



Component resolved diagnostics for hymenoptera venom allergy

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Purpose of review

Component-resolved diagnostics makes use of defined allergen molecules to analyse IgE-mediated sensitizations at a molecular level. Here, we review recent studies on the use of component-resolved diagnostics in the field of Hymenoptera venom allergy (HVA) and discuss its benefits and limitations.

Recent findings

Component resolution in HVA has moved from single molecules to panels of allergens. Detection of specific immunoglobulin E (sIgE) to marker and cross-reactive venom allergens has been reported to facilitate the discrimination between primary sensitization and cross-reactivity and thus, to provide a better rationale for prescribing venom immunotherapy (VIT), particularly in patients sensitized to both honeybee and vespid venom. Characterization of IgE reactivity to a broad panel of venom allergens has allowed the identification of different sensitization profiles that in honeybee venom allergy were associated with increased risks for side effects or treatment failure of VIT. In contrast, component resolution so far has failed to provide reliable markers for the discrimination of sensitizations to venoms of different members of Vespidae.

Summary

Component-resolved diagnostics allows a better understanding of the complexity of sensitization and cross-reactivities in HVA. In addition, the enhanced resolution and precision may allow identification of biomarkers, which can be used for risk stratification in VIT. Knowledge about the molecular composition of different therapeutic preparations may enable the selection of appropriate preparations for VIT according to individual sensitization profiles, an approach consistent with the goals of personalized medicine.

Keywords

allergy, anaphylaxis, insect venom, risk stratification, specific immunoglobulin E diagnostics

INTRODUCTION

The diagnosis of Hymenoptera venom allergy (HVA) is based on the clinical history of a systemic/anaphylactic sting reaction and the detection of sensitization to relevant insect venoms by skin testing and/or detection of specific IgE antibodies in a serum sample [1^{*}]. In addition, cellular tests such as the basophil activation test (BAT) can be used not only in cases with a clear history but also with negative or unclear results of skin or in-vitro IgE tests. Depending on the geographical region, different insect species are more or less likely to be involved. The most frequent Hymenoptera sting reactions in central and northern Europe are caused by yellow jacket (*Vespula* spp.) and honeybee (*Apis mellifera*), whereas in southern Europe and the Americas, other wasps (e.g. *Polistinae*) are relevant. In addition, systemic sting reactions can be caused by ants, such as the jumper ant (*Myrmecia*) in Australia, the Asian needle ant (*Pachycondyla*) in Asia and the fire ant (*Solenopsis*) in the Americas.

HYMENOPTERA VENOM ALLERGENS

Whole venom preparations used for the detection of IgE-mediated sensitization contain a plethora of different components (such as proteins,

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KEY POINTS

- Currently, 75 venom allergens from 31 Hymenoptera species have been identified and officially accepted as allergens (www.allergen.org).
- Component resolution in routine diagnostics of HVA allows improved discrimination between primary sensitization and cross-reactivity, particularly in yellow jacket and honeybee venom allergy.
- Component-resolved diagnostics provides additional information on the complexity of the IgE and IgG4 immune response to Hymenoptera venom and allows characterization of individual sensitization profiles.
- Molecular sensitization profiles can be used for risk stratification that may lead to improved patient-related outcomes in VIT.

lipoproteins, glycoproteins, lipids etc.). The progress of molecular biology over the last decades has allowed a detailed characterization of relevant Hymenoptera venom allergens from different culprit insects. The currently known Hymenoptera venom allergens are summarized in Table 1. The list contains 75 allergens from 31 species and for some of these allergens, additional isoforms have been described. In the last decades, mainly the prototypical venom proteins (phospholipases, hyaluronidases and antigen 5) of several species were identified and accepted by the WHO/International Union of Immunology Societies' allergen nomenclature subcommittee as novel allergens (www.allergen.org).

The latest additions to the official list of hymenoptera venom allergens are Poly p 2, a hyaluronidase of *Polybia paulista*; Pol d 3, a dipeptidylpeptidase IV (DPPIV) of *Polistes dominula* and Pac c 3, an antigen 5 from *Pachycondyla chinensis*, the Asian needle ant. Poly p 2 from *Polybia paulista*, a clinically relevant social wasp that frequently causes stinging accidents in southeast Brazil, seems to have more pronounced IgE reactivity than the yellow jacket hyaluronidases Ves v 2 [3]. Pol d 3 from *Polistes dominula* is a member of the cross-reactive DPPIV protein family found in the majority of species and initially identified in honeybee venom (HBV) and yellow jacket venom (YJV). Pac c 3 was recently produced in recombinant form and was shown to exhibit significant IgE reactivity in patients with anaphylaxis [4[■]]. ImmunoCAP inhibition studies further showed the high degree of cross-reactivity to Ves v 5.

Additional potential allergens not yet included in the official allergen list have recently been described or in some cases evaluated as recombinant proteins. Poly p 1, a phospholipase A1 from *Polybia paulista* was cloned and produced in bacteria and

assessed with regard to IgE reactivity for diagnostic purposes [5[■]]. The main venom components of the ectoparasitic ant-like bethylid wasp were recently described [6]. Notably, the most abundant components were acid phosphatase and antigen 5. Identifying an acid phosphatase – a marker allergen found in HBV – in wasp venom might open novel questions about composition of venoms from even highly diverse species.

SPECIFIC IgE TO WHOLE VENOM PREPARATIONS

Skin and/or sIgE tests with whole venom preparations are regarded as the gold standard in the diagnostics of HVA. Today, a number of companies offer test systems for the detection of sIgE to insect venoms. Results are usually expressed as kU_A/l of allergen-specific IgE based on calibration against a heterologous IgE standard curve [7]. The international cut-off for sIgE detection historically has been set to $0.35 kU_A/l$. However, the lower limit of quantification (LoQ), that is the analytical sensitivity, of the most widely used modern autoanalyser-based singleplex IgE assays has been accepted by the regulatory authorities as $0.1 kU_A/l$ [8]. Thus, IgE antibody levels between 0.1 and $0.35 kU_A/l$ should be reported by the laboratory and must be interpreted by the clinician within the context of the patient's history, clinical symptoms and total serum IgE concentrations.

By using the cut-off value of $0.35 kU_A/l$, positive IgE test results to HBV have been reported in 90–100% of patients with HVA [9,10[■]]. With the same cut-off level, 83–97% of YJV-allergic patients have been reported to test positive for IgE to YJV [9,10[■],11]. Interestingly, sIgE against Ves v 5 could be detected in patients with a clear history of YJV anaphylaxis who were negative for sIgE to YJV, suggesting that the whole venom preparations had a shortage of Ves v 5 immunoreactivity [12–15]. Spiking of YJV with recombinant Ves v 5 increased sensitivity from 83 to 97% [11,15,16]. This improved YJV reagent has been available for routine diagnostics on the ImmunoCAP platform since 2012. In patients with low total IgE and a clear history of anaphylaxis, careful evaluation is needed as sIgE can sometimes be hard to detect [17,18]. Here, IgE antibody levels between 0.1 and $0.35 kU_A/l$ should be considered and interpreted by the clinician as indicated above.

MULTIPLE SENSITIZATIONS, CROSS-REACTIVITY AND COMPONENT-RESOLVED DIAGNOSTICS

Testing sIgE to whole venom preparations of different Hymenoptera has one major limitation: In cases

Table 1. Current list of Hymenoptera venom allergens as of July 2017 (www.allergen.org)

Allergen	Name/function	MW (kDa)	% DW	Potential N-glycosylation	Diagnostic availability
Honeybees (<i>Apis mellifera</i>, <i>Apis cerana</i>, <i>Apis dorsata</i>)					
Api m 1, Api c 1, Api d 1	Phospholipase A ₂	17	12	1	+ (rApi m 1)
Api m 2	Hyaluronidase	45	2	3	+ (rApi m 2)
Api m 3	Saure phosphatase	49	1–2	2	+ (rApi m 3)
Api m 4	Melittin	3	50	–	+ (rApi m 4)
Api m 5	Dipeptidylpeptidase IV	100	<1	6	+ (rApi m 5)
Api m 6	Protease inhibitor	8	1–2	–	–
Api m 7	Protease	39		3	–
Api m 8	Carboxylesterase	70		4	–
Api m 9	Carboxypeptidase	60		4	–
Api m 10	CRP/Icarapin	55	<1	2	+ (rApi m 10)
Api m 11	MRJP 8,9	60,65		3,6	–
Api m 12	Vitellogenin	200		1	–
Bumble bees (<i>Bombus terrestris</i>, <i>Bombus pennsylvanicus</i>)					
Bom t 1, Bom p 1	Phospholipase A ₂	16		1	–
Bom t 4, Bom p 4	Protease	27		–	–
Yellow Jackets (<i>Vespa vulgaris</i>*, <i>Vespa flavopilosa</i>, <i>Vespa germanica</i>, <i>Vespa maculifrons</i>, <i>Vespa pennsylvanica</i>, <i>Vespa squamosa</i>, <i>Vespa vidua</i>)					
Ves v 1, Ves m 1, Ves s 1	Phospholipase A ₁	34	6–14	–	+ (rVes v 1)
Ves v 2, Ves m 2	Hyaluronidase	45	1–3	2	–
Ves v 3	Dipeptidylpeptidase IV	100		6	–
Ves v 5, Ves f 5, Ves g 5, Ves m 5, Ves p 5, Ves s 5, Ves vi 5	Antigen 5	23	5–10	–	+ (rVes v 5)
Ves v 6	Vitellogenin	200		4	–
Hornets (<i>Vespa crabro</i>, <i>Vespa magnifica</i>, <i>Vespa mandarina</i>)					
Vesp c 1, Vesp ma 1, Vesp m 1	Phospholipase A ₁	34		–	–
Vesp ma 2	Hyaluronidase	35		–	–
Vesp c 5, Vesp ma 5, Vesp m 5	Antigen 5	23		1	–
Bald-faced hornet (e.g. <i>Dolichovespula maculata</i>, <i>Dolichovespula arenaria</i>)					
Dol m 1	Phospholipase A ₁	34		2	–
Dol m 2	Hyaluronidase	42		2	–
Dol m 5, Dol a 5	Antigen 5	23		–	–
European paper wasps (<i>Polistes dominula</i>, <i>Polistes gallicus</i>)					
Pol d 1, Pol g 1	Phospholipase A ₁	34		1	+ (rPol d 1)
Pol d 3	Dipeptidylpeptidase IV	100		4	–
Pol d 4	Protease	33		6	–
Pol d 5, Pol g 5	Antigen 5	23		–	+ (rPol d 5)
American paper wasps (<i>Polistes annularis</i>, <i>Polistes exclamans</i>, <i>Polistes fuscatus</i>, <i>Polistes metricus</i>)					
Pol a 1, Pol e 1	Phospholipase A ₁	34		–	–
Pol a 2	Hyaluronidase	38		2	–
Pol e 4	Protease	?		–	–
Pol a 5, Pol e 5, Pol f 5, Pol m 5	Antigen 5	23		–	–
South American paper wasps (<i>Polybia paulista</i>, <i>Polybia scutellaris</i>)					
Poly p 1	Phospholipase A ₁	34		–	–
Poly p 2	Hyaluronidase	33		2	–
Poly p 5, Poly s 5	Antigen 5	21		–	–

Table 1 (Continued)

Allergen	Name/function	MW (kDa)	% DW	Potential N-glycosylation	Diagnostic availability
Ants					
Fire ants (<i>Solenopsis invicta</i>, <i>Solenopsis richteri</i>, <i>Solenopsis saevissima</i>)					
Sol i 1	Phospholipase A ₁	18		3	–
Sol i 2, Sol r 2, Sol s 2		14		–	–
Sol i 3, Sol r 3, Sol s 3	Antigen 5	24–26		2	–
Sol i 4		12		–	–
Jumper ant (<i>Myrmecia pilosula</i>)					
Myr p 1		7.5, 5.5		–	–
Myr p 2	Pilosulin-3	8.5, 2.4		–	–
Myr p 3	Pilosulin-4.1			–	–
Asian needle ant (<i>Pachycondyla chinensis</i>)					
Pac c 3	Antigen 5	23		–	–

Modified with permission from [2].

*Of note, the *Vespula vulgaris* antigen Ves v 4, a protease, has been cloned and recombinantly expressed, but has so far not officially been confirmed as an allergen.

of double- or triple-positive test results against different venoms, the assays do not allow discrimination between cross-reactivity and primary sensitization to multiple venoms. According to the assessment of the current American Stinging Insect Hypersensitivity Practice Parameter [19[¶]], immunological cross-reactivity is extensive between hornet and YJV, somewhat less extensive between yellow jacket and hornet with wasp venoms and less common between honeybee and *Vespidea* venoms. Data from European studies, however, suggest that there is quite a substantial cross-reactivity also between honeybee and yellow jacket venom [10[¶],20–22]. In our own patient cohort ($n=815$) from the south/west region of Germany, 45% of patients with anaphylactic sting reactions display positive sIgE to both HBV and YJV [1[¶],20].

Double positivity may either reflect true double sensitization to both venoms or may be caused by IgE antibodies to cross-reactive carbohydrate determinants (CCD), which are present in the majority of hymenoptera venom allergens [21,23,24] or to homologous peptide sequences in proteins present in both venoms.

Analysis of single allergens in HBV and YJV allowed the identification of allergens that are present only in one or the other venom and may therefore, serve as so-called marker allergens to unequivocally identify a primary sensitization to a given venom. In the discrimination between primary HBV and YJV sensitization, Ves v 1 and Ves v 5 serve as marker allergens for YJV sensitization whereas Api m 1, Api m 3, Api m 4 and Api m 10 for HBV sensitization.

Other allergens are likely to be cross-reactive based on sequence homology, such as the

hyaluronidases Api m 2 and Ves v 2, the dipeptidyl-peptidases Api m 5 and Ves v 3 and the vitellogenins Api m 12 and Ves v 6 [25–28]. Significantly, the cross-reactivity of the hyaluronidases appears to be mostly based on IgE reactivity to CCD epitopes as demonstrated with YJV hyaluronidases (Ves v 2a, Ves v 2b) with and without CCDs [28]. In contrast, IgE reactivity to CCD-free HBV hyaluronidase Api m 2 is quite prevalent in HBV-allergic patients ranging from 43 to 52% [29,30^{¶¶},31^{¶¶}]. Structural data demonstrate that, despite high sequence identity, Api m 2 and Ves v 2 display very little homology when it comes to allergen surface epitopes [32]. Thus, IgE reactivity to CCD-free Api m 2 may also with a grain of salt be interpreted as indicator for a primary HBV sensitization.

RECOMBINANT VENOM ALLERGENS FOR ROUTINE DIAGNOSTICS OF HYMENOPTERA VENOM ALLERGY

Recently, recombinant venom allergens that are devoid of CCD reactivity have been introduced for routine sIgE diagnostics to improve the discrimination between primary sensitization and cross-reactivity [10[¶],13,22]. The first study on the use of rApi m 1 and rVes v 5 in IgE diagnostics reported a frequency of sensitization of 97% to Api m 1 among HBV and 87% to Ves v 5 among YJV sensitized patients, using a then available liquid-phase detection system [10[¶]]. Subsequent studies, using the currently available test systems and marker allergens for YJV, reported sensitization frequencies for rVes v 5 as 84.5–90% and for rVes v 1 as 33–54%. The combination of sIgE measurements in rVes v 5 and

rVes v 1 allowed for the detection of 92–98% of YJV-allergic patients [11,15,20,33,34]. Additional measurements of sIgE to Ves v 2 and Ves v 3 were suggested to further increase the ability to detect YJV sensitization [12], which, however, could not be confirmed by a follow-up study [35].

In contrast to the initial report [10[■]], the frequency of sensitization to rApi m 1 in HBV-allergic patients was found to be lower in subsequent studies that used currently available test systems. Reported frequencies range from 58 to 80% [13,31[■],36,37], leaving a considerable gap in the ability to detect HBV sensitization by using component-based diagnostic tools. Thus, lack of sensitization to Api m 1 in patients suspected of having HBV allergy has been regarded as insufficient to rule out primary HBV sensitization.

The difference in Api m 1 sensitization rates (ranging from 58 to 80%) has been suggested to reflect regional differences [14] or differences in the definition of the patient population [13]. In addition, the sensitivity of Api m 1 may partly depend on the test system used. Recently, direct comparison of sIgE levels with Api m 1 measured on the Immulite fluid phase test system and the ImmunoCAP solid phase test system suggested a higher sensitivity for the Immulite system [38[■],39[■]]. It was speculated that IgE binding capacity of the recombinant Api m 1 used in the ImmunoCAP system may be diminished because of altered protein folding [38[■],39[■]]. However, this notion was contradicted by a direct comparison of IgE binding with purified natural Api m 1, which was found to be identical in CCD-negative sera [40]. A more likely cause is an apparent difference in the heterologous calibration system between the two assays, as suggested by one study [39[■]]. Indeed, two comparative studies using chimeric IgE antibodies to different allergens provided convincing evidence that the Immulite system tends to overestimate the actual levels of sIgE to a given allergen as approximately three to five-fold [41,42], hence explaining the higher frequency of test results exceeding the cut-off level chosen.

Aside from Api m 1, additional major allergens have been reported in HBV allergy. Analysis of sIgE reactivity to a panel of HBV allergens (Api m 1–5, Api m 10) allowed the detection of 94% of patients with HBV allergy and demonstrated that patients with HBV allergy display a broad spectrum of sensitization profiles [31[■]]. Particularly interesting was the finding that HBV-specific marker allergens rApi m 3 and rApi m 10 allowed the detection of primary HBV sensitizations in about two thirds of Api m 1 negative sera [43], demonstrating that these

components help to reduce the diagnostic gap in detecting HBV sensitization.

HBV allergens currently available for routine sIgE diagnostics include rApi m 1, rApi m 2, rApi m 3, Api m 4, rApi m 5 and rApi m 10 (Table 2). By using this panel of allergens, a recent study [29] reported a combined sensitization frequency of only 79% among the HBV-allergic patients studied and concluded that the currently available allergens are still not sufficient to reliably identify HBV sensitization. The lower frequency of sensitization to at least one of the HBV components observed in this study, as compared with the study by Kohler *et al.* [31[■]] may have been due to differences in the patient populations, in particular the number of HBV monosensitized and HBV and YJV double-sensitized patients. We previously demonstrated that in patients with HBV allergy, concomitant sensitization to YJV was associated with higher levels of both total and HBV-specific IgE, as well as higher levels of sIgE to all HBV allergens tested [31[■]], suggesting effects that were independent of serological cross-reactivity. Accordingly, HBV monosensitized patients mostly displayed lower sIgE levels and recognition of fewer allergens, whereas double-sensitized patients often recognized multiple HBV allergens and with higher sIgE levels [31[■]]. The same trend was observed in a separate population of patients with YJV allergy, suggesting that this might reflect a more advanced state of atopic immune deviation in the double-sensitized population [31[■]].

Even more difficult than resolution between HBV and YJV sensitization is the discrimination between sensitization to yellow jacket and *Polistes* venoms, which is of particular relevance in Mediterranean countries and the Americas. Due to a high degree of IgE cross-reactivity, unequivocal discrimination is rarely achieved. Significantly, *Polistes* venom proteins are devoid of CCD reactivity [44], so that cross-reactivity is mostly caused by homologous proteins as described for the hyaluronidases (Ves v 2 and the homologous protein in *Polistes* venom), for the dipeptidylpeptidases (Ves v 3, Pol d 3), the group 5 antigens (Ves v 5, Pol d 5) and to a lesser extent for group 1 antigens (Ves v 1, Pol d 1). Among these venoms, which is the most likely primary sensitizer may however be indicated by the relative level of sensitization to them. To this end, the combination of group 1 and group 5 allergens were reported to identify the most probable sensitizing insect in two thirds of the patients studied, whereas the hyaluronidases (Ves v 2 and the homologous protein in *Polistes* venom) did not provide any additional value [45]. Currently available allergens for routine sIgE diagnostics to yellow jacket and *Polistes* venom include rVes v 1, rVes v 5, rPol d 1 and rPol d 5 (Table 2).

Table 2. Selected test systems for sIgE detection to Hymenoptera venom allergens as of July 2017

Allergen	Manufacturer	Test system	Significance
Honeybee			
Api m 1	Thermo Fisher	Singleplex, multiplex	Marker allergen for HBV sensitization, allows discrimination between HB and YJ/ <i>Polistes</i> venom sensitization
	Siemens	Singleplex	
Api m 2	Euroimmun	Multiplex	Major HBV allergen. Due to limited cross-reactivity with hyaluronidases of YJV or <i>Polistes</i> venom in the absence of CCDs, IgE to Api m 2 can be used as indicator for HBV sensitization
	Dr Fooke Laboratories	Singleplex	
	Macro Array Diagnostics (ALEX and Faber test)	Multiplex	
	Thermo Fisher	Singleplex	
Api m 3	Thermo Fisher	Singleplex	Marker allergen for HBV sensitization, allows discrimination between HB and YJ/ <i>Polistes</i> venom sensitization
Api m 4	Macro Array Diagnostics (Faber test)	Multiplex	Marker allergen for HBV sensitization, allows discrimination between HB and YJ/ <i>Polistes</i> venom sensitization
Api m 5	Thermo Fisher	Singleplex	Major HBV allergen. High cross-reactivity with dipeptidylpeptidase of YJV and <i>Polistes</i> venom prevents its use as marker allergen
Api m 10	Thermo Fisher	Singleplex	Marker allergen for HBV sensitization, allows discrimination between HB and YJ/ <i>Polistes</i> venom sensitization
	Euroimmun	Multiplex	
	Dr Fooke Laboratories	Singleplex	
	Macro Array Diagnostics (ALEX)	Multiplex	
Yellow jacket			
Ves v 1	Thermo Fisher	Singleplex	Marker allergen for YJV sensitization, allows discrimination between YJV and HBV sensitization; n.b. cross-reactivity with phospholipase of <i>Polistes</i> venom
	Euroimmun	Multiplex	
Ves v 5	Thermo Fisher	Singleplex, multiplex	Marker allergen for YJV sensitization, allows discrimination between YJV and HBV sensitization; n.b. high cross-reactivity with antigen 5 of <i>Polistes</i> venom
	Siemens	Singleplex	
	Euroimmun	Multiplex	
	Dr Fooke Laboratories	Singleplex	
Macro Array Diagnostics (ALEX)	Multiplex		
European paper wasp			
Pol d 1	Euroimmun	Multiplex	Marker allergen for <i>Polistes</i> venom sensitization, allows discrimination between <i>Polistes</i> and HBV sensitization; n.b. cross-reactivity with phospholipase of YJV
Pol d 5	Thermo Fisher	Singleplex	Marker allergen for <i>Polistes</i> venom sensitization, allows discrimination between <i>Polistes</i> and HBV sensitization; n.b. high cross-reactivity with phospholipase of YJV
	Euroimmun	Multiplex	
	Macro Array Diagnostics (ALEX)	Multiplex	

CCD, cross-reactive carbohydrate determinant; HBV, honeybee venom; YJV, yellow jacket venom.

Additional members of the Vespidae family play a prominent role in other areas of the world, such as the genus *Polybia* in South America. *Polybia* belong to the subfamily of *Polistinae* and a number of allergens have recently been cloned and characterized. These *Polybia* allergens display a high degree of sequence homology to their counterparts in yellow jacket and *Polistes* venoms. Again, no unique marker allergen has been identified, which would likely allow reliable discrimination between *Polistinae* and *Vespinae* venom sensitization.

Finally, cross-reactivity is also observed between ant venom and YJV. In particular, the antigen 5 allergens exhibit significant degree of sequence

identity, rendering differentiation of sensitization to venom of *Formicoidea* and *Vespoidea* superfamily members difficult.

In conclusion, the currently available recombinant Hymenoptera venom allergens are useful for the identification of sensitizations to YJV and HBV allergens, not confounded by CCD reactivity, even if a minority (5–20%) of HBV-allergic patients is not sensitized to any of the available HBV allergens. Although Ves v 1 and Ves v 5 negative results exclude YJV sensitization with a high likelihood, negative results to the HBV-specific allergens Api m 1, Api m 2, Api m 3, Api m 4 and Api m 10 do not necessarily exclude HBV sensitization. Here,

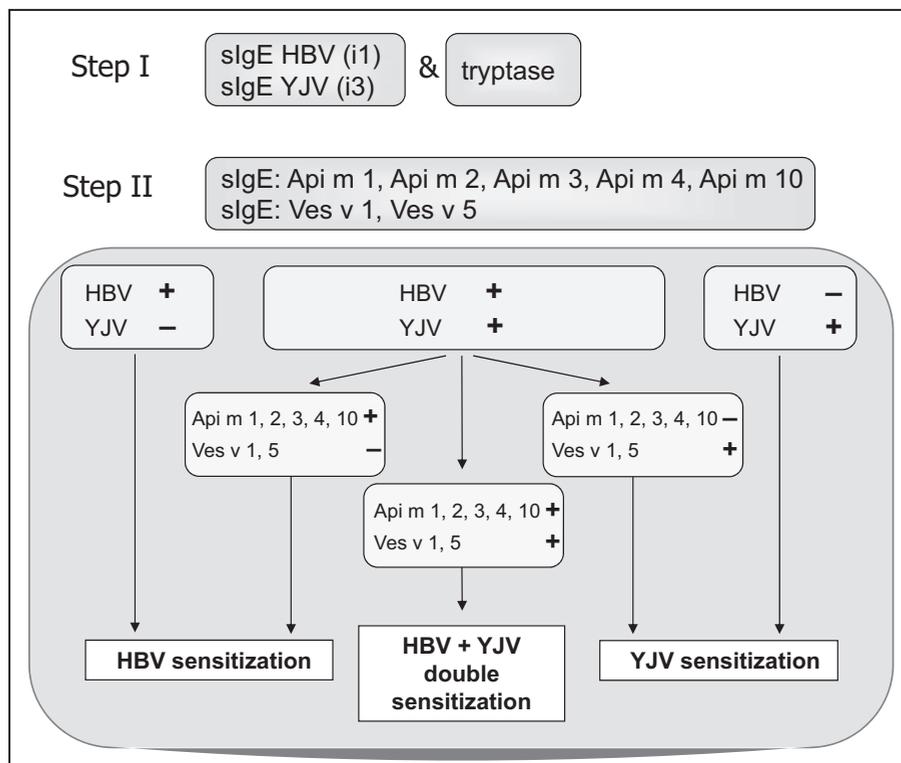


FIGURE 1. Two-step diagnostic algorithm for an improved discrimination between yellow jacket venom and honeybee venom sensitization. Step I: Baseline in-vitro diagnostics; Step II: Component-resolved diagnostics in cases of honeybee venom and yellow jacket venom double-positive cases, or in cases of discrepancies between history, skin test and serology. Please refer to Table 2 for availability of single allergens for routine diagnostics by different manufacturers. HBV, honeybee venom; YJV, yellow jacket venom. Modified with permission from [2].

consideration of low (0.1–0.35 kU_A/l) IgE levels to HBV marker allergens, comparison of IgE levels with cross-reactive allergens or the identification of additional HBV marker allergens would be helpful towards optimizing the diagnostic precision. No major marker allergens that would support definitive discrimination of sensitizations to venoms of different Vespidae subfamilies have so far been identified.

Based on the available allergens, a diagnostic algorithm has been suggested for an improved discrimination between YJV and HBV sensitization (Fig. 1, modified from [2]).

COMPONENT-RESOLVED DIAGNOSTICS FOR PERSONALIZED RISK STRATIFICATION IN VENOM IMMUNOTHERAPY

Component-resolved diagnostics not only supports improved diagnostic precision in HVA but also enables detailed characterization of sensitization profiles of individual patients. Particularly in HBV allergy, patients display a wide spectrum of sensitization

profiles [31^{***}], which might be associated with different risks in venom immunotherapy (VIT).

In this context, it was of interest that some of the newly identified major allergens in HBV, namely Apm 3 and Apm 10, were reported to be under-represented or absent in a number of therapeutic preparations [46^{***}]. This observation prompted us to ask whether treatment failure in honeybee VIT may be associated with certain sensitization profiles. In a retrospective study of VIT-treated HBV-allergic patients, comparison of sIgE levels with HBV and individual allergens identified predominant sensitization to Apm 10 (>50% of sIgE to HBV) as the best predictor of treatment failure with an odds ratio 8.44. No such signal was obtained for dominant sensitization to any of the other allergens [30^{***}]. In this study, again some of the therapeutic HBV preparations analysed displayed lack of Apm 10, whereas their Apm 1 content was comparable with that of crude HBV [30^{***}]. A follow-up analysis of the allergen composition of four different therapeutic venom preparations confirmed the previously reported lack or underrepresentation of Apm 10 [47^{***}]. In addition, shortage of Apm 3 and Apm 5

was observed in some preparations. Of note, although some allergens are present in large quantities (Api m 1, Api m 2 and Api m 4 with 10%, 3% and >40% of venom dry weight, respectively), Api m 3, Api m 5 and Api m 10 belong to the low abundance allergens with less than 1% of the venom dry weight. Currently, we do not fully understand the role of Api m 10 in HBV allergy and tolerance induction during VIT. However, the high prevalence of Api m 10 sensitization (>50%), the shortage of Api m 10 in widely used therapeutic HBV preparations and the significant association of dominant Api 10 sensitization and treatment failure strongly suggest that Api m 10 is a relevant allergen and that this kind of component-resolved diagnostics may be useful for the risk stratification in honeybee VIT. Even though prospective studies are still lacking, the clinical implication would be that HBV-allergic patients with dominant sensitization to Api m 10 are at increased risk for treatment failure in honeybee VIT and should preferably be treated with HBV preparations demonstrated to contain an adequate amount of Api m 10, sufficient to induce a robust IgG4 response in treated patients.

In a recent study, component-resolved diagnostics using nApi m 1, rApi m 2 and Api m 4 demonstrated a high prevalence of Api m 4 sensitization among HBV-allergic patients who experienced systemic reactions during the induction of honeybee VIT [48]. A subsequent prospective study stratified HBV-allergic patients according to their sIgE to Api m 4 into two groups (<0.98 or >0.98 KU_A/l) and confirmed higher rates of systemic reactions during the VIT induction phase in the latter group. In addition, this group was characterized by increased baseline skin reactivity, increased base line HBV sIgE and more persistent responses in intradermal testing during VIT [49].

This data supports the concept that component-resolved diagnostics will enable us to define different endotypes of HBV allergy and based on the individual's sensitization profile, allow a personalized risk stratification as well as an optimization of treatment protocols.

UNRESOLVED/OPEN ISSUES

For the use in clinical routine, test reagents ideally should allow a definite discrimination between sensitization to one or the other Hymenoptera venom. Marker allergens such as Ves v 5, Ves v 1 and Api m 1, Api m 3, Api m 4 and Api m 10 allow such discrimination between YJV and HBV sensitization in the majority of cases. However, the limited sensitization prevalence to HBV marker allergens only allows detection of 80–90% of HBV-allergic patients

[29,30,31]. To further reduce this diagnostic gap, it should be helpful to take advantage of the low end of the assays' measuring range and consider IgE levels down to the LoQ of 0.1 kU_A/l, particularly in patients with low total IgE. In addition, direct quantitative comparison of IgE levels with corresponding cross-reactive HBV and YJV allergens such as Api m 2 and Ves v 2, Api m 5 and Ves v3 or Api 12 and Ves v 6 might prove useful towards identifying the primary sensitizer. The best example are the hyaluronidases Api m 2 and Ves v 2. Api m 2 is a major allergen in HBV allergy, in contrast, IgE reactivity to Ves v 2 is mostly CCD-related and only few patients with YJV allergy display CCD-independent reactivity to Ves v 2 [27,28]. The same approach could be useful in the discrimination between sensitizations to Vespidae and *Polistinae* venoms [45]. However, so far no manufacturer offers test reagents that allow this kind of direct comparison of IgE reactivity with cross-reactive Hymenoptera venom allergens.

The BAT by using whole venom preparations has been demonstrated to be helpful in the investigation of double-sensitized patients or in patients with a clear history of sting reactions but negative sIgE and skin tests [17,50]. The BAT may be of similar diagnostic advantage by using single CCD-free allergens, as recently demonstrated in YJV-allergic patients [12]. So far, component resolution in BAT has only been performed in academic research settings. For routine testing, however, a stringent standardization of allergen preparations, stability and test procedures would be required. Provided this can be achieved, BAT may be helpful to close diagnostic gaps that sIgE determinations leave open.

Finally, data obtained so far on the potential of component-resolved diagnostics for improved risk stratification in VIT is still limited. Prospective randomized studies are needed to put current hypotheses to the test. This is especially true for the retrospective data on Api m 10 regarding the VIT response in patients with HBV allergy.

CONCLUSION

Component resolution provides for a better understanding of the complexity of sensitization and cross-reactivities in HVA. In addition, it has opened up new avenues for identification of biomarkers that may allow risk stratification for VIT responses. The continuously expanding field of venom allergens will permit enhanced resolution and precision in the diagnostic testing of patients with suspected HVA. In addition, improved methods of monitoring therapeutic outcomes and detailed knowledge about the molecular composition of different

therapeutic preparations will enable the selection of appropriate venom preparations for VIT according to the individual sensitization profile. This will help move VIT from a generalized towards a precision-targeted immunotherapy approach, consistent with all other efforts to achieve the goals of personalized medicine.

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