity to nesting places for stinging insects, visual identification of the insect, the presence of a stinger) 17/42 multiple-positive patients had SAR to the honeybee, 12/42 to wasp, and 6/42 to the hornet. Seven of 42 patients could not identify the insect (Table 3).

13/17 (76.5%) multiple-positive patients with a history of SAR due to honeybee sting displayed positivity of sIgE-rApi m1. All multiple-positive patients with a history of SAR due to wasp and hornet sting concomitant displayed positivity of sIgE-rVes v5 (Table 3).

Table 1. Comparison of demographic characteristics of patients with *Hymenoptera* venom allergy

	Multiple-positivity to HBV/WV or HBV/WV/HV extracts (n=42)	Single-positivity to HBV or WV or HV extract (n=40)
Age (years)	48.9±14.2	43.8±6.46
Female (%)	6 (14.3)	14 (35)
Male (%)	36 (85.7)	26 (65)
Beekeepers (%)	7 (16.7)	5 (12.5)
Degree of severity sting reactions (%)		
I	3 (7.1)	10 (25)
II	3 (7.1)	11 (27.5)
III	19 (45.2)	4 (10)
IV	17 (40.5)	15 (37.5)

HBV: honeybee venom; WV: wasp venom; HV: hornet venom

Table 2. Serum-specific IgE (sIgE) to recombinant major honeybee and wasp allergens and bromelain in 82 patients with a history of systemic anaphylactic reactions (SAR) to *Hymenoptera* sting

sIgE (ImmunoCAP)	Multiple-positivity to HBV/WV or HBV/WV/HV extracts (n=42)	Single- positivity to HBV extract (n=12)	Single-positivity to WV extract (n=18)	Single- positivity to HV extract (n=10)
rApi m1 and Ves v5 +	11 (26.2%)	0	0	0
rApi m1 +	10 (23.8%)	10 (83.3%)	0	0
rVes v5 +	18 (42.8%)	1 (8.3%)	17 (94.4%)	10 (100%)
rApi m1 and sIgE-Ves v5 -	3 (7.1%)	1 (8.3%)	1 (5.6%)	0
bromelain (CCD) +	17 (40.5%)	4 (33.3%)	0	1 (10%)

rApi m1: recombinant phospholipase A2, rVes v5: recombinant antigen 5, HBV: honeybee venom, WV: wasp venom, HV: hornet venom, CCD: cross-reactive carbohydrate determinants

Table 3. Serum-specific IgE (sIgE) to recombinant major honeybee and wasp allergens in 42 patients with multiple-positivity for venom extracts and history of systemic anaphylactic reactions (SAR) due to certain kinds of *Hymenoptera* sting

History of insect sting	positive sIgE to rApi m1 and rVesv5 (n=11)	positive sIgE to rApi m1 (n=10)	positive sIgE to rVes v5 (n=18)	negative sIgE to rApi m1 and rVesv5 (n=3)
Honeybee (HBV/WV+) (n=17)	3 (17.6%)	10 (58.8%)	1 (5.9%)	3 (17.6%)
Wasp (HBV/WV+) (n=12)	3 (25%)	0	9 (75%)	0
Hornet (HBV/WV/HV+) (n= 6)	2 (33.3%)	0	4 (66.7%)	0
Unidentified (HBV/WV/HV+) (n=7)	3 (42.9%)	0	4 (57.1%)	0

rApi m1: recombinant phospholipase A2, rVes v5: recombinant antigen 5, HBV: honeybee venom, WV: wasp venom, HV: hornet venom

According to the history of stinging insects, positive CCD-sIgE were more frequently found in patients with a history of honeybee sting 14/29 (48.3%) than in patients with a history of wasp sting 2/30 (6.7%) (p<0.001) and hornet sting 4/16 (25%) (p=0.049) (Table 4)No significant differences were found in the mean concentration of sIgE to venom extracts and recombinant major allergens determined by ImmunoCAP between patients with a history of mild and patients with a history of severe SAR (Table 5).

Table 4. Specific IgE (sIgE) to cross-reactive carbohydrate determinants (CCD) in 82 patients with *Hymenoptera* venom allergy

History of insect sting	Multiple-positivity to HBV/WV or HBV/WV/HV extracts (n=42)		Single-positivity to HBV or WV or HV extract (n=40)	
	CCD + (n=17)	CCD - (n=25)	CCD+ (n=5)	CCD- (n=35)
Honeybee (n=29)	10	7	4	8
Wasp (n=30)	2	10	0	18
Hornet (n=16)	3	3	1	9
Unidentified (n=7)	2	5	-	-

CCD: cross-reactive carbohydrate determinants, HBV: honeybee venom, WV: wasp venom, HV: hornet venom

Table 5. Specific IgE (sIgE) to venom extracts and recombinant allergens in 82 patients with a history of mild and more severe systemic anaphylactic reactions (SAR) to Hymenoptera sting

sIgE (ImmunoCAP)	I and II degrees of sting reactions (n=27)	III and IV degrees of sting reactions (n=55)	*p*
HBV extract (mean ±SD) (n=29)	16.27±2.16	13.31±1.51	0.718
WV extract (mean ±SD) (n=30)	10.56±1.37	7.71±1.09	0.558
HV extract (mean ±SD) (n=16)	3.07±2.92	3.49±7.67	0.915
rApi m1 (mean ±SD) (n=23)	4.98±6.30	2.11±2.91	0.374
rVes v5 (mean ±SD) (n=29)	5.56±6.76	6.32±1.20	0.840

HBV: honeybee venom, WV: wasp venom, HV: hornet venom, rApi m1: recombinant phospholipase A2, rVes v5: recombinant antigen 5

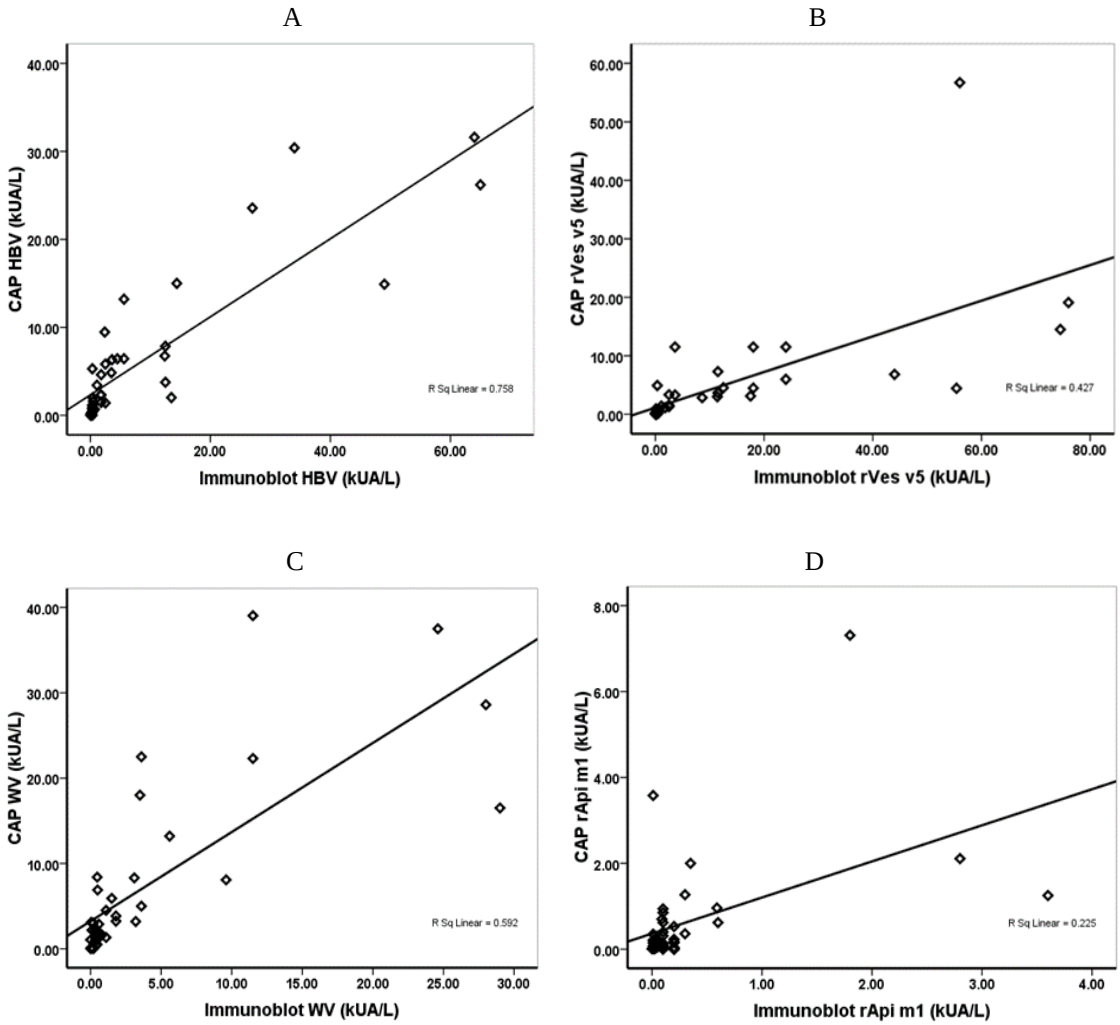

Figure 1. Correlation between concentrations of specific IgE (sIgE) to venom extracts and recombinant allergens determined by ImmunoCAP and Immunoblot in 39 patients with allergy to *Hymenoptera* sting. (A) honeybee venom (HBV) extract (B) recombinant antigen 5 (rVes v5) (C) wasp venom(WV) extract (D) recombinant phospholipase A2 (rApi m1)

The highly significant positive correlation between the levels of sIgE determined by two methods was found for the HBV extract (r, 0.886; $p<0.0001$) (Figure 1A) and rVes v5 (r, 0.886; $p<0.0001$) (Figure 1B), followed by the correlation for WV extract (r, 0.808; $p<0.0001$) (Figure 1C) and finally for rApi m1 (r, 0.349; $p=0.029$) (Figure 1D).

In addition, when tested by ImmunoCAP and Immunoblot, the specificity of sIgE to recombinant allergens (rApi m1 and rVes v5) was excellent and significantly higher than the specificity of sIgE to venom extracts (HBV and WV extracts) (Table 6).

Table 6. Sensitivity and specificity of specific IgE (sIgE) to venom extracts and major recombinant allergens determined by ImmunoCAP and Immunoblot, according to an accurate history of honeybee or wasp sting

sIgE	Sensitivity n (%)		Specificity n (%)	
	ImmunoCAP (n=59)	Immunoblot (n=30)	ImmunoCAP (n=59)	Immunoblot (n=30)
HBV extract	29/29 (100)	12/13 (92.3)	16/30 (53.3)	8/17 (47.1)
WV extract	30/30 (100)	12/17 (70.6)	14/29 (48.3)	5/13 (38.5)
rApi m1	23/29 (79.3)	5/13 (38.5)	27/30 (90)	16/17 (94)
rVes v5	29/30 (96.7)	15/17 (88.2)	24/29 (82.8)	9/13 (69.2)

HBV: honeybee venom, WV: wasp venom, rApi m1: recombinant phospholipase A2, rVes v5: recombinant antigen 5

In a group of patients with a history of honeybee sting, ImmunoCAP had higher sensitivity for HBV extract and rApi m1 (100% and 79.3%, respectively) than Immunoblot (92.3% and 38.5%, respectively). Also, in patients with a history of a wasp sting, ImmunoCAP had a higher sensitivity for WV extract and rVes v5 (100% and 96.7%, respectively) than Immunoblot (70.6% and 88.2%, respectively) (Table 6). According to an accurate history of insect sting, ImmunoCAP for rApi m1 (90%) had higher specificity than ImmunoCAP for HBV extract (53.3%), and ImmunoCAP for rVes v5 (82.8%) had higher specificity than ImmunoCAP for WV extract (48.3%).

DISCUSSION

Our study demonstrates the importance of a new molecular methodology in the daily clinical practice of an allergist who considers SAR caused by *Hymenoptera* venoms. IgE-testing to recombinant CCD-free allergens is necessary for the adequate selection of venoms for VIT, especially in patients with multiple-positivity to venom extracts. A total of 51% of patients in our study group had multiple-positivity to HBV/WV or HBV/WV/HV extracts. We have demonstrated that the use of venom extracts gives false-positive results and significantly reduces the specificity of IgE-testing. According to the accurate history of insect sting, the specificity of IgE-testing to rApi m1 and rVesv5 was much higher than IgE-testing to HBV and WV. The correlation between both tests was highly significant for HBV, WV, and, rVes V5. We emphasize that this is the first study on the diagnostic importance of recombinant allergens in patients with multiple sensitivity to venom extracts in the southeastern part of Europe.

The precise identification of sensitization to the relevant insect is of great importance for initiating adequate immunotherapy in *Hymenoptera* venom allergic patients. Although skin testing represents the first level of approach for the diagnosis of IgE-mediated allergy, *in vitro* tests are the most important diagnostic step before the introduction of long-lasting VIT.[3,4] The gold standard for diagnosis of insect venom allergy is skin testing with venom extracts.[15] However, a skin test may show an unexplained variability and inconclusive multiple-positive results.[16] Also, the quality and standardization of allergens for skin tests may influence the interpretation of the results.

According to the findings of our study, SAR appeared more frequently in males than in female patients ($p=0.029$) which was in line with previously published results.[17] The majority of our patients had more severe SAR ($p<0.001$) and the severity of SAR was independent of the concentration of sIgE to venom extracts and recombinant major allergens measured by ImmunoCAP ($p>0.05$). These data were previously found by other authors.[17]

According to the literature, 3% to 7.5% of people develop SAR after an insect sting.[3] The chance of a systemic reaction to a sting varies between 30% and 65% in adults with previous systemic reactions, depending on the severity of previous reactions.[4] According to the recent guidelines of the European Academy of Allergy and Clinical Immunology (EAACI), VIT is recommended in candidates with a positive history of severe SAR, beekeepers, honeybee allergic patients, patients with mast cell disorders, or elevated baseline serum tryptase.[18] Decision process for VIT introduction is very important for the efficacy of the therapy and should be adjusted for each patient. Molecular diagnostics, using recombinant CCD-free allergens, enables detection of genuine sensitization, and thus in many patients improves the selection of the appropriate VIT.[11,19] Reliable identification of the stinging insect is usually difficult. Similarly, in our group of patients, 7/82 (8.5%) could not identify the insect. The use of venom extracts for the detection of sIgE could lead to "false-positive" results due to their cross-reactivity. Up to 75% of determined multiple-positivity by *in vitro* tests with honeybee and wasp venoms are caused by CCD-sIgE to common protein epitopes of homologous allergens.[2] Multiple-positivity could be avoided by measuring CCD-sIgE.[19,20] Our results showed that determination of sIgE to recombinant major allergens is especially useful for the patients, with multiple-positivity to venom extracts and those who could not identify the insect.

Cross-reactivity can be attributed to CCD frequently present in allergens of insects and plants and common glycoprotein epitopes of homologous allergens present in *Hymenoptera* venoms as described for hyaluronidases (Api m2 and Ves v2) and dipeptidyl-peptidases (Api m5 and Ves v3) which are known to share around 50% sequence identity.[21,22] As a result of that, diagnostics tests based on the detections of antibodies to venom extracts, although very sensitive, do not have adequate specificity.[23] Our study confirmed data from previous studies showing that 50-60% of patients with *Hymenoptera* venom allergy have multiple-positivity. A large number of patients with SAR after honeybee, wasp, or hornet sting were found to have positive sIgE to all three venom extracts. It may be explained by either a presence of CCD-sIgE or genuine sensitization.[2]

In our patients, positive CCD-sIgE was more frequent in multiple-positive than single-positive patients ($p<0.001$). Therefore, CCD-sIgE significantly contributes to cross-reactivity determined in these patients.[11,19] However, the simultaneous presence of CCD-sIgE and IgE to cross-reactive protein epitopes could make the decision on the selection of relevant venom for immunotherapy even more difficult.[5] Also, the analysis of CCD-sIgE demonstrated that our patients with a history of honeybee sting more frequently had positive CCD-sIgE than patients with a history of wasp sting ($p<0.001$). Overall, this can be explained by the fact that all major honeybee allergens are glycosylated, while the two major wasp allergens are not glycosylated.[5] The concept that multiple-positivity to venom extracts comes from CCD-sIgE was previously confirmed.[2,19] On the other hand, genuine positivity may be caused by sensitization to a previously well-tolerated sting.[11] In this regard, 26.2% of our patients showed sensitization to rApi m1 and rVes v5. Taken together, a better solution for the detection of genuine positivity may be the inhibition test with CCD and venom extracts by ImmunoCAP or Immunoblot.[24]

New molecular-allergy testing based on the detection of sIgE against several non-glycosylated allergens may help in determining the true sensitization and result in an adequate therapeutic approach.[7,9,11,12] Many patients with genuine sensitization may be identified; using the rApi m1 and rVes v5 that are available for routine *in vitro* diagnostics, and more importantly not related structurally.[25] According to the latest guidelines, VIT is not recommended in patients with incidentally detected sensitization to insects without clinical symptoms.[18]

By using different combinations of the most important recombinant honeybee allergens, the sensitivity of IgE-testing reaches 94. 4%.[13] Combination of recombinant wasp allergens rVes v5 and rVes v1 (phospholipase A1), the sensitivity of IgE-testing reaches even up to 100%.[11,26,27]

Our results of ImmunoCAP measurement identified a very high sensitivity for WV extract and rVes v5 in patients with a history of wasp sting (100% and 96.7%, respectively), and even higher sensitivity for rVes v5 in patients with a history of hornet sting (100%). Previous studies have reported lower sensitivity for rVes v5 ranging from 86.5% to 93%[2,7,8,12] in wasp allergic patients. Also, good correlation levels of sIgE were revealed for HV extract and rVesv5 measured by immunoCAP.[28] As we already know, the cross-

reactivity that occurs between the venoms of different Vespidae (*Vespula vulgaris* and *Vespa crabo*) is strong, mainly due to the similarities of venom composition and structure of the allergens.[28] The antigen 5 has been recognized as the major and most potent allergen in wasp and hornets. Given this consideration, identification of sIgE-rVes v5 can help in serological confirmation of sensitization to the hornet. According to the literature, 92.6% of patients sensitized to hornet have positive sIgE-rVes v5.[29]

The sensitivity of sIgE-testing for HBV extract (100%) and sIgE-rApi m1 (79.3%) in honeybee allergic patients are similar or higher in compression with previously published results.[8,11,30] The sensitivity of sIgE-rApi m1 of 83.3% in single-positive and 76.5% in multiple-positive patients confirmed Api m1 as one of the main honeybee allergens. Four of five patients who were negative to both recombinant allergens had a positive history of honeybee sting. Clinical diagnosis of honeybee allergy would be more precise with commercial availability of several honeybee allergens such as rApi m1, rApi m2, rApi m4 (melittin), rApi m5, and rApi m10 (icarpin).[13]

Nevertheless, despite a good correlation between levels of sIgE to venom extracts and recombinant allergens measured by both tests, our results showed that ImmunoCAP had a higher sensitivity and specificity than Immunoblot. In addition, according to the accurate history of insect sting, sensitivity and specificity for rApi m1 and rVes v5 are more than 75%.

This study's main limitation is the incomplete availability of several honeybee allergens. Major allergen rApi m1 that was used is enough for 80% of patients, but for 20% of patients with a history of honeybee sting, additional IgE-testing is necessary to other recombinant honeybee allergens. Also, a relatively small number of patients were tested by Immunoblot, but our data represent the first IgE correlation analysis between ImmunoCAP and Immunoblot for HBV, WV, rApi m1, and rVes v5.

In conclusion, this study has shown that SAR is more frequent in males. The majority of patients had severe SAR. The severity of SAR was not dependent on the level of sIgE to venom extracts and recombinant allergens. Multiple-positivity to venom extracts had more than 50% of patients. Severe SAR and CCD-sIgE were more frequent in multiple-positive than single-positive patients. Serum CCD-sIgE was more frequent in honeybee than wasp and hornet allergic patients. There is a significant correlation between levels of sIgE to venom extracts and recombinant allergens by ImmunoCAP and Immunoblot. The ImmunoCAP had a higher sensitivity and specificity than Immunoblot for diagnosis of honeybee and wasp allergy. We have demonstrated that the use of whole venom extracts gives false-positive results and significantly reduces the specificity of the IgE test.

The clinical history and molecular allergy testing are essential for the adequate choice of long-lasting venom immunotherapy, especially in patients with multiple sensitivities to venom extracts and in patients who could not identify the insect which caused SAR.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Ruëff F, Przybilla B, Bilo´ MB, Müller U, Scheipl F, Aberer W, et al. Predictors of severe systemic anaphylactic reactions in patients with Hymenoptera venom allergy: importance of baseline serum tryptase-a study of the European Academy of Allergology and Clinical Immunology Interest Group on Insect Venom Hypersensitivity. J Allergy Clin Immunol. 2009;124(5):1047-54.
2. Müller UR, Johansen N, Peterson AB, Fromberg-Neilsen J, Haeberli G. Hymenoptera venom allergy: Analysis of double positivity of honey bee and vespula venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. Allergy. 2009;64(4):543-8.
3. Blank S, Biló MB, Ollert M. Component-resolved diagnostics to direct in venom immunotherapy: Important steps towards precision medicine. Clin Exp Allergy. 2018;48(4):354–64.
4. Biló MB. Anaphylaxis caused by Hymenoptera stings: from epidemiology to treatment. Allergy. 2011;95(2):35-7.
5. Ollert M, Blank S. Anaphylaxis to Insect Venom Allergens: Role of Molecular Diagnostics. Curr Allergy Asthma Rep. 2015;15(5):26.

6. King TP, Kochoumian L, Lam T. Immunochemical observations of antigen 5, a major venom allergen of hornets, yellowjackets and wasps. MolImmunol. 1987;24(8):857–64.
7. Schrautzer C, Bokanovic D, Hemmer W, Lang R, Hawranek T, Schwarz I, et al. Sensitivity and specificity of Hymenoptera allergen components depend on the diagnostic assay employed. J Allergy Clin Immunol. 2016;137(5):1603-5.
8. Hofman SC, Pfender N, Weckesser S, Huss-Marp J, Jakob T. Added value of IgE detection to rApi m1 and rVes v5 in patients with Hymenoptera venom allergy. J Allergy Clin Immunol. 2011;127(1):265-7.
9. Monsalve RI, Vega A, Marqués L, Miranda A, Fernández J, Soriano V, et al. Componenet-resolved diagnosis of vespid venom allergic individuals: phospholipases and antigen 5s are necessary to identify Vespula and Polistes sensitization. Allergy. 2012;67(4):528-36.
10. Korosec P, Valenta R, Mittermann I, Celesnik N, Erzen R, Zidarn M, et al. Low sensitivity of commercially available rApi m1 for diagnosis of honey bee venom allergy. J Allergy Clin Immunol. 2011;128(3):671-3.
11. Müller U, Schmid-Grendelmeier P, Hausmann O, Helbling A. IgE to recombinant allergens Api m 1, Ves v 1, and Ves v 5 distinguish double sensitization in venom allergy. Allergy. 2012;67(8):1069–73.
12. Šelb J, Kogovšek R, Šilar M, Košnik M, Korošec P. Improved recombinant Api m1- and Ves v5-based IgE testing to dissect bee and yellow jacket allergy and their correlations with the severity of the sting reaction. Clin Exp Allergy. 2016;46(4):621-30.
13. Köhler J, Blank S, Müller S, Bantleon F, Frick M, Huss-Marp J, et al. Component resolution reveals additional major allergens in patients with honeybee venom allergy. J Allergy Clin Immunol. 2014;133(5):383-9.
14. Mueller HL. Diagnosis and treatment of insect sensitivity. Asthma Res 1966; 3 (4): 331-3.
15. Krishna MT, Ewan PW, Diwakar L, Durham SR, Frew AJ, Leech SC, et al. Diagnosis and management of hymenoptera venom allergy: British Society for Allergy and Clinical Immunology (BSACI) guidelines. Clin Exp Allergy. 2011;41(9):1201-20.
16. Hoffman DR. Allergens in Hymenoptera venom. XXV: The amono acids equences of antigen 5 molecules and the structural basis of antigenic cross-reactivity. J Allergy Clin Immunol.1993;92(5):707-16.
17. Fehr D, Micalleto S, Moehr T, Schmid-Grendelmeier P. Risk factors for severe systemic sting reactions in wasp (*Vespula* spp.) and honeybee (*Apismellifera)* venom allergic patients. ClinTransl Allergy. 2019;11(2):9-54.
18. Sturm GJ, Varga E-M, Roberts G, Mosbech H, Bilo MB, Akdis CA, et al. EAACI guidelines on allergen immunotherapy: Hymenoptera venom allergy. Allergy. 2018;73(4):744–64.
19. Eberlein B, Krischan L, Darsow U, Ollert M, Ring J. 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(1):155–61.
20. Jovanovic D, Peric-Popadic A, Andrejevic S, Jovanovic I, Bonaci-Nikolic B. Triple IgE-positivity to hornet, wasp and bee venom in the patient with SAR: diagnostic and therapeutic approach. Vojnosanit Pregl. 2019;76(2):839-42.
21. Jin C, Focke M, Leonard R, Jarisch R, Altmann F, Hemmer W. Reassessing the role of hyaluronidase in yellow jacket venom allergy. J Allergy Clin Immunol. 2010;125(1):184–90.
22. Blank S, et al. Identification, recombinant expression and characterization of the 100 kDa high molecular weight Hymenoptera venom allergens Api m5 and Ves v3. J Immunol.2010;184(9):5403-13.
23. Watanabe M, Hirata H, Arima M, Hayashi Y, Chibana K, Yoshida N, et al. Measurement of Hymenoptera venom specific IgE by the IMMULITE 3gAllergy in subjects with negative or positive result by ImmunoCAP. Asia Pac Allergy. 2012; 2(3):195-202.
24. Hemmer W, Focke M, Kolarich D, Wilson B I, Almann F, Wöhrl S, et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honey bee and yellow jacket venom in patients with stinging-insect allergy. J Allergy Clin Immunol.2001;108(6):1045-52.
25. King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. Int Arch Allergy Immunol. 2000;123(8):99-106.
26. Sturm GJ, Biló MB, Bonadonna P, Hemmer W, Caruso B, Bokanovic D, Aberer W. 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(3):818-9.
27. Korošec P, Valenta R, Mittermann I, Čelsnik N, Šilar M, Zidarn M, Košnik M . High sensitivity of CAP-FEIA rVes v5 and rVes v1 for diagnosis of Vespula venom allergy. J Allergy ClinImmunol. 2012;129(3):1406-8.

28. King TP, Lu G, Gonzales M, Qian N, SoldatovaL. Yellow jacket venom allergens, hyaluronidase and phospholipase: sequence similarity and antigenic cross-reactivity with hornet and wasp homologs and possible implications for clinical allergy. J Allergy Clin Immunol. 1996;98(4):588-600.
29. Yoshida N, Hirata H, Watanabe M, Sugiyama K, Arima M, Fukushima Y, Ishii Y. Improved sensitivity to venom specific-immunoglobulin E by spiking with the allergen component in Japanese patients suspected of Hymenoptera venom allergy. Allergol Int. 2015;64(3):248–52.
30. Sturm GJ, Hemmer W, Hawranek T, Lang R, Ollert M, Spillner E, et al. Detection of IgE to recombinant Api m1 and rVes v5 is valuable but not sufficient to distinguish bee from wasp venom allergy. J Allergy Clin Immunol. 2011;128 (1):247-8.