



The role of component-resolved diagnosis in Hymenoptera venom allergy

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Purpose of review

Component-resolved diagnostics (CRD) is a new tool aiming at detecting IgE-mediated sensitizations against individual, relevant allergens. Here, we discuss recent literature on molecular diagnosis in the field of Hymenoptera venom allergy (HVA) as well as CRD strengths and weaknesses.

Recent findings

CRD, using single molecules or panels of allergens, may discriminate between primary sensitization and cross-reactivity in patients with double/multiple positivity in diagnostic tests with whole extracts, allowing the specialist to choose the most suitable venom for specific immunotherapy (VIT), avoiding unnecessary VIT and reducing the risk of side effects. Future availability of the cross-reactive recombinant pairs of allergens of different species may further increase the diagnostic performance. CRD may be useful in patients with negative allergy tests and a proven history of a previous systemic reaction, including those with mast cell disorders, who could benefit from VIT. In honeybee venom allergy, different sensitization profiles have been identified, which could be associated with a greater risk of VIT failure or treatment side effects.

Summary

CRD is undoubtedly an innovative diagnostic method that leads to a more precise definition of the sensitization profile of the HVA patient. Together with a better knowledge of the molecular composition of different venom extracts, CRD may contribute to optimize patient-tailored therapy.

Keywords

allergy diagnostics, anaphylaxis, Hymenoptera venom allergy, systemic reactions, venom immunotherapy

INTRODUCTION

Hymenoptera stings can induce systemic and occasionally fatal allergic reactions [1], and are the most common cause of anaphylaxis in European adult patients [2]. Fortunately, subcutaneous venom immunotherapy (VIT) is currently the most effective form of allergen-based immunotherapy, which is designed to reduce the risk of a subsequent systemic reaction and to improve health-related quality of life [3^{••}]. Moreover, its carry-over effect may last up to several years after its completion [4].

However, successful VIT depends on a correct diagnosis aiming at classifying the type of reaction, confirming the IgE-mediated pathogenesis and identifying the allergy-eliciting insect [5]. In the last years, increasing knowledge about the venom composition on a molecular basis and availability of component-resolved diagnostics (CRD) have vastly augmented our ability to solving many diagnostic issues, thus, leading to a more precise VIT prescription [6[•],7[•]], reducing the costs, the risk of potential side effects and the possibility of de-novo sensitization due to unnecessary VIT.

THE ALLERGEN SOURCES

The allergy-relevant Hymenoptera belong to the families *Apoidea* (*Apis* and *Bombus* species) and *Vespoidea* (*Vespula*, *Vespa*, *Polistes*, *Dolichovespula*, *Polybia*, *Solenopsis*, *Myrmecia* and *Pachychondyla* species) [5]. Honeybees (*Apis mellifera*) and *Vespula* spp. (called wasps in Europe, yellow jackets in the USA)

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KEY POINTS

- CRD is helpful in case of multiple sensitizations or negative allergy tests in patients with a proven history of a previous systemic reaction, including those with mast cell disorders.
- Currently, CRD allows the discrimination between primary sensitization and cross-reactivity in double-sensitivity to *Apis mellifera* and *Vespula* ssp. venoms; CRD currently offers only limited value in case of double/multiple positivity to vespid venoms.
- The detailed knowledge about sensitization profiles on a molecular basis might open new options to identify patients who are at an increased risk of treatment failure or of side effects.

are the most important elicitors of HVA in Europe. In southern Europe, also hornets (*Vespa crabro* and others) are a frequent cause of allergic reactions. The species *Polistes dominula* and *Polistes gallicus* are European paper wasps and of particular relevance in Mediterranean areas of Europe; *P. dominula* has also spread to the northeastern United States and been reported in Australia. The species *Polistes exclamans*, *Polistes annularis* and *Polistes fuscatus* are indigenous to North America and not present in Europe [5]. *Polybia* wasps, particularly *Polybia paulista*, are present in South America [8].

Systemic reactions to the venoms of stinging ants are of particular importance in America, where the red fire ant (*Solenopsis invicta*) is constantly expanding its habitat [9,10], Australia (jumper ant, *Myrmecia pilosula*) [11] and Asia (Asian needle ant, *Pachycondyla chinensis*) [12].

RELEVANT ALLERGENIC MOLECULES

Hymenoptera venoms are complex cocktails of various substances such as biogenic amines, basic peptides and higher molecular weight proteins, all of which may contribute to sensitization, allergic symptoms and success of VIT.

The best characterized venom is that of the honeybee *Apis mellifera* with more than 100 identified proteins [13]. Twelve honeybee venom (HBV) allergens are currently included in the official allergen nomenclature database (www.allergen.org) (Table 1) [14]. Among them, Api m 1, Api m 2, Api m 3, Api m 5 and Api m 10 can be defined as major allergens for which more than 50% of patients show IgE reactivity. The majority of the other HBV allergens seem to be of minor importance. The venom allergens of different honeybee species are similar and also bumblebee venom closely resembles bee venom [15].

Also, the venoms of most *Vespoidea* species seem to be closely related [15]. Prominent yellow jacket venom (YJV) allergens include phospholipase A1 (Ves v 1), and antigen 5 (Ves v 5) (Table 2).

Other allergens are homologues of HBV allergens and, hence, exhibit cross-reactivity. These include 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 [16–19] (Fig. 1). However, a significant part of in-vitro cross-reactivities can be attributed to clinically irrelevant IgE antibodies that are directed against cross-reactive carbohydrate determinants (CCDs) [20], induced by pollen exposure and insect stings. Most Hymenoptera venom allergens are glycoproteins with one or more of such N-linked carbohydrate structures.

Table 1. Current list of Apidae allergens as of June 2019 (www.allergen.org)

Allergen	Name/function	MW (kDa)	Allergen	Name/function	MW (kDa)
Bees (<i>Apis mellifera</i> , <i>A. cerana</i> , <i>A. dorsata</i>)			Bumblebees (<i>Bombus pennsylvanicus</i> , <i>B. terrestris</i>)		
Api m 1	Phospholipase A2	17	Bom p 1, Bom t 1	Phospholipase A2	16
Api m 2	Hyaluronidase	45	Bom p 4, Bom t 4	Protease	27
Api m 3	Acid phosphatase	49			
Api m 4	Melittin	3			
Api m 5	Dipeptidyl peptidase IV	100			
Api m 6	Protease inhibitor	8			
Api m 7	CUB serine protease	39			
Api m 8	Carboxylesterase	70			
Api m 9	Serine carboxypeptidase	60			
Api m 10	Icarapin	55			
Api m 11.0101	Major royal jelly protein 8	60			
Api m 11.0201	Major royal jelly protein 9	65			
Api m 12	Vitellogenin	200			

Table 2. Current list of Vespinae allergens as of June 2019 (www.allergen.org)

Allergen	Name/function	MW (kDa)	Allergen	Name/function	MW (kDa)
Yellow jackets (<i>Vespula vulgaris</i> , <i>V. flavopilosa</i> , <i>V. germanica</i> , <i>V. maculifrons</i> , <i>V. pensylvanica</i> , <i>V. squamosa</i> , <i>V. vidua</i>)			White-faced hornets, Yellow hornets (<i>Dolichovespula maculata</i> , <i>D. arenaria</i>)		
Ves v 1, Ves m 1, Ves s 1	Phospholipase A1	34	Dol m 1	Phospholipase A1	34
Ves v 2.0101, Ves m 2	Hyaluronidase	45	Dol m 2	Hyaluronidase	42
Ves v 2.0201	Hyaluronidase (inactive)	-	Dol m 5, Dol a 5	Antigen 5	23
Ves v 3	Dipeptidyl peptidase IV	100			
Ves v 5, Ves f 5, Ves g 5, Ves m 5, Ves p 5, Ves s 5, Ves vi 5	Antigen 5	23			
Ves v 6	Vitellogenin	200	Hornets (<i>Vespa crabro</i> , <i>V. magnifica</i> , <i>V. mandarinia</i>)		
			Vesp c 1, Vesp m 1	Phospholipase A1	34
			Vesp ma 2	Hyaluronidase	35
			Vesp c 5, Vesp ma 5, Vesp m 5	Antigen 5	23

The cross-reactivity between hyaluronidases (Api m 2 and Ves v 2) appears to be mostly based on CCD epitopes [19]. Moreover, in contrast to HBV Api m 2, the relevance of YJV hyaluronidases (Ves v 2.0101 and Ves v 2.0201) as allergens seems to be limited [18,19].

A detailed proteomic analysis of pure *P. domini* venom (PDV) led to the identification of 47 proteins (unpublished data). At the moment, the most important allergens are phospholipase A1 (Pol d 1), dipeptidyl peptidase IV (Pol d 3) and antigen 5 (Pol d 5). Moreover, a serine protease (Pol d 4) and a hyaluronidase (Pol d 2) have been identified (Table 3). Unlike YJV and HBV, venoms of different *Polistes* species have been demonstrated to be devoid of CCD reactivity and, hence, allow CCD interference-free diagnostics [21].

An overview of the currently identified ant venom allergens is given in Table 4.

SENSITIZATION TO INDIVIDUAL MOLECULES AND ITS CLINICAL RELEVANCE

Nowadays, advanced recombinant strategies allow the production of correctly folded allergens, devoid of carbohydrate-based cross-reactivity, which help to distinguish between genuine sensitization and clinically irrelevant cross-reactivity [19]. Thus, reliable data on sensitization rates are available for many HBV and vespid venom allergens.

Table 5 summarizes the sensitization rates to individual HBV allergens [17,22[■],23–25,26[■],27–29]. For some HBV allergens, less information

concerning sensitization rates is available, not excluding that they might be of particular relevance for some patients.

In contrast to the first historical study [28], which showed a high diagnostic sensitivity of Api m 1 (97%), later on reported frequencies of Api m 1 sensitization ranged from 58 to 80% [22[■],23–25,26[■],27–29], thus, reflecting possible regional differences [30], differences in studied populations [24] or different test systems used [31,32].

Only the combination of six HBV allergens (Api m 1–5 and 10) had a diagnostic sensitivity of approximately 95% for HBV-allergic patients, whereby 74% of patients were sensitized to more than one allergen (39 different sensitization profiles) [26[■]]. Moreover, in the same study, 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, suggesting a more advanced state of Th2 immune deviation in the double-sensitized population [26[■]]. However, in a recent study using five bee allergens (Api m 1–3, 5 and 10) on the same assay platform, the diagnostic sensitivity for patients with allergy to HBV only and for those allergic to HBV and YJV was different with 71.6 and 92.7%, respectively [33[■]].

Even though the number of available allergens may be insufficient for the correct diagnosis of all HBV-allergic patients [32,34[■]], the current panel of commercially available recombinant allergens allows a more accurate diagnosis than the extract-based sIgE determination (Fig. 2).

So far, no data are available on the correlation between certain molecular sensitization profiles to

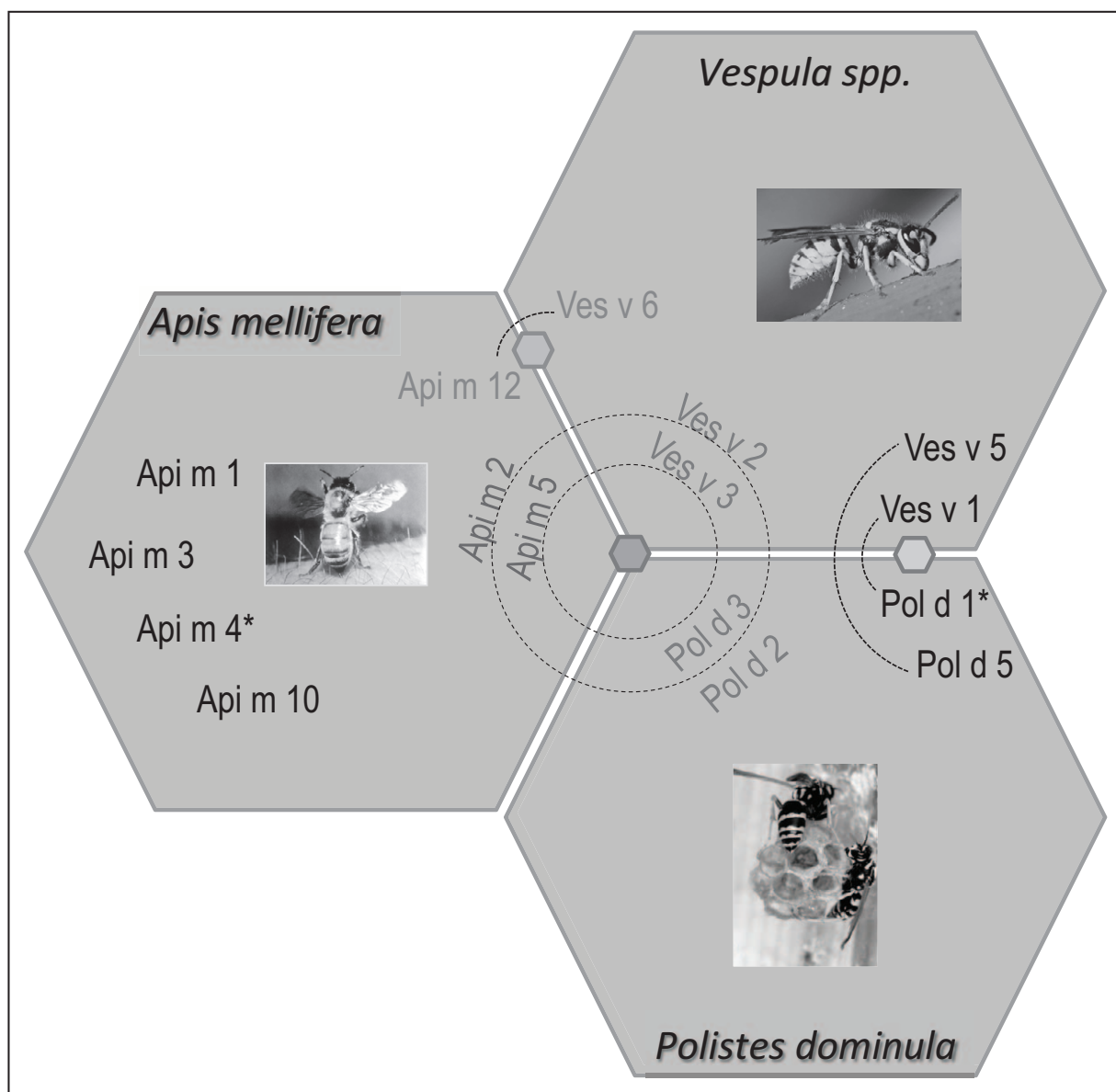


FIGURE 1. Schematic representation of recombinant marker and cross-reactive allergens of *Apis mellifera*, *Vespula* spp. and *Polistes dominula* (see the text). *The allergens Api m 4 and Pol d 1 (in brackets) are only available for selected multiplex sIgE test platforms.

HBV allergens and severity of the sting reaction [32,35[¶]], but first data have emerged that indicate a correlation between a specific sensitization profile and VIT efficacy [22^{¶¶}] as well as VIT side effects [36[¶]]. A retrospective multicentre study suggests that a predominant sensitization (>50% of sIgE to HBV) to Api m 10 represents a relevant risk factor for treatment failure of VIT [22^{¶¶}]. Moreover, it was demonstrated that some of the relevant low abundance allergens of HBV (Api m 3, Api m 5 and Api m 10) are under-represented or even lacking in certain therapeutic extracts compared with crude HBV [22^{¶¶},37,38]. However, the current lack of results from prospective clinical studies makes it difficult

to exclude other predominant sensitizations as risk factors for treatment failure in specific patients and to conclude that the therapeutic failure of HBV VIT is really due to the lack of Api m 10 or other important allergens in some therapeutic preparations [3^{¶¶}].

Sensitization rates to individual YJV allergens are summarized in Table 6 [17,18,24,28,39–44]. The diagnostic sensitivity of a combination of the YJV recombinant allergens rVes v 5 and rVes v 1 has been reported to be as high as 92–98% [17,18,24,28,39–44]. Therefore, commercially available CRD using these allergens is valuable and can be used to exclude unspecific sensitization due to CCDs

Table 3. Current list of Polistinae allergens as of June 2019 (www.allergen.org)

Allergen	Name/function	MW (kDa)	Allergen	Name/function	MW (kDa)
European paper wasps (<i>Polistes dominula</i> , <i>P. gallicus</i>)			American paper wasps (<i>Polistes annularis</i> , <i>P. exclamans</i> , <i>P. fuscatus</i> , <i>P. metricus</i>)		
Pol d 1, Pol g 1	Phospholipase A1	34	Pol a 1, Pol e 1	Phospholipase A1	34
Pol d 2	Hyaluronidase	50	Pol a 2	Hyaluronidase	38
Pol d 3	Dipeptidyl peptidase IV	100	Pol e 4	Serine protease	33
Pol d 4	Serine protease	33	Pol a 5, Pol e 5, Pol f 5,	Antigen 5	23
Pol d 5, Pol g 5	Antigen 5	23	Pol m 5		
			POLYBIA WASPS (<i>Polybia paulista</i> , <i>P. scutellaris</i>)	Phospholipase A1	34
			Poly p 1	Hyaluronidase	33
			Poly p 2	Antigen 5	21
			Poly p 5, Poly s 5		

Table 4. Current list of Formicidae allergen as of June 2019 (www.allergen.org)

Allergen	Name/function	MW (kDa)	Allergen	Name/function	MW (kDa)
Fire ants (<i>Solenopsis invicta</i> , <i>S. geminata</i> , <i>S. richteri</i> , <i>S. saevissima</i>)			Australian jumper ant (<i>Myrmecia pilosula</i>)		
Sol i 1	Phospholipase A1	18	Myr p 1	Pilosulin-1	7.5, 5.5
Sol i 2, Sol g 2, Sol r 2, Sol s 2	-	14	Myr p 2	Pilosulin-3	8.5, 2.4
Sol i 3, Sol g 3, Sol r 3, Sol s 3	Antigen 5	24-26	Myr p 3	Pilosulin-4.1	-
Sol i 4, Sol g 4	-	12			
			Asian needle ant (<i>Pachycondyla chinensis</i>)		
			Pac c 3	Antigen 5	23

(Fig. 2). In contrast to HBV VIT, the high concentration of the two most relevant allergens Ves v 1 and Ves v 5 in commercially available extracts might be one reason for the higher success rate of VIT with YJV than with HBV [6[■]].

Sensitization rates to individual *Polistes* spp. venom allergens are reported in Table 7. For the

diagnosis of *P. dominula* venom allergy, so far, only the major allergen Pol d 5 [45[■]] is commercially available (and Pol d 1 for selected multiplex sIgE test platforms), thus, clearly underlining to the need for additional allergens to be included in the CRD of PDV-allergic patients in the future [43,46].

Component-resolved diagnostics and the issue of double/multiple sensitization

Diagnosis is complicated by sensitization to multiple venoms in patients who have not identified the stinging Hymenoptera. In-vitro double sensitization to the venoms of *Apis mellifera* and *Vespa* species is found in 25–40% of cases and may be due to true double sensitization, cross-reactivity between epitopes present in both venoms (Fig. 1), CCDs and asymptomatic sensitization [47,48]. As the clinical relevance of IgE directed against CCDs seem to be low [49], their measurement using CCD marker molecules such as MUXF or bromelain together with CRD using CCD-free allergens often helps

Table 5. Sensitization rates to individual honeybee venom allergens

Allergen	Name/function	Sensitization rate (%)
Api m 1	Phospholipase A2	57–97
Api m 2	Hyaluronidase	47.9–52.2
Api m 3	Acid phosphatase	50
Api m 4	Melittin	23–42.5
Api m 5	Dipeptidyl peptidase IV	58.3–61.7
Api m 10	Icarapin	61.8–72.2
Api m 12	Vitellogenin	50

Adapted from [17,22^{■■},23–25,26[■],27–29].

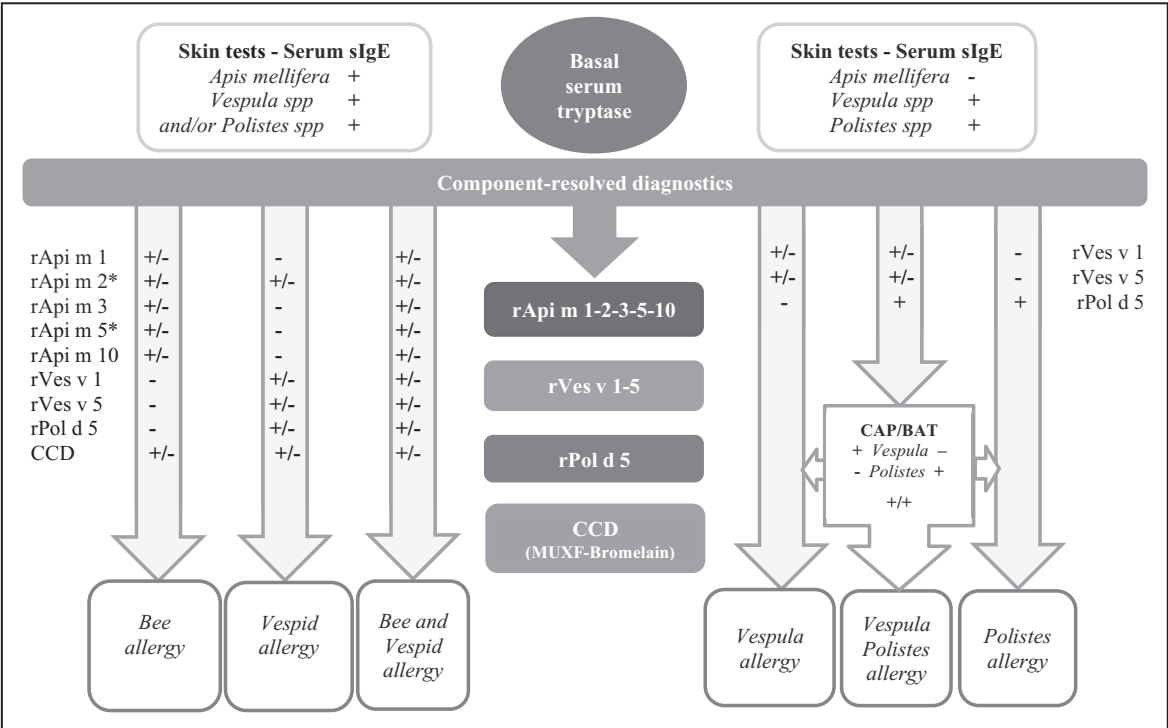


FIGURE 2. Workflow for Hymenoptera venom allergy diagnosis in *Apis-Vesputa* and *Vesputa-Polistes* double sensitizations. *The allergens Api m 2 and Api m 5 are potentially cross-reactive with their homologues from vespid venom (not available for CRD) and, hence, a positive test result does not necessarily exclude allergy to vespid venom.

Table 6. Sensitization rates to individual yellow jacket venom allergens

Allergen	Name/function	Sensitization rate (%)
Ves v 1	Phospholipase A1B	33.3–50
Ves v 2a (0101)	Hyaluronidase	5
Ves v 2b (0201)	Hyaluronidase	20–25
Ves v 3	Dipeptidylpeptidase IV	50–62.8
Ves v 5	Antigen 5	84.5–100
Ves v 6	Vitellogenin	39

Adapted from [17,18,24,28,39–44].

Table 7. Sensitization rates to individual *Polistes dominula/annularis* venom allergens

Allergen	Name/function	Sensitization rate (%)
Pol d 1	Phospholipase A1	87 (nPol d 1)
Pol d 2	Hyaluronidase	?
Pol d 3	Dipeptidylpeptidase IV	?
Pol d 4	Serine protease	?
Pol d 5	Antigen 5	69.72 (nPol d 5)
Pol a 5	Antigen 5	44 (r Pol a 5)

Adapted from [43].

physicians to discriminate between primary sensitization and cross-reactivity in patients with double-positive tests with whole venom extracts [6[¶]].

However, a recent study [34[¶]] showed that 55 and 70% of patients with in-vitro double sensitization to HBV/YJV extracts had sIgE against MUXF and were double sensitized to CCD-free recombinant venom components, respectively. These data are in contrast to the assumption that in-vitro venom extract double sensitization is mostly due to IgE antibodies against CCDs.

Of note, none of the cross-reactive recombinant pairs (rApi m 2/rVes v 2, rApi m 5/rVes v 3 and rApi m 12/rVes v 6) are commercially available (with the exception of Api m 2), thus, preventing physicians from identifying a primary sensitizer in cases of sensitization to those allergens.

Because cross-reactivity between Api m 2 and Ves v 2 is mainly attributed to CCDs and Ves v 2 represents only a minor allergen of YJV, while Api m 2 is an important major allergen of HBV [18,22^{¶¶},26[¶],33[¶]], also the measurement of sIgE to Api m 2 might be beneficial to diagnose HBV allergy.

In Southern Europe, double *Vesputa-Polistes* sensitization is more frequent than *Apis-Vesputa/Polistes* sensitization, and cross-reactivity between allergens

of these two species often poses diagnostic difficulties [43]. As so far only Ves v 1, Ves v 5 and Pol d 5 are available for routine CRD on commonly used assay platforms, some studies showed that the use of Ves v 5 and Pol d 5 seems to be helpful in clinical practice, provided that the difference in specific IgE levels between the two molecules is particularly significant [50,51]. However, a more recent multicentre study did not find any agreement between CAP-inhibition test results and double sIgE values of Ves v 5 over Pol d 5 or vice versa [52].

It was also demonstrated that measuring the levels of sIgE to the four major allergens phospholipases A1 (Pol d 1 and Ves v 1) and antigens 5 (Pol d 5 and Ves v 5) of PDV and YJV allowed a reliable identification of the allergy-eliciting venom in 69% of allergic patients with double sensitization [43]. A subsequent study showed that the detection of sIgE against the same four allergens could determine the correct venom for immunotherapy in the majority, but not in all patients [53]. Therefore, the commercial availability of additional cross-reactive allergens such as at least Pol d 1 might be beneficial for uncovering primary sensitization in vespid venom allergic individuals.

On the basis of the commercially available allergens for singleplex assays, a workflow for diagnosis in HBV and vespid venom and YJV and PDV double sensitizations is suggested in Fig. 2.

So far, no bumblebee, ant and hornet recombinant major allergens are commercially available for CRD.

Finally, in patients with double or multiple-positive results and inconclusive in-vivo or in-vitro test results with recombinant allergens, the CAP-inhibition test as well as the basophil activation test (BAT) may be helpful [43,54].

Component-resolved diagnostics in patients with negative test results but a history of systemic reaction and in patients with mast cell disorders

Negative serological test results using extracts may be found in 9–16.6% of YJV and in 1–13% of HBV-allergic patients, respectively [28,44,55]. Potential reasons might be the refractory period following the sting reaction, spontaneous desensitization over time, the use of inappropriate venom extracts and the presence of mast cell diseases. In patients with a documented history but negative test results, some studies underlined the potential to diagnose such cases using recombinant Ves v 5 (from 42.1% up to 84%) or Ves v 1 [24,30,39,44,56]. Consequently, one of the diagnostic YJV sIgE tests (i3, ImmunoCAP

system) was spiked with recombinant Ves v 5, which led to a higher sensitivity of up to 96% [40,44].

Up to 7% of adult patients with Hymenoptera venom allergy may suffer from a clonal mast cell disease [57] and negative sIgE and negative skin tests have been reported in up to 15% of patients with systemic mastocytosis and history of a systemic reaction to insect stings [58], thus, restricting them from VIT. In a recent study on high-risk patients with YJV allergy and elevated baseline serum tryptase and/or mastocytosis, in about 8% of them, the use of CRD (Ves v 1 and Ves v 5) together with a decrease of the threshold sIgE level to 0.1 kU_A/l was useful for detecting very low levels of sIgE [59]. More recently, other authors confirmed that the diagnostic sensitivity improved, but specificity did not markedly change by lowering the cutoff in mastocytosis and YJV-allergic patients [60]. Here, it was demonstrated that a cutoff of 0.17 kU_A/l gives an acceptable sensitivity and specificity (83.6 and 85.0%, respectively). Indeed, sIgE levels between 0.1 and 0.35 kU_A/l should be considered relevant in patients with a clear clinical history, irrespective of the presence of mast cell diseases [61–63].

The role of BAT as a diagnostic tool in patients with mast cell disorders and negative venom-specific IgE and skin test results remains controversial [64,65].

Open issues and future perspectives

Limitations of CRD rely on the current inability to make a molecular diagnosis in 100% of allergic patients because of the lack of some relevant allergens on the available immunoassay platforms. On the contrary, increasing the number of allergens lowers the specificity and augments the number of patients who would be considered really double sensitized [54]. Moreover, as observed for venom preparations, asymptomatic sensitization may be detected by CRD, posing the question of how to effectively manage these individuals. Probably, defining the patients' molecular sensitization profile is not a sufficient tool to discover the clinical relevance of double sensitization to the whole extract [34].

We cannot exclude that future studies using CRD may identify biomarkers able to distinguish between asymptomatic and symptomatic sensitization, between different degrees of systemic reaction severity, as well as biomarkers for VIT efficacy [22], VIT tolerance [36] and relapse after discontinuing the treatment. Eventually, such new tools of investigation could lead to a more precise definition of the sensitization profile of the allergic patient and to a real 'tailored therapy' [66].

CONCLUSION

The clinical history of the allergic reaction(s), skin testing and detection of sIgE to venom preparations are still the mainstays of the diagnostic procedure in HVA [67]. The use of CRD is indicated in patients with a proven history of a previous systemic reaction and negative results in standard diagnostic tests and in cases of polysensitization to different venoms, as it may help the specialist to choose the most suitable venom for VIT. However, the decision for VIT not only relies on CRD results but should also take into account the severity of the reaction and the patient's general health status.

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