

REVIEW

Component-resolved diagnostics to direct in venom immunotherapy: Important steps towards precision medicine

S. Blank¹  | M. B. Bilò² | M. Ollert^{3,4}

¹Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany

²Allergy Unit, Department of Internal Medicine, University Hospital of Ancona, Ancona, Italy

³Department of Infection and Immunity, Luxembourg Institute of Health (LIH), Esch-sur-Alzette, Luxembourg

⁴Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis, University of Southern Denmark, Odense, Denmark

Correspondence

Simon Blank, Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Munich, Germany.
Email: simon.blank@tum.de

Summary

Stings of Hymenoptera can induce IgE-mediated systemic and even fatal allergic reactions. Venom-specific immunotherapy (VIT) is the only disease-modifying and curative treatment of venom allergy. However, choosing the correct venom for VIT represents a necessary prerequisite for efficient protection against further anaphylactic sting reactions after VIT. In the past, therapeutic decisions based on the measurement of specific IgE (sIgE) levels to whole venom extracts were not always straightforward, especially when the patient was not able to identify the culprit insect. In the last years, the increasing knowledge about the molecular structure and relevance of important venom allergens and their availability as recombinant allergens, devoid of cross-reactive carbohydrate determinants, resulted in the development of an advanced component-resolved diagnostics (CRD) approach in venom allergy. Already to date, CRD has increased the sensitivity of sIgE detection and enabled the discrimination between primary sensitization and cross-reactivity, particularly in patients with sensitization to both honeybee and vespid venom. Hence, CRD in many patients improves the selection of the appropriate immunotherapeutic intervention. Moreover, the detailed knowledge about sensitization profiles on a molecular level might open new options to identify patients who are at increased risk of side-effects or not to respond to immunotherapy. Therefore, increasing potential of CRD becomes evident, to direct therapeutic decisions in a personalized and patient-tailored manner. Reviewed here are the state of the art options, recent developments and future perspectives of CRD of Hymenoptera venom allergy.

1 | INTRODUCTION

Allergy to insect stings is one of the most severe IgE-mediated hypersensitivity reactions with regard to the high risk of severe and even fatal anaphylaxis. The only curative treatment which is effective in reducing the risk of subsequent systemic reactions and to improve the patients' quality of life is venom immunotherapy (VIT). VIT is reported to be effective in 77%-84% of patients treated with honeybee venom and in 91%-96% of patients receiving vespid venom.¹ However, successful VIT depends on the use of the correct venom for therapy and, hence, the identification of the allergy-eliciting Hymenoptera species is a prerequisite for a successful therapy.

As there are numerous allergy-eliciting Hymenoptera species with overlapping phenotypes that are hard to discriminate, many patients, primary care doctors and even allergy specialists are not able to correctly distinguish between different species.² Unnecessary treatment with more than one or even with the wrong venom can lead to de novo sensitizations,³ potentially increased risk of side-effects, missing or limited protection to further stings, increases in treatment costs and even fatal outcome in the worst case scenario.

In northern and central Europe, the most common elicitors of venom allergy are honeybees (*Apis mellifera*) and yellow jackets (*Vespa spp.*). In southern Europe and America, additionally, allergy to paper wasps (*Polistes spp.*) is common. Particularly, *Polistes dominula*,

known to be domestic in southern Europe, is an invasive species which increasingly spreads to more moderate climate zones of Europe.⁴ *Polistes dominula* has also entered the United States.⁵ Therefore, allergy to *Polistes dominula* might also gain importance as an elicitor of venom anaphylaxis in other areas.

For many patients, it is hard to discriminate between different members of the *Apidae* and *Vespidae* families, so that correct diagnosis often depends on an extensive and sometimes challenging diagnostic work-up by the allergy specialist. The diagnosis of Hymenoptera venom allergy comprises the clinical history of a systemic sting reaction and the proof of sensitization to the relevant venom by skin testing and/or the detection of venom-specific IgE antibodies in the serum of the patients.^{1,6,7} Additionally, cellular tests such as the basophil activation test (BAT) can be used in cases of unclear or even negative skin and specific IgE (sIgE) test results.⁸⁻¹⁰ Especially, when the patient was not able to identify the allergy-eliciting insect, in clinical practice, the correct diagnosis is not always straightforward due to inherent limitations of skin testing and whole venom-based in vitro diagnosis.¹¹

Many of the diagnostic limitations are due to the use of whole venom preparations. However, in the last years, increasing knowledge about the venom composition on a molecular level and the availability of recombinant allergens with advanced characteristics have facilitated the development of an advanced molecular or component-resolved diagnostics (CRD) approach to Hymenoptera venom allergy,¹² which has largely contributed to solving many diagnostic challenges.^{11,13} Hence, CRD of venom allergy has created the basis for a more reliable identification of the venom responsible for the allergic reaction and provides appropriate and evidence-based guidance for therapeutic decisions. Here, we will give examples for the advantages and limitations of component-resolved diagnostics and for potential therapeutic implications with a focus on honeybee venom (HBV), yellow jacket venom (YJV) and *Polistes dominula* venom (PDV) allergy.

2 | WHICH ALLERGENS ARE IMPORTANT IN VENOM ALLERGY?

Hymenoptera venoms are complex mixtures of various components which together mediate the venomous effects, including small molecular weight substances such as biogenic amines, several small peptides and many proteins and, hence, potential allergens. An overview about allergens of HBV, YJV and PDV is given in Table 1.

The venom of the honeybee *Apis mellifera* is certainly the best characterized Hymenoptera venom.^{14,15} So far, 12 allergens of HBV are included in the official WHO/International Union of Immunology Societies' allergen nomenclature list.¹⁶ In fact, only two of these allergens make up substantial amounts of the venom, the major allergen phospholipase A2 (Api m 1) and the minor peptidic allergen melittin (Api m 4) with 12% and 50% of the venom dry weight, respectively.¹⁷ All other HBV allergens are contained in the venom in much lower quantities. Despite their low abundance in HBV, in

TABLE 1 Allergens of HBV, YJV and PDV

Allergen	Name/Function	MW [kDa]	% of DW	Available for CRD
Honeybee (<i>Apis mellifera</i>)				
Api m 1	Phospholipase A2	17	12	S ^{a,b,c} , M ^{a,d,e}
Api m 2	Hyaluronidase	45	2	S ^{a,b,c} , M ^{d,e}
Api m 3	Acid phosphatase	49	1-2	S ^a
Api m 4	Melittin	3	50	M ^e
Api m 5	Dipeptidyl peptidase IV	100	<1	S ^a
Api m 6	Protease inhibitor	8	1-2	
Api m 7	Protease	39		
Api m 8	Carboxylesterase	70		
Api m 9	Carboxypeptidase	60		
Api m 10	Icarapin	55	<1	S ^{a,c} , M ^{d,e}
Api m 11.0101	Major Royal Jelly Protein 8	55		
Api m 11.0201	Major Royal Jelly Protein 8	60		
Api m 12	Vitellogenin	200		
Yellow jacket (<i>Vespa vulgaris</i>)				
Ves v 1	Phospholipase A1	35	6-14	S ^a , M ^d
Ves v 2.0101	Hyaluronidase	45	1-3	
Ves v 2.0201	Hyaluronidase (inactive)	45		
Ves v 3	Dipeptidyl peptidase IV	100		
Ves v 5	Antigen 5	25	5-10	S ^{a,b,c} , M ^{a,d,e}
Ves v 6	Vitellogenin	200		
European paper wasp (<i>Polistes dominula</i>)				
Pol d 1	Phospholipase	34		M ^d
Pol d 2	Hyaluronidase	45		
Pol d 3	Dipeptidyl peptidase IV	100		
Pol d 4	Protease	33		
Pol d 5	Antigen 5	23		S ^a , M ^{d,e}

CRD, Component-resolved Diagnostics; DW, Dry weight; M, Multiplex assay; S, Singleplex assay.

^aThermo Fisher Scientific, ^bSiemens Healthcare Diagnostics, ^cDr. Focke Laboratories, ^dEuroimmun, ^eMacro Array Diagnostics.

addition to Api m 1, a role as relevant major allergen was also confirmed for Api m 2 (hyaluronidase), Api m 3 (acid phosphatase), Api m 5 (dipeptidyl peptidase IV) and Api m 10 (icarapin) with sIgE reactivity in the range of 47.9%-52.2%, 49.6%-50%, 58.3%-61.7% and 61.8%-72.2% of allergic patients' sera, respectively.^{18,19} For the other HBV allergens, less information about sensitization rates is available.²⁰⁻²²

Prominent YJV allergens include phospholipase A1 (Ves v 1), hyaluronidase (Ves v 2.0101) and antigen 5 (Ves v 5).^{23,24} Moreover, a second hyaluronidase (Ves v 2.0201) carrying an inactivating

mutation in the active site of the enzyme was identified, which seems to be the predominant isoform.^{25,26} YJV also contains a dipeptidyl peptidase IV (Ves v 3) which shows high homology to HBV Api m 5.²⁷ The sensitization rates of YJV-allergic patients to Ves v 1, Ves v 3 and Ves v 5 are 33.3%-54%,²⁸⁻³³ 50%-62.8%^{27,28} and 84.5%-100%, respectively.^{28,30,31,33-35} In contrast to HBV hyaluronidase Api m 2, which is a major allergen, the YJV homologue Ves v 2 seems to be of restricted relevance and sensitization was reported in 5%-25% of YJV-allergic patients.³⁶

The allergen composition of PDV is very similar to that of YJV, and the most important allergens are phospholipase A1 (Pol d 1), dipeptidyl peptidase IV (Pol d 3) and antigen 5 (Pol d 5) with sensitization rates of 87%,³⁷ 66.7%³⁸ and 69%-72%,³⁷ respectively. Moreover, PDV contains a serine protease (Pol d 4) and a hyaluronidase (Pol d 2), for which no data about their clinical relevance are available so far.

3 | DIAGNOSTIC SENSITIVITY OF COMPONENT-RESOLVED sIgE DIAGNOSTICS

In the last years, it has become more and more evident that testing for sIgE reactivity against several single allergens is evolving as a superior tool to support classical allergy testing. In contrast to venom extract-based sIgE testing, which only provides information whether there is sensitization to the whole venom or not, the component-resolved approach additionally allows for exactly determining which allergens are relevant for a given patient in an insect venom extract (Figure 1). The Hymenoptera venom allergens currently available on various diagnostic platforms for either singleplex or multiplex testing are listed in Table 1.

The diagnostic sensitivity of a combination of the YJV allergens Ves v 1 and Ves v 5 lies between 92% and 98%.^{29,30,33,36,39,40} Although diagnostic sensitivity of these two commercially available

allergens is quite high and similar or even higher than whole YJV (using the cut-off of 0.35 kU_A/L 83%-97% of YJV-allergic patients are tested positive for IgE to YJV extract),^{33,35,41} in the cited studies, CRD failed to diagnose 2%-8% of subjects with clear history of YJV allergy, suggesting that more allergen components for in vitro diagnostics are needed. Indeed, one study using a combination of routine ImmunoCAPs for Ves v 1 and Ves v 5 and ELISAs for Ves v 2 and Ves v 3 demonstrated a diagnostic sensitivity of 100%.²⁸ In contrast, these results were not confirmed in the following study using ImmunoCAPs for all allergens.⁴² More recently, however, Michel et al⁴³ used Ves v 1 and Ves v 5 (Immulate system) to diagnose 27 patients with YJV allergy and 53 patients with YJV allergy and mastocytosis and/or elevated baseline serum tryptase. In this study, a diagnostic sensitivity of 100% was reached in the first group using the cut-off of 0.35 kU_A/L and in the second group using the cut-off of 0.1 kU_A/L.

For the diagnosis of PDV allergy, so far, only the major allergen Pol d 5 is available on the most widely used singleplex IgE assay (ImmunoCAP), thus clearly pointing to the need of adding additional allergens such as Pol d 1 or others for enabling a more accurate CRD of PDV-allergic patients.^{37,44}

Component-resolved diagnostics of HBV allergy is even more complex compared to vespid venom allergy in terms of diagnostic sensitivity. In a first study, using Api m 1 on a liquid-phase IgE assay system no longer available, a diagnostic sensitivity as high as 97% was reported.³⁵ However, in follow-up studies applying currently available immunoassay platforms, sensitivity of Api m 1 was lower and ranged between 58% and 80%.^{19,34,45-47} These differences in sensitization rates were attributed to differential inclusion criteria of the patient population, geographical differences and to sensitivity aspects of the immunoassay platforms used.^{34,40,48-50} However, what these studies demonstrated foremost was the need for including additional allergens to properly diagnose HBV allergy by CRD. After the clinical relevance of several more HBV allergens was demonstrated,¹⁹ all these molecules (Api m 2, Api m 3, Api m 4, Api m 5, Api m 10) became available for CRD in the clinical routine. The

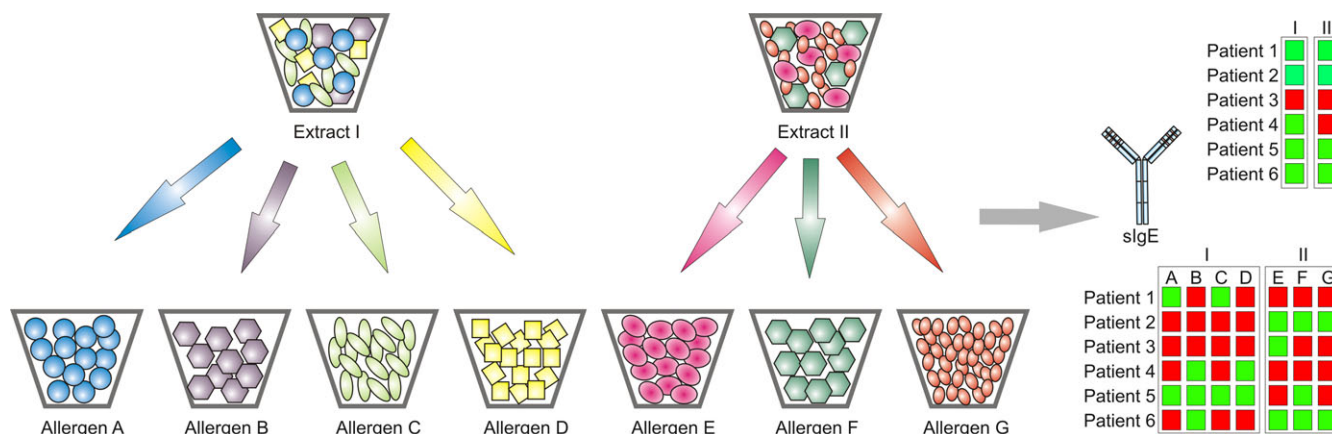


FIGURE 1 Concept of component-resolved diagnostics (CRD). Venom extract-based diagnostics provide the information if a patient is sensitized to the whole venom or not. In contrast, in CRD, sIgE to a number of important isolated allergen molecules is measured. Hence, CRD gives information about the sensitization profile of the patient on a molecular level and allows determining exactly which allergens are relevant for a given patient

first study that used this combination of allergens to diagnose 144 patients with confirmed history of HBV allergy demonstrated a diagnostic sensitivity of 94.4%.¹⁹ However, a recent study using the allergens Api m 1, Api m 2, Api m 3, Api m 5 and Api m 10 on the same assay platform showed differences in the diagnostic sensitivity for patients with allergy to HBV only and for those, allergic to HBV and YJV.⁵⁰ In patients monosensitized to HBV and double sensitized to HBV and YJV, the diagnostic sensitivity was 71.6% and 92.7%, respectively. Certainly, these data demonstrate the need for additional studies, using well-defined patient populations, to evaluate if the sensitivity of the available sIgE tests and the number of available allergens is sufficient for appropriate CRD of patients allergic to HBV. Nevertheless, the extension of the panel of commercially available HBV allergens has created clear added clinical benefit as approximately two-thirds of patients with negative sIgE to Api m 1 can be diagnosed using Api m 3 and Api m 10.⁵¹ It is of note that using the cut-off of 0.35 kU_A/L, 85%-100% of HBV-allergic patients are tested positive for IgE to HBV extract.^{35,41,52}

4 | CRD TO DIRECT IN MULTIPLE sIgE SENSITIZATION

Double-positive sIgE test results are commonly observed in venom-allergic patients, but clinical relevance of these results in terms of systemic symptoms is rare.⁵³ However, as many patients are not able to identify the culprit insect, a clinically relevant allergy to both venoms cannot be excluded. Therefore, this often leads to unnecessary VIT with both venoms resulting in higher costs, potentially increased risk of side-effects and the possibility of de novo sensitization.

Major reasons that are responsible for these clinically irrelevant double-positive sIgE test results are as follows: i) IgE antibodies directed to protein epitopes on homologous allergens, which are present in both venoms, ii) sIgE to cross-reactive carbohydrate determinants (CCDs) and iii) additionally, clinically irrelevant test results may be caused by asymptomatic sensitization as discussed in the section "Limitations and future perspectives of component-resolved diagnostics."

Cross-reactive carbohydrate determinants are present on the majority of HBV and YJV allergens,⁵⁴ and they most frequently cause double sensitization to these venoms.⁵⁵ IgE antibodies directed against CCDs were shown to be of high affinity,⁵⁶ but their clinical relevance seems to be low, meaning that they cause no clinical symptoms.⁵⁷ However, they clearly interfere with diagnostic approaches, as they lead to positive test results with any allergen source displaying CCDs. While measuring sIgE to CCD marker molecules such as MUXF or bromelain is able to confirm the presence of CCD-specific IgE as reason for multiple positive test results, the detection of these antibodies does not rule out a clinically relevant sensitization to protein epitopes on allergens of different venoms.^{12,55,58} In CRD, the CCD problem was solved by the introduction of recombinant allergens that display the full protein epitope spectrum of the native allergens but are free of CCDs.^{26,59} Thus, positive sIgE test results using these

engineered allergens in CRD indicate sensitization to protein epitopes only and not to CCD epitopes.^{45,60} Hence, CRD with CCD-free allergens offers clear advantages to adequately diagnose patients exhibiting CCD-specific IgE antibodies.¹¹

Additionally, double-positive sIgE test results can be caused by homologous allergens which are present in different venoms. So far, three homologous allergen pairs have been described in HBV and YJV: the hyaluronidases (Api m 2 and Ves v 2), the dipeptidyl peptidases IV (Api m 5 and Ves v 3) and the vitellogenins (Api m 12 and Ves v 6) (Figure 2). However, it was suggested that cross-reactivity of the hyaluronidases is mainly caused by CCDs.^{26,36} This is further supported by the identical sensitization rates to CCD-free Api m 2 in HBV monosensitized and HBV and YJV double-sensitized patients.⁵⁰ In contrast, the sensitization to CCD-free Api m 5 is more than doubled in patients double sensitized to HBV and YJV, indicating a high degree of cross-reactivity on the level of protein epitopes.⁵⁰ Much less detail is known about the cross-reactivity of the vitellogenins.²¹

The potential of CRD becomes evident by the fact that HBV and YJV in addition to homologous allergens also contain differentiating marker allergens which are present in either HBV or YJV. So far, identified marker allergens are Api m 1, Api m 3, Api m 4 and Api m 10 in HBV and Ves v 1 and Ves v 5 in YJV (Figure 2). Hence, in addition to the use of allergens in a CCD-free context, the other major advantage of CRD is that it allows the diagnostic application of these differentiating allergens as single components, thus excluding any perturbation of sIgE test results by cross-reactive allergens as it is the case in extract-based sIgE testing. Due to the fact that cross-reactivity between Api m 2 and Ves v 2 is mainly attributed to CCDs and that Ves v 2 represents only a minor allergen³⁶ of YJV, while Api m 2 is an important major allergen of HBV,^{18,19,50} also the measurement of sIgE to Api m 2 might be beneficial to diagnose HBV allergy. However, a clear shortage of the currently commercially available allergens for CRD is the unavailability of potentially cross-reactive allergens of YJV (and of PDV) such as hyaluronidases and dipeptidyl peptidases IV.

While there is intriguing evidence that the availability of molecular allergens for CRD on commercially available immunoassay platforms represents an advanced tool to discriminate between sensitization to HBV and YJV/PDV, this is not the case when differential diagnosis between *Vespula* spp. and *Polistes* spp. is required. In the south of Europe double sensitization to YJV and PDV is more frequent than that to YJV/PDV and HBV.⁶¹⁻⁶³ A definite discrimination is rarely possible due to a high degree of cross-reactivity of these venoms. Unlike HBV and YJV, venoms of different *Polistes* species have been demonstrated to be devoid of any CCD reactivity and, hence, allow CCD interference-free diagnostics.⁶⁴ However, due to the structural similarity and, therefore, cross-reactivity of the so far identified important allergens of vespid venoms such as phospholipases A1, antigens 5 and dipeptidyl peptidases IV (Figure 2), differentiation between allergy to the stings of the different vespid species is still challenging.^{37,65,66} Unique marker allergens that would allow reliable discrimination are not available and, to our opinion, will be hard to find due to the close phylogenetic distance of the species. A recent study reported that the measurement of the relative

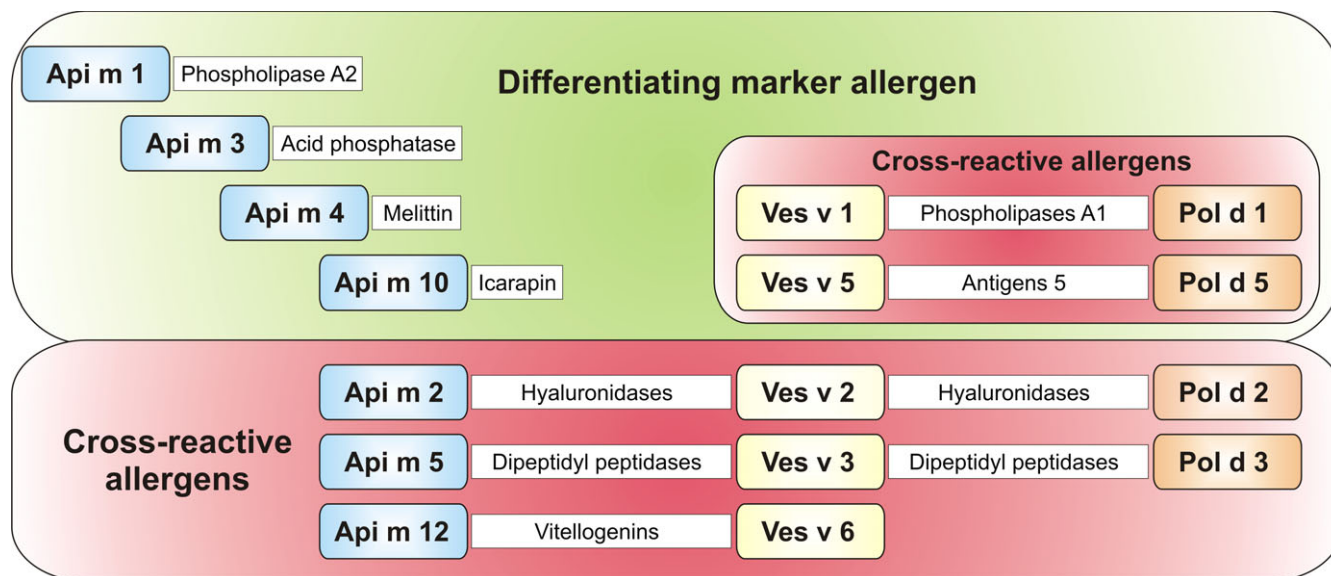


FIGURE 2 Established differentiating and cross-reactive allergens of HBV, YJV and PDV. While allergens are identified that enable a differentiation between HBV (Api m 1, Api m 3, Api m 4 and Api m 10) and YJV/PDV (Ves v 1/Pol d 1 and Ves v 5/Pol d 5) sensitization, the so far known allergens of YJV and PDV exhibit cross-reactivity

levels of sIgE to the phospholipases A1 (Ves v 1 and Pol d 1) and antigens 5 (Ves v 5 and Pol d 5) of YJV and PDV is required to identify the most probable sensitizing species in 69% of allergic patients with double sensitization.³⁷ In a smaller population, the detection of sIgE to Pol d 1, Pol d 5, Ves v 1 and Ves v 5 seemed to be able to determine the venom for VIT in the majority of patients.⁶⁷ However, in many cases, the results will be difficult to interpret and, moreover, not all required allergens are available for the most commonly used sIgE assay platforms. Recently, the ability of CRD with the commercially available allergen components to identify the allergy-triggering venom in patients with double-positive tests to *Polistes spp.* and *Vespula spp.* venom was compared to CAP-inhibition assays.^{68,69} A good concordance between the results was found when the value of sIgE in kU_A/L to Ves v 5 was about twice of those to Pol d 5 and vice versa, a fact, clearly demonstrating the limitations of currently available CRD to distinguish between allergy to these species. Therefore, the availability of additional YJV and PDV allergens for CRD will clearly add value for proper and advanced diagnosis of vespid venom-allergic patients.

Based on the commercially available allergens, diagnostics algorithms for sIgE testing to discriminate between HBV and vespid venom allergy as well as between YJV and PDV allergy are suggested in Figure 3A and Figure 3B, respectively.

5 | CRD TO DIRECT IN PATIENTS WITH SENSITIZATION THAT IS DIFFICULT TO PROOF

According to current guidelines, in addition to a reported history of a systemic sting reaction, the proof of sIgE sensitization by either

skin test or in vitro diagnostics is a prerequisite for the prescription of potentially life-saving VIT.¹ However, there are patients for whom this proof is difficult to achieve.

A prominent example is a study that demonstrated that only 83.4% (cut-off 0.35 kU_A/L) of patients with systemic reaction to YJV could be diagnosed with the conventional YJV ImmunoCAP, while sensitization was verified in 96% of patients using the individual allergens Ves v 1 and Ves v 5.³³ Interestingly, amongst the patients negative for whole YJV, only one was tested positive for Ves v 1, whereas 84.4% showed a positive test for Ves v 5, suggesting a shortage of available Ves v 5 epitopes in the whole venom preparation despite the abundance of this allergen in YJV. Spiking of the YJV ImmunoCAP with recombinant Ves v 5 increased the diagnostic sensitivity to 96.8%.^{33,70} The improved YJV ImmunoCAP is available for routine diagnosis since 2012. By fact, such developments with impact on routine patient management have only become possible through the advancement of the molecular knowledge on venom allergens and, subsequently, through availability of recombinant allergens on sIgE assay platforms.

Another problem of diagnostics with whole Hymenoptera venoms stems from the unequal distribution of relevant allergens in the venom preparation and the instability of certain allergens. In HBV, only one (Api m 1) of five relevant allergens is present in the venom in substantial amounts, while only trace amounts of the others are detectable. Moreover, an intrinsic instability and rapid degradation of the important major allergen Api m 10 was reported recently.⁷¹ Such a predominance of particular allergens in the venom extract is very likely to create a diagnostic information bias by favouring the detection of high abundant allergens. However, such problems can easily be overcome by the use of recombinant allergens in CRD, which are available in nearly unlimited amounts and stable form.

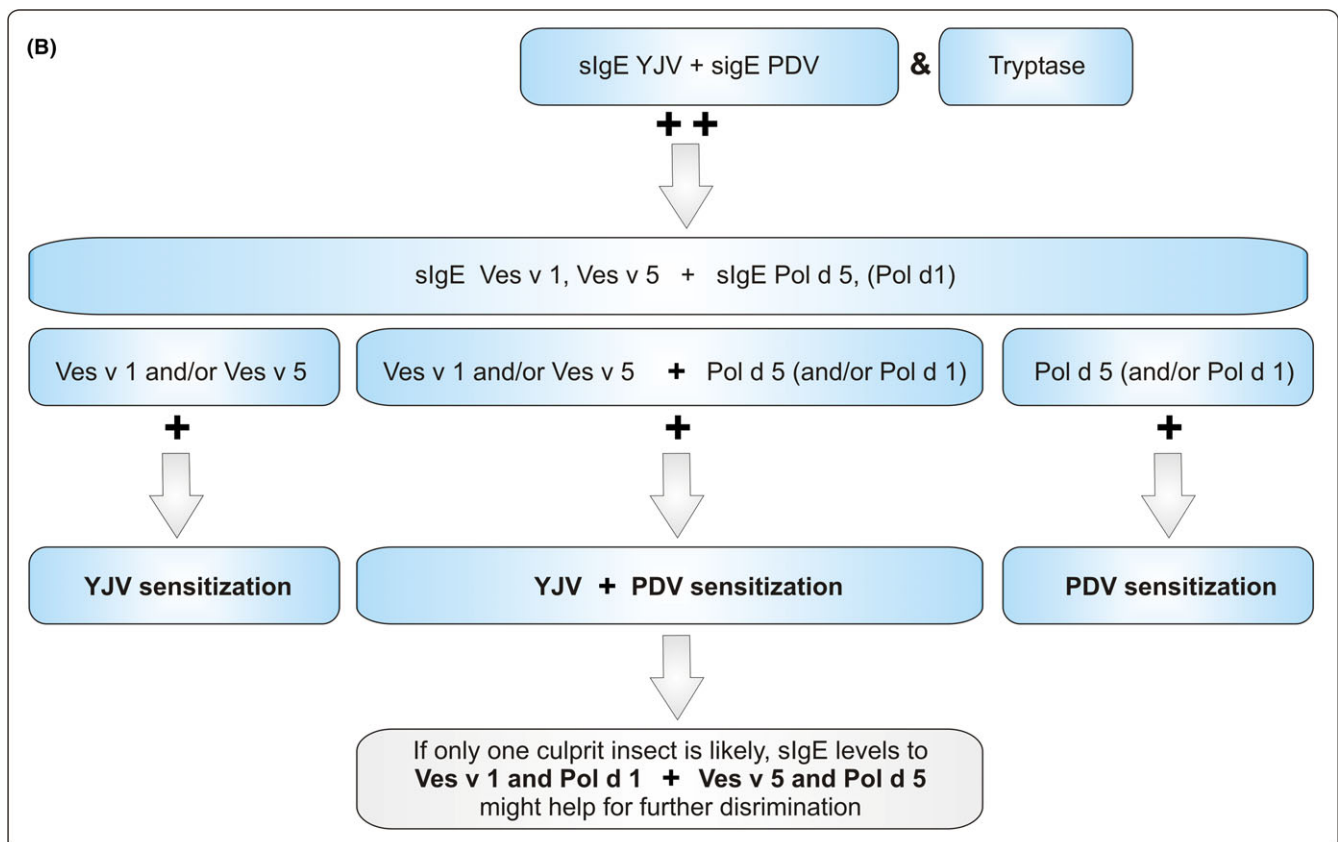
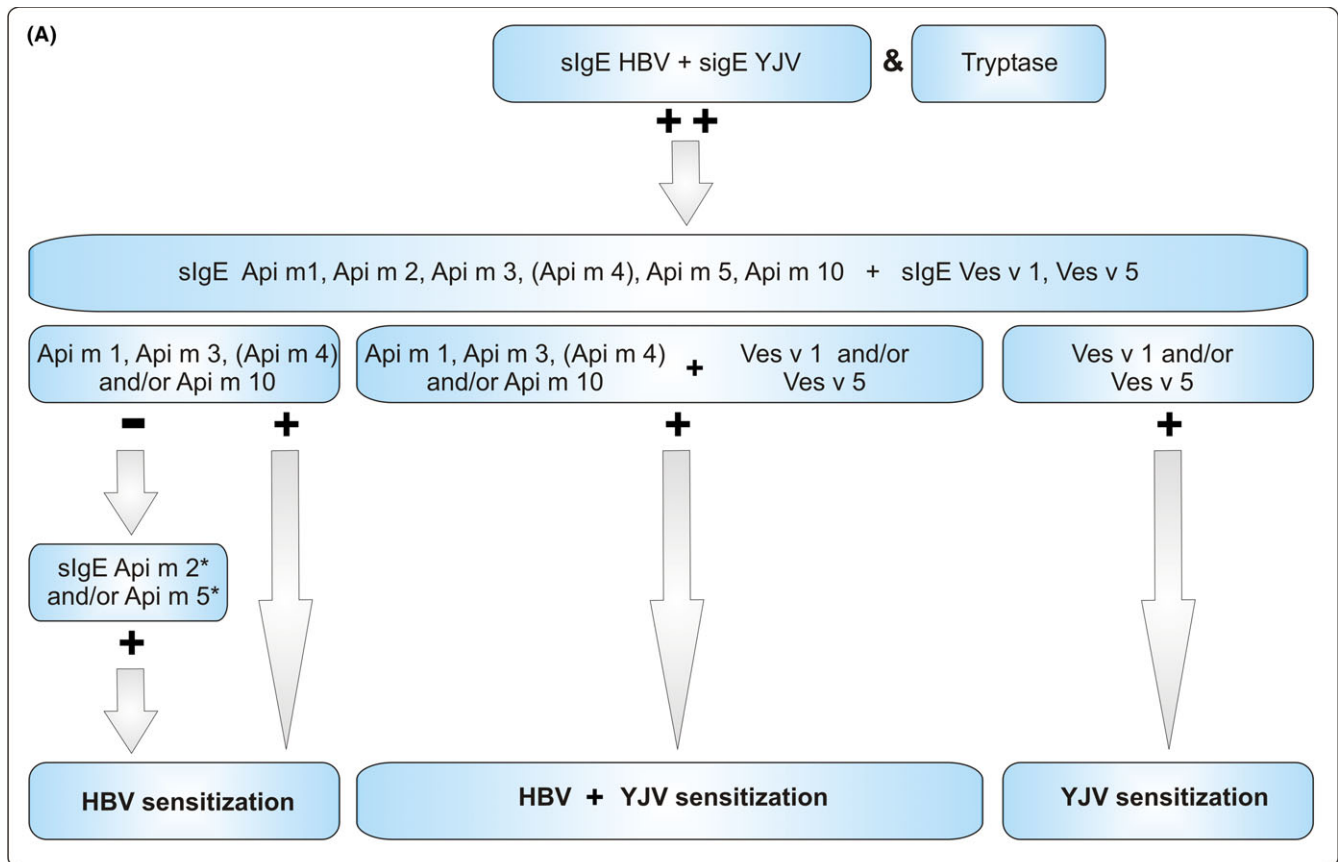


FIGURE 3 Diagnostic algorithm for in vitro diagnostics of (A) HBV and vespid venom allergy and (B) YJV and PDV allergy. The diagnostic algorithm shown in (A) with the corresponding PDV allergens Pol d 1 and Pol d 5 can also be used to discriminate between HBV and PDV allergy. In addition, to discriminate in the case of double-positive test results, CRD might also be useful in cases of negative results despite a convincing history of venom allergy or in cases of discrepancies between history, skin tests and venom extract-based testing. A plus indicates a positive and a minus a negative test result. Of note, the allergens Api m 4 and Pol d 1 (in brackets) are only available for selected multiplex sIgE test platforms. *The allergens Api m 2 and Api m 5 are potentially cross-reactive with their homologues from YJV and PDV (not available for CRD) and, hence, a positive test result does not necessarily exclude allergy to vespid venom

Recently, it was demonstrated that diagnostic sensitivity using recombinant allergens also differs on different sIgE assay systems,^{48,49,72} a fact that is most likely not due to the quality of the allergens used,⁴⁵ but rather to the difference in the calibration approach used for the different systems, resulting in an overestimation of the levels of sIgE in one system.⁷³⁻⁷⁵

In patients with mast cell disorders and venom allergy, negative IgE and negative skin tests are quite common, restricting them from VIT.⁷⁶ In a population of high-risk patients with YJV allergy and elevated baseline serum tryptase and/or mastocytosis, it was demonstrated that the use of CRD and decreasing the threshold sIgE level to 0.1 kU_A/L may be needed to avoid otherwise undetectable sIgE to YJV extract in about 8% of such patients.⁴³ Although 0.35 kU_A/L has commonly been used as the lower threshold for sIgE detection, sIgE concentrations can be measured today with high accuracy on the major singleplex sIgE immunoassay platforms with the lower end threshold of 0.1 kU_A/L. Thus, sIgE levels between 0.1 and 0.35 kU_A/L should be considered in the context of venom-allergic patients with a clear clinical history.^{52,77-79} Moreover, the sIgE to total IgE ratio has particular clinical importance, because any defined level of sIgE will have a different significance if produced in the midst of a high total serum IgE as opposed to a low total serum IgE.⁸⁰

6 | CRD TO DIRECT IN IMMUNOTHERAPY-DO SENSITIZATION PROFILES MATTER?

Apart from being helpful for routine diagnostics, component-resolved analyses have helped us to learn a lot about sensitization profiles of patients, particularly in HBV allergy. A total of 39 different sensitization profiles were identified in 144 patients with confirmed HBV allergy using six allergens (Api m 1-5 and Api m 10).¹⁹ Currently, it is a matter of debate whether some of these profiles might be associated with a risk of therapeutic failure of VIT. Although VIT is effective in 77%-96% of patients in preventing sting anaphylaxis, therapy failures occur significantly more often in HBV compared to YJV VIT.^{1,81} Successful allergen immunotherapy is dose-dependent.⁸² This is also the case in VIT, where a therapeutic maintenance dosage of 50 µg is significantly less effective than 100 µg.^{1,83} Furthermore, VIT therapeutic maintenance dosages are recommended to be doubled from 100 µg to 200 µg in patients who developed systemic allergic reactions after a field sting or a sting challenge while under 100 µg maintenance VIT.^{1,84} Thus, it can be hypothesized that therapy success of VIT might be associated with the concentration of relevant allergens a patient is sensitized to in the therapeutic venom

extract (Figure 4A). In HBV, Api m 1 is the only major allergen that is present in substantial amounts, while all other relevant allergens make up only 0.6%-2% of the venom dry weight.^{59,85} In contrast, in YJV, the two most relevant allergens Ves v 1 and Ves v 5 are present in relatively high and, moreover, in nearly equimolar amounts, which implies that these differences in the allergen content between the two venoms might be one reason for the higher success rate of VIT with YJV than with HBV.

Additionally, it was demonstrated that some of the relevant low-abundance allergens of HBV such as Api m 3, Api m 5 and Api m 10 are under-represented or are even lacking in certain therapeutic extracts compared to crude HBV, while their Api m 1 content was comparable.^{18,59,71} Intriguingly, a recent retrospective multicentre study demonstrated that a predominant sensitization (>50% of sIgE to HBV) to Api m 10 represents a relevant risk factor for treatment failure of VIT (odds ratio of 8.44; univariate analysis not considering other identified confounders for VIT failure),¹⁸ thus making Api m 10 a candidate for the model proposed for VIT outcome based on individual allergen concentration in the therapeutic extract (Figure 4B). Thus far, none of the other allergens such an association was identified. Another study demonstrated only minimal IgG4 induction against Api m 10 and Api m 3 during VIT in contrast to allergens that are present in high amounts in the extract.¹⁹ Although IgG4 induction per se cannot be correlated with therapeutic success, this indicates differences in the immunological response or even unresponsiveness to those under-represented allergens. However, due to the current lack of results from prospective clinical studies, it remains to be further proven if therapeutic failure of HBV VIT is indeed linked to the lack of Api m 10 or other allergens in particular therapeutic extracts.

The frequency of systemic adverse events during VIT in large multicentre studies ranges from 8% to 20%, whereby VIT with HBV is less safe compared to vespid venom.¹ Interestingly, recent studies have shown that Api m 4 sensitization might be a useful marker to identify a particular phenotype of HBV allergy that is associated with a higher risk of systemic reactions during the up-dosing phase of VIT.^{86,87} However, it has to be taken into account that in these studies, the number of patients was very small and that no other known risk factors for side-effects were considered. So far, no data are available on the correlation between certain sensitization profiles to vespid allergens and the severity of disease, as well as side-effects or therapy outcome of VIT.

Although it will remain an important topic of future clinical research to analyse the relationship between sensitization profiles and particular phenotypes of the disease or outcome of VIT during

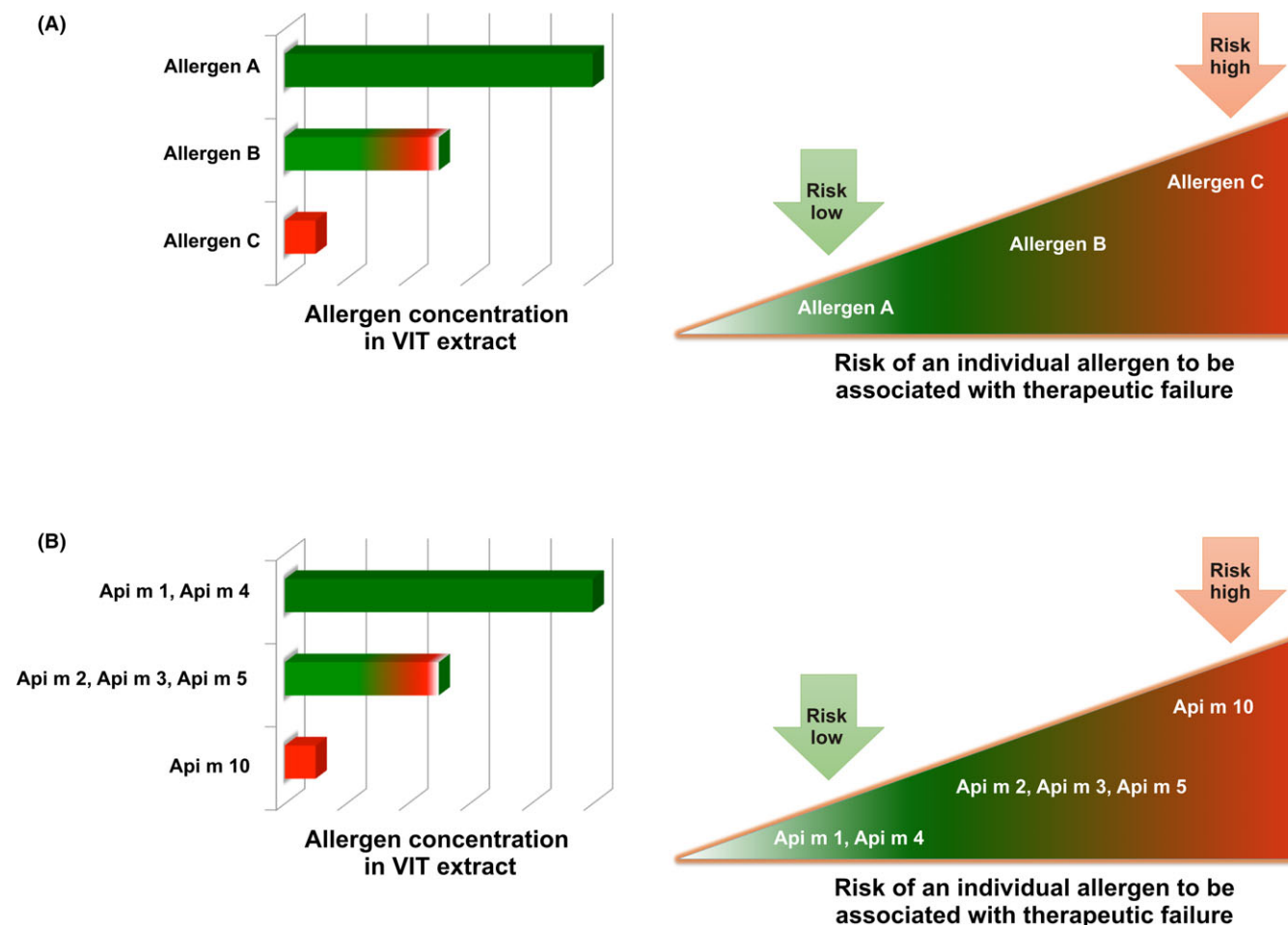


FIGURE 4 Proposed model for the association between therapeutic success of venom-specific immunotherapy (VIT) and the concentration of the allergens, relevant for a patient, in the VIT extract. A, General Model. B, Evidence-based model for success of HBV VIT. Of note, this model might be particularly relevant if a patient is predominantly or exclusively sensitized to under-represented allergens and, thus, sensitization to under-represented allergens per se is no risk factor for therapeutic failure. Therefore, a low amount of an individual allergen in one specific extract might be relevant if the patient is dominantly sensitized to it

the treatment as well as after stopping VIT, the available data already now demonstrate a potential of CRD for an individual risk assessment of the patient and to direct therapeutic decisions as a major step forward towards a personalized medicine approach in VIT.

7 | LIMITATIONS AND FUTURE PERSPECTIVES OF COMPONENT-RESOLVED DIAGNOSTICS

Even though skin testing with whole extracts still plays a key role in the diagnosis of Hymenoptera venom allergy, CRD is undoubtedly a major advancement in the field. In fact, CRD is helpful to discriminate between true allergy to different venoms and cross-reactivity, allowing physicians to optimize venom selection for immunotherapy. CRD is also helpful in identifying patients with Hymenoptera venom-induced anaphylaxis having negative test results to whole venom extracts. CRD with CCD-free allergens and CCD markers is always

preferable, taking into account that sIgE to CCDs does not exclude clinically relevant allergy to different venoms.

Currently available allergens of honeybee and vespid venoms allow a molecular diagnosis in the vast majority of patients, but not in 100% of them and, moreover, not all allergens are available for one assay system. New recombinant molecules are needed to improve the diagnosis of *Polistes spp.*-allergic patients, especially in the case of double positivity to both *Polistes spp.* and *Vespula spp.* venom, to prevent unnecessary double VIT.

Although CRD of Hymenoptera venom allergy has opened many new options for routine diagnostics and clinical research, as any diagnostic approach, it also has clear limitations. For instance, as observed for venom extracts, sIgE sensitization can also be incidentally found in patients without clinical history of a sting reaction.⁸⁸ For such patients, it is likely that such a positive sensitization test result will have no clinical significance. However, the possibility of a reaction to a future sting cannot be fully excluded. To date, no indications are currently available on how to effectively manage these cases. Finally, as also true for venom extracts, sIgE levels to

venom components do not reflect the severity of the sting reaction.

More work has to be carried out to assess whether CRD may detect biomarkers for VIT efficacy, the risk assessment of side-effect and relapse after stopping the treatment. There is one report that hints to the possibility that allergen-based assays might present tools for assessing long-term tolerance in patients treated with VIT.⁸⁹ Moreover, some studies show that patients exclusively sensitized to allergens that are under-represented in therapeutic extracts might be at risk to respond less well to VIT. Therefore, it is necessary to have accurate methods to investigate the exact molecular composition of the different VIT extracts for a "tailored therapy" based on the patient's sensitization profile.⁷¹ This progress, together with a greater diagnostic accuracy through a further improvement of CRD, can allow physicians to move further towards precision and personalized medicine even in the field of Hymenoptera venom allergy.

CONFLICT OF INTEREST

SB has received speaker's honorarium and/or travel support from ALK-Abelló, Bencard Allergie GmbH, Teomed AG and Thermo Fisher Scientific; has received consultancy fees as an advisory board member and research support from Bencard Allergie GmbH and Allergy Therapeutics. MBB has received speaker's honorarium and/or travel support from ALK-Abelló and Thermo Fisher Scientific; has received consultancy fees as an advisory board member from ALK-Abelló. MO has received speaker's honorarium and/or travel support from Thermo Fisher Scientific and Siemens Healthcare; has received consultancy fees as scientific advisor on the advisory board of Bencard Allergie GmbH and of Hycor Diagnostics.

ORCID

S. Blank  <http://orcid.org/0000-0002-5649-1078>

REFERENCES

- Sturm GJ, Varga EM, Roberts G, et al. EAACI guidelines on allergen immunotherapy: hymenoptera venom allergy. *Allergy*. 2017; doi: 10.1111/all.13262. [Epub ahead of print].
- Baker TW, Forester JP, Johnson ML, Sikora JM, Stolfi A, Stahl MC. Stinging insect identification: are the allergy specialists any better than their patients? *Ann Allergy Asthma Immunol*. 2016;116:431-434.
- Juarez C, Blanca M, Miranda A, et al. Specific IgE antibodies to vespids in the course of immunotherapy with *Vespula germanica* administered to patients sensitized to *Polistes dominulus*. *Allergy*. 1992;47:299-302.
- Höcherl N, Tautz J. Nesting behavior of the paper wasp *Polistes dominula* in Central Europe—a flexible system for expanding into new areas. *Ecosphere*. 2015;6:1-11.
- Cervo R, Zacchi F, Turillazzi S. *Polistes dominulus* (Hymenoptera, Vespidae) invading North America: some hypotheses for its rapid spread. *Insectes Soc*. 2000;47:155-157.
- Golden DB, Demain J, Freeman T, et al. Stinging insect hypersensitivity: a practice parameter update 2016. *Ann Allergy Asthma Immunol*. 2017;118:28-54.
- Bilo BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. Hypersensitivity EIGoV, Diagnosis of Hymenoptera venom allergy. *Allergy*. 2005;60:1339-1349.
- Balzer L, Pennino D, Blank S, et al. Basophil activation test using recombinant allergens: highly specific diagnostic method complementing routine tests in wasp venom allergy. *PLoS One*. 2014;9:e108619.
- 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;130:155-161.
- Korosec P, Silar M, Erzen R, et al. Clinical routine utility of basophil activation testing for diagnosis of hymenoptera-allergic patients with emphasis on individuals with negative venom-specific IgE antibodies. *Int Arch Allergy Immunol*. 2013;161:363-368.
- Ollert M, Blank S. Anaphylaxis to insect venom allergens: role of molecular diagnostics. *Curr Allergy Asthma Rep*. 2015;15:527.
- Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. EAACI molecular allergology user's guide. *Pediatr Allergy Immunol*. 2016;27(Suppl 23):1-250.
- Jakob T, Muller U, Helbling A, Spillner E. Component resolved diagnostics for hymenoptera venom allergy. *Curr Opin Allergy Clin Immunol*. 2017;17:363-372.
- Peiren N, Vanrobaeys F, de Graaf DC, Devreese B, Van Beeumen J, Jacobs FJ. The protein composition of honeybee venom reconsidered by a proteomic approach. *Biochim Biophys Acta*. 2005;1752:1-5.
- Van Vaerenbergh M, Debyser G, Devreese B, de Graaf DC. Exploring the hidden honeybee (*Apis mellifera*) venom proteome by integrating a combinatorial peptide ligand library approach with FTMS. *J Proteomics*. 2014;99:169-178.
- Radauer C, Nandy A, Ferreira F, et al. Update of the WHO/IUIS Allergen Nomenclature Database based on analysis of allergen sequences. *Allergy*. 2014;69:413-419.
- Spillner E, Blank S, Jakob T. Hymenoptera allergens: from venom to "venome". *Front Immunol*. 2014;5:77.
- Frick M, Fischer J, Helbling A, et al. Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom immunotherapy. *J Allergy Clin Immunol*. 2016;138:1663-1671. e9.
- Kohler J, Blank S, Muller S, et al. Component resolution reveals additional major allergens in patients with honeybee venom allergy. *J Allergy Clin Immunol*. 2014;133:1383-1389. 89 e1-6.
- Blank S, Bantleon FI, McIntyre M, Ollert M, Spillner E. The major royal jelly proteins 8 and 9 (Api m 11) are glycosylated components of *Apis mellifera* venom with allergenic potential beyond carbohydrate-based reactivity. *Clin Exp Allergy*. 2012;42:976-985.
- Blank S, Seismann H, McIntyre M, et al. Vitellogenins are new high molecular weight components and allergens (Api m 12 and Ves v 6) of *Apis mellifera* and *Vespula vulgaris* venom. *PLoS One*. 2013;8:e62009.
- Michel Y, McIntyre M, Ginglinger H, et al. The putative serine protease inhibitor Api m 6 from *Apis mellifera* venom: recombinant and structural evaluation. *J Invest Allergol Clin Immunol*. 2012;22:476-484.
- King TP, Spangfort MD. Structure and biology of stinging insect venom allergens. *Int Arch Allergy Immunol*. 2000;123:99-106.
- Muller UR. Recombinant Hymenoptera venom allergens. *Allergy*. 2002;57:570-576.
- Kolarich D, Leonard R, Hemmer W, Altmann F. The N-glycans of yellow jacket venom hyaluronidases and the protein sequence of its major isoform in *Vespula vulgaris*. *FEBS J*. 2005;272:5182-5190.
- Seismann H, Blank S, Braren I, et al. Dissecting cross-reactivity in hymenoptera venom allergy by circumvention of alpha-1,3-core fucosylation. *Mol Immunol*. 2010;47:799-808.
- Blank S, Seismann H, Bockisch B, et al. Identification, recombinant expression, and characterization of the 100 kDa high molecular

- weight Hymenoptera venom allergens Api m 5 and Ves v 3. *J Immunol.* 2010;184:5403-5413.
28. Cifuentes L, Vosseler S, Blank S, et al. Identification of Hymenoptera venom-allergic patients with negative specific IgE to venom extract by using recombinant allergens. *J Allergy Clin Immunol.* 2014;133:909-910.
 29. Ebo DG, Faber M, Sabato V, Leysen J, Bridts CH, De Clerck LS. Component-resolved diagnosis of wasp (yellow jacket) venom allergy. *Clin Exp Allergy.* 2013;43:255-261.
 30. Korosec P, Valenta R, Mittermann I, et al. High sensitivity of CAP-FEIA rVes v 5 and rVes v 1 for diagnosis of *Vespula* venom allergy. *J Allergy Clin Immunol.* 2012;129:1406-1408.
 31. Mittermann I, Zidarn M, Silar M, et al. Recombinant allergen-based IgE testing to distinguish bee and wasp allergy. *J Allergy Clin Immunol.* 2010;125:1300-1307. e3.
 32. Seismann H, Blank S, Cifuentes L, et al. Recombinant phospholipase A1 (Ves v 1) from yellow jacket venom for improved diagnosis of hymenoptera venom hypersensitivity. *Clin Mol Allergy.* 2010;8:7.
 33. Vos B, Kohler J, Muller S, Stretz E, Rueff F, Jakob T. Spiking venom with rVes v 5 improves sensitivity of IgE detection in patients with allergy to *Vespula* venom. *J Allergy Clin Immunol.* 2013;131:1225-1227. 27. e1.
 34. Hofmann SC, Pfender N, Weckesser S, Huss-Marp J, Jakob T. Added value of IgE detection to rApi m 1 and rVes v 5 in patients with Hymenoptera venom allergy. *J Allergy Clin Immunol.* 2011;127:265-267.
 35. Muller UR, Johansen N, Petersen AB, Fromberg-Nielsen J, Haeberli G. Hymenoptera venom allergy: analysis of double positivity to honey bee and *Vespula* venom by estimation of IgE antibodies to species-specific major allergens Api m1 and Ves v5. *Allergy.* 2009;64:543-548.
 36. 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;125:184-190. e1.
 37. Monsalve RI, Vega A, Marques L, et al. Component-resolved diagnosis of vespid venom-allergic individuals: phospholipases and antigen 5s are necessary to identify *Vespula* or *Polistes* sensitization. *Allergy.* 2012;67:528-536.
 38. Schiener M, Hilger C, Eberlein B, et al. The high molecular weight dipeptidyl peptidase IV Pol d 3 is a major allergen of *Polistes dominula* venom. *Scientific Reports.* 2018;8:1318.
 39. Bokanovic D, Schwarz I, Wutte N, Komericki P, Aberer W, Sturm GJ. Specificity of conventional and Ves v 5-spiked venom decreases with increasing total IgE. *J Allergy Clin Immunol.* 2014;134:739-741.
 40. Sturm GJ, Bilo MB, Bonadonna P, et al. Ves v 5 can establish the diagnosis in patients without detectable specific IgE to wasp venom and a possible north-south difference in Api m 1 sensitization in Europe. *J Allergy Clin Immunol.* 2012;130:817. author reply 18-9.
 41. Leimgruber A, Lantin JP, Frei PC. Comparison of two in vitro assays, RAST and CAP, when applied to the diagnosis of anaphylactic reactions to honeybee or yellow jacket venoms. Correlation with history and skin tests. *Allergy.* 1993;48:415-420.
 42. Rafei-Shamsabadi D, Muller S, Pfutzner W, Spillner E, Rueff F, Jakob T. Recombinant allergens rarely allow identification of Hymenoptera venom-allergic patients with negative specific IgE to whole venom preparations. *J Allergy Clin Immunol.* 2014;134:493-494.
 43. Michel J, Brockow K, Darsow U, et al. Added sensitivity of component-resolved diagnosis in hymenoptera venom-allergic patients with elevated serum tryptase and/or mastocytosis. *Allergy.* 2016;71:651-660.
 44. Dalmau Duch G, Gazquez Garcia V, Gaig Jane P, Galan Nieto A, Monsalve Clemente RI. Importance of controlled sting challenge and component-resolved diagnosis in the success of venom immunotherapy. *J Invest Allergol Clin Immunol.* 2012;22:135-136.
 45. Jakob T, Kohler J, Blank S, et al. Comparable IgE reactivity to natural and recombinant Api m 1 in cross-reactive carbohydrate determinant-negative patients with bee venom allergy. *J Allergy Clin Immunol.* 2012;130:276-278. author reply 78-9.
 46. Korosec P, Valenta R, Mittermann I, et al. Low sensitivity of commercially available rApi m 1 for diagnosis of honeybee venom allergy. *J Allergy Clin Immunol.* 2011;128:671-673.
 47. Sturm GJ, Hemmer W, Hawranek T, et al. Detection of IgE to recombinant Api m 1 and rVes v 5 is valuable but not sufficient to distinguish bee from wasp venom allergy. *J Allergy Clin Immunol.* 2011;128:247-248; author reply 48.
 48. Cifuentes L, Blank S, Pennino D, et al. Reply: to PMID 24290287. *J Allergy Clin Immunol.* 2014;134:494-495.
 49. Schrautzer C, Bokanovic D, Hemmer W, et al. Sensitivity and specificity of Hymenoptera allergen components depend on the diagnostic assay employed. *J Allergy Clin Immunol.* 2016;137:1603-1605.
 50. Arzt L, Bokanovic D, Schrautzer C, et al. Questionable diagnostic benefit of the commercially available panel of bee venom components. *Allergy.* 2017;72:1419-1422.
 51. Frick M, Muller S, Bantleon F, et al. rApi m 3 and rApi m 10 improve detection of honey bee sensitization in Hymenoptera venom-allergic patients with double sensitization to honey bee and yellow jacket venom. *Allergy.* 2015;70:1665-1668.
 52. Ollert M, Weissenbacher S, Rakoski J, Ring J. Allergen-specific IgE measured by a continuous random-access immunoanalyzer: interassay comparison and agreement with skin testing. *Clin Chem.* 2005;51:1241-1249.
 53. Sturm GJ, Kranzelbinder B, Schuster C, et al. Sensitization to Hymenoptera venoms is common, but systemic sting reactions are rare. *J Allergy Clin Immunol.* 2014;133:1635-1643. e1.
 54. Hemmer W, Focke M, Kolarich D, et al. Antibody binding to venom carbohydrates is a frequent cause for double positivity to honeybee and yellow jacket venom in patients with stinging-insect allergy. *J Allergy Clin Immunol.* 2001;108:1045-1052.
 55. Sturm GJ, Jin C, Kranzelbinder B, et al. Inconsistent results of diagnostic tools hamper the differentiation between bee and vespid venom allergy. *PLoS One.* 2011;6:e20842.
 56. Jin C, Hantusch B, Hemmer W, Stadlmann J, Altmann F. Affinity of IgE and IgG against cross-reactive carbohydrate determinants on plant and insect glycoproteins. *J Allergy Clin Immunol.* 2008;121:185-190. e2.
 57. Altmann F. Coping with cross-reactive carbohydrate determinants in allergy diagnosis. *Allergo J Int.* 2016;25:98-105.
 58. Jappe U, Raulf-Heimsoth M, Hoffmann M, Burow G, Hubsch-Muller C, Enk A. In vitro hymenoptera venom allergy diagnosis: improved by screening for cross-reactive carbohydrate determinants and reciprocal inhibition. *Allergy.* 2006;61:1220-1229.
 59. Blank S, Seismann H, Michel Y, et al. Api m 10, a genuine *A. mellifera* venom allergen, is clinically relevant but underrepresented in therapeutic extracts. *Allergy.* 2011;66:1322-1329.
 60. Muller 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 from crossreaction in venom allergy. *Allergy.* 2012;67:1069-1073.
 61. Blanca M, Garcia F, Miranda A, et al. Determination of IgE antibodies to *Polistes dominulus*, *Vespula germanica* and *Vespa crabro* in sera of patients allergic to vespids. *Allergy.* 1991;46:109-114.
 62. Grant JA, Rahr R, Thuesen DO, Lett-Brown MA, Hokanson JA, Yunginger JW. Diagnosis of *Polistes* wasp hypersensitivity. *J Allergy Clin Immunol.* 1983;72:399-406.
 63. Severino MG, Campi P, Macchia D, et al. European *Polistes* venom allergy. *Allergy.* 2006;61:860-863.
 64. Blank S, Neu C, Hasche D, Bantleon FI, Jakob T, Spillner E. *Polistes* species venom is devoid of carbohydrate-based cross-reactivity and

- allows interference-free diagnostics. *J Allergy Clin Immunol*. 2013;131:1239-1242.
65. Caruso B, Bonadonna P, Severino MG, et al. Evaluation of the IgE cross-reactions among vespid venoms. A possible approach for the choice of immunotherapy. *Allergy*. 2007;62:561-564.
 66. Schiener M, Eberlein B, Moreno-Aguilar C, et al. Application of recombinant antigen 5 allergens from seven allergy-relevant Hymenoptera species in diagnostics. *Allergy*. 2017;72:98-108.
 67. Galindo-Bonilla PA, Galan-Nieto A, Alfaya-Arias T, Garcia-Rodriguez C, de la Roca-Pinzon F, Feo-Brito F. Component-resolved diagnosis in vespid venom-allergic individuals. *Allergol Immunopathol*. 2015;43:398-402.
 68. Caruso B, Bonadonna P, Bovo C, et al. Wasp venom allergy screening with recombinant allergen testing. Diagnostic performance of rPol d 5 and rVes v 5 for differentiating sensitization to *Vespula* and *Polistes* subspecies. *Clin Chim Acta*. 2016;453:170-173.
 69. Savi E, Peveri S, Makri E, Pravettoni V, Incorvaia C. Comparing the ability of molecular diagnosis and CAP-inhibition in identifying the really causative venom in patients with positive tests to *Vespula* and *Polistes* species. *Clin Mol Allergy*. 2016;14:3.
 70. Huss-Marp J, Raulf M, Jakob T. Spiking with recombinant allergens to improve allergen extracts: benefits and limitations for the use in routine diagnostics: part 19 of the Series Molecular Allergology. *Allergo J Int*. 2015;24:236-243.
 71. Blank S, Etzold S, Darsow U, et al. Component-resolved evaluation of the content of major allergens in therapeutic extracts for specific immunotherapy of honeybee venom allergy. *Hum Vaccin Immunother*. 2017;13:2482-2489.
 72. Selb J, Kogovsek R, Silar M, Kosnik M, Korosec P. Improved recombinant Api m 1- and Ves v 5-based IgE testing to dissect bee and yellow jacket allergy and their correlation with the severity of the sting reaction. *Clin Exp Allergy*. 2016;46:621-630.
 73. Jakob T, Spillner E. Comparing sensitivity of Hymenoptera allergen components on different diagnostic assay systems: comparing apples and oranges? *J Allergy Clin Immunol*. 2017;139:1066-1067.
 74. Szecsi PB, Stender S. Comparison of immunoglobulin E measurements on IMMULITE and ImmunoCAP in samples consisting of allergen-specific mouse-human chimeric monoclonal antibodies towards allergen extracts and four recombinant allergens. *Int Arch Allergy Immunol*. 2013;162:131-134.
 75. Wood RA, Segall N, Ahlstedt S, Williams PB. Accuracy of IgE antibody laboratory results. *Ann Allergy Asthma Immunol*. 2007;99:34-41.
 76. Gonzalez-de-Olano D, Alvarez-Twose I, Vega A, Orfao A, Escribano L. Venom immunotherapy in patients with mastocytosis and hymenoptera venom anaphylaxis. *Immunotherapy*. 2011;3:637-651.
 77. Hamilton RG, Oppenheimer J. Serological IgE analyses in the diagnostic algorithm for allergic disease. *J Allergy Clin Immunol Pract*. 2015;3:833-840. quiz 41-2.
 78. Hamilton RG. Clinical laboratories worldwide need to report IgE antibody results on clinical specimens as analytical results and not use differential positive thresholds. *J Allergy Clin Immunol*. 2015;136:811-812.
 79. Hamilton RG, Matsson PNJ, Chan S, et al. Analytical performance characteristics, quality assurance, and clinical utility of immunological assays for human immunoglobulin E antibodies of defined allergen specificities. CLSI report I/LA20. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
 80. Hamilton RG, Williams PB; Specific IgE Testing Task Force of the American Academy of Allergy, Asthma & Immunology; American College of Allergy, Asthma and Immunology. Human IgE antibody serology: a primer for the practicing North American allergist/immunologist. *J Allergy Clin Immunol*. 2010;126:33-38.
 81. Rueff F, Przybilla B, Bilo MB, et al. Clinical effectiveness of hymenoptera venom immunotherapy: a prospective observational multicenter study of the European academy of allergology and clinical immunology interest group on insect venom hypersensitivity. *PLoS One*. 2013;8:e63233.
 82. Secrist H, DeKruyff RH, Umetsu DT. Interleukin 4 production by CD4 + T cells from allergic individuals is modulated by antigen concentration and antigen-presenting cell type. *J Exp Med*. 1995;181:1081-1089.
 83. Golden DB, Kagey-Sobotka A, Valentine MD, Lichtenstein LM. Dose dependence of Hymenoptera venom immunotherapy. *J Allergy Clin Immunol*. 1981;67:370-374.
 84. Rueff F, Wenderoth A, Przybilla B. Patients still reacting to a sting challenge while receiving conventional Hymenoptera venom immunotherapy are protected by increased venom doses. *J Allergy Clin Immunol*. 2001;108:1027-1032.
 85. Müller UR. *Insektenstichallergie: Klinik, Diagnostik und Therapie*. Stuttgart, New York: Gustav Fischer Verlag; 1988.
 86. Ruiz B, Serrano P, Moreno C. IgE-Api m 4 is useful for identifying a particular phenotype of bee venom allergy. *J Invest Allergol Clin Immunol*. 2016;26:355-361.
 87. Ruiz B, Serrano P, Verdu M, Moreno C. Sensitization to Api m 1, Api m 2, and Api m 4: association with safety of bee venom immunotherapy. *Ann Allergy Asthma Immunol*. 2015;114:350-352.
 88. Passalacqua G, Melioli G, Bonifazi F, et al. The additional values of microarray allergen assay in the management of polysensitized patients with respiratory allergy. *Allergy*. 2013;68:1029-1033.
 89. Mobs C, Muller J, Rudzio A, et al. Decline of Ves v 5-specific blocking capacity in wasp venom-allergic patients after stopping allergen immunotherapy. *Allergy*. 2015;70:715-719.

How to cite this article: Blank S, Bilò MB, Ollert M. Component-resolved diagnostics to direct in venom immunotherapy: Important steps towards precision medicine. *Clin Exp Allergy*. 2018;48:354-364. <https://doi.org/10.1111/cea.13090>