



Marker allergens in Hymenoptera venom allergy — Characteristics and potential use in precision medicine

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Abstract

Background A comprehensive diagnostic work-up is essential to ensure adequate patient management for the potentially life-threatening condition of Hymenoptera venom allergy (HVA). This includes an unambiguous identification of the allergy-relevant venom as prerequisite for successful venom-specific immunotherapy (VIT). If the clinical history does not allow the identification of the culprit insect, diagnosis is often hampered by positive test results to various venoms. Modern component-resolved diagnostics (CRD) applying marker allergens of Hymenoptera venoms has created new opportunities which facili-

tate therapeutic decisions and may allow personalized risk stratification for individual patients.

Methods Comprehensive literature search and critical analysis of recently published studies on Hymenoptera venom allergens and CRD.

Results and discussion Changing the research focus from whole venom extracts to individual allergenic molecules led to the development of CRD in HVA. The currently available CRD is a valuable tool to resolve cross-reactivity and primary sensitization, particularly in honeybee and vespid venom allergy. Hence, CRD has simplified therapeutic decisions in case of multiple positive test results, especially in patients who were not able to identify the culprit insect or in cases of discrepancies between clinical history and classical diagnostic results. Moreover, there is first evidence that sensitization to particular allergens might serve as biomarkers to predict risk for severe side-effects during VIT or even for VIT failure. To date, a clear limitation of CRD is the currently available allergen panel which does not allow a definite resolution of allergy to different vespid species such as yellow jackets and European paper wasps.

Keywords Component-resolved diagnostics · Hymenoptera venom allergy · Marker allergen · Molecular allergology · Precision medicine · Venom-specific immunotherapy

Abbreviations

Ag5	Antigen 5
BAT	Basophil activation test
CCD	Cross-reactive carbohydrate determinant
CRD	Component-resolved diagnostics
DPP IV	Dipeptidyl peptidases IV
DS	Double-sensitized
HBV	Honeybee venom
HVA	Hymenoptera venom allergy

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MS	Monosensitized
PDV	<i>Polistes dominula</i> venom
PLA1	Phospholipase A1
PLA2	Phospholipase A2
sIgE	Specific IgE
tIgE	Total IgE
VIT	Venom-specific immunotherapy
YJV	Yellow jacket venom

Introduction

Hymenoptera venom allergy (HVA) may be a potentially severe and even fatal deviation of the immune response to insect stings compared to reactions in healthy individuals. Venom allergy can be effectively cured by venom-specific immunotherapy (VIT) which is the only available treatment that is able to shift the immune balance from allergic inflammation towards immune tolerance. However, an unambiguous identification of the culprit insect and an assessment of the patients' sensitization and individual risks are prerequisites for efficient therapy and adequate patient management.

Thus, proving sensitization to a certain venom by skin testing and/or specific IgE (sIgE) measurements is imperative for the initiation of potential life-saving VIT [1]. In clinical routine, therapeutic decisions are frequently hampered by multiple positive, or even negative, test results to different venoms, especially when the patient was not able to identify the culprit insect. In addition to primary sensitization to different venoms, multiple positive test results may result from clinically irrelevant cross-reactivity. This might result in unnecessary treatment with more than one or application of the wrong venom. Specific IgE inhibition assays with venom extracts [2] or cellular tests such as the basophil activation test (BAT) [3] can be helpful in many cases to confirm the diagnosis when skin tests or sIgE measurements show their limits. However, although recommended in the diagnostic guidelines, these tests are still not available for routine diagnostics in all clinics as the interpretation of results requires specific expertise.

In the last decades, research in HVA has shifted from venom extracts to individual venom allergens [4]. In more recent times, the evolving knowledge of relevant venom allergens has led to the development of molecular or component-resolved diagnostics (CRD) in HVA [4–9]. In contrast to extract-based sIgE diagnosis that measures sIgE levels to native whole venom extracts, in CRD, levels of sIgE to single allergens of the venoms are determined. Thus, CRD not only provides information about whether a patient is sensitized to the whole venom, but also which allergens of the venoms are relevant for a patient. Sensitization profiles obtained in this way can help to discriminate between cross-reactivity and primary sensitization to different venoms. Additionally, allergens for CRD can be recombinantly produced without cross-

reactive carbohydrate determinants (CCDs). CCDs are carbohydrate epitopes on allergens; more precisely, posttranslational modifications involving a core α 1,3-linked fucose [10]. These N-linked glycosylations are present on various plant and insect allergens, while being absent in humans, and can lead to false-positive results in extract-based diagnostic approaches. sIgE directed against CCDs is present in 20–30% of allergic patients [11, 12]. However, sensitization to CCDs is not clinically relevant. There is either no cross-linking of Fc ϵ RI or tolerance is induced by frequent intake of CCD-carrying proteins through food, both theories being part of ongoing research. Still, the use of allergen source extracts or native allergens in diagnosing CCD-sensitized patients is not practicable. Positive test results can be the consequence of true sensitization to, for example, Hymenoptera venom or due to a silent sensitization to CCDs. Applying recombinant, CCD-free allergens in CRD to exclude clinically irrelevant sensitization to CCDs has proven its worth [6, 7].

Reviewed here are characteristics and cross-reactivity of Hymenoptera venom marker allergens as well as their role in diagnostics and as sensitizing venom components. Additionally, the potential of individual allergens to act as biomarker for personalized risk stratification as well as limitations of currently available CRD and future needs to improve precision medicine in HVA are discussed. The review is limited to currently commercially available allergens, including those of honeybee (*Apis mellifera*) venom (HBV), yellow jacket (*Vespula vulgaris*) venom (YJV), and European paper wasp (*Polistes dominula*) venom (PDV) (Table 1).

Sensitization rates to marker allergens of Hymenoptera venoms

In addition to several other characteristics, the rates of sIgE sensitization of allergic patients to Hymenoptera venom allergens are discussed in the following sections. It should be kept in mind that these sensitization rates depend on a plethora of factors. For instance, given an equal quality and purity of the allergen preparation, sensitization rates depend on the inclusion criteria of the assessed patient population such as positive sIgE or skin tests to the respective venom extracts or an unambiguous identification of the allergy-eliciting insect by the patient [13, 14]. Moreover, differences can be observed in monosensitized (MS) and double-sensitized (DS) patients, as it was demonstrated that sensitization rates to individual HBV and YJV allergens are lower in patients MS to the respective venoms compared to HBV/YJV-DS patients [15, 16]. This effect is probably independent of cross-reactivity, since it can be observed for allergens for which no homologous allergen is known in other Hymenoptera venoms. Additionally, DS patients have higher total IgE (tIgE) levels, higher sIgE

Table 1 Characteristics and significance of Hymenoptera venom allergens currently available for routine component-resolved diagnostics as of August 2020

Allergen	Sensitization rate ^a (%)	Available for routine CRD	Significance
Honeybee venom (<i>Apis mellifera</i>)			
Api m 1 Phospholipase A2	57–97	S ^{d,e,f} , M ^{g,h,i}	Marker allergen for HBV sensitization; Allows discrimination between HBV and YJV/PDV sensitization
Api m 2 Hyaluronidase	28–60	S ^{d,e,f} , M ^g	Due to limited cross-reactivity with Ves v 2 and Pol d 2 in the absence of CCDs, potential marker for HBV sensitization ^b
Api m 3 Acid phosphatase	28–63	S ^d	Marker allergen for HBV sensitization; Allows discrimination between HBV and YJV/PDV sensitization; Valuable marker allergen to diagnose HBV allergy in Api m 1-negative patients
Api m 4 Melittin	17–54	M ⁱ	Marker allergen for HBV sensitization; Allows discrimination between HBV and YJV/PDV sensitization; Putative marker allergen for severe VIT side-effects
Api m 5 Dipeptidyl peptidase IV	16–70	S ^d	High cross-reactivity with Ves v 3 and Pol d 3 prevents its use as marker allergen ^c
Api m 10 Icarapin	35–73	S ^{d,f} , M ^{g,h}	Marker allergen for HBV sensitization; Allows discrimination between HBV and YJV/PDV sensitization; Valuable marker allergen to diagnose HBV allergy in Api m 1-negative patients; Dominant Api m 10 sensitization is a putative marker for risk of VIT failure
Yellow jacket venom (<i>Vespa vulgaris</i>)			
Ves v 1 Phospholipase A1	39–66	S ^d , M ^{g,h}	Marker allergen for YJV sensitization; Allows discrimination between YJV and HBV sensitization; High cross-reactivity with Pol d 1 prevents its use as marker allergen to discriminate between YJV and PDV sensitization
Ves v 5 Antigen 5	82–98	S ^{d,e,f} , M ^{g,h}	Marker allergen for YJV sensitization; Allows discrimination between YJV and HBV sensitization; High cross-reactivity with Pol d 5 prevents its use as marker allergen to discriminate between YJV and PDV sensitization
European paper wasp venom (<i>Polistes dominula</i>)			
Pol d 1 Phospholipase A1	87	M ^g	Marker allergen for PDV sensitization; Allows discrimination between PDV and HBV sensitization; High cross-reactivity with Ves v 1 prevents its use as marker allergen to discriminate between PDV and YJV sensitization
Pol d 5 Antigen 5	72	S ^d , M ^{g,h}	Marker allergen for PDV sensitization; Allows discrimination between PDV and HBV sensitization; High cross-reactivity with Ves v 5 prevents its use as marker allergen to discriminate between PDV and YJV sensitization
CCDs cross-reactive carbohydrate determinants, CRD component-resolved diagnostics, HBV honeybee venom, M multiplex sIgE assay system, PDV <i>Polistes dominula</i> venom, S singleplex sIgE assay system, YJV yellow jacket venom			
^a Defined by different assay systems and in patient populations with different inclusion criteria. References for sensitization rates are given in the main text			
^b Cross-reactivity with Ves v 2 and Pol d 2 cannot fully excluded			
^c Api m 5 monosensitization may occur in HBV-allergic patients			
^d Thermo Fisher Scientific			
^e Siemens Healthcare Diagnostics			
^f Dr. Focke Laboratories			
^g Euroimmun			
^h Macro Array Diagnostics			
ⁱ Faber test (different suppliers)			

levels to venom extracts and higher levels of sIgE to single allergens, suggesting a more advanced state of allergic immune deviation in DS patients [7].

Geographical differences may also influence sIgE sensitization rates [17]. For instance, such differences might be influenced by the prevailing climate and, thus, distribution and frequency of different insect species or geographic circumstances such as forestation.

Finally, sensitization rates can vary substantially depending on the test used for sIgE detection. For instance, assessing the same patient population, sev-

eral studies found higher sensitization rates using the Immulite™ platform (Siemens Healthcare Diagnostics, Eschborn, Germany) compared to using the ImmunoCAP™ system (Thermo Fisher Scientific, Uppsala, Sweden) [16, 18–20]. These differences are most likely due to different calibration approaches, resulting in an overestimation of sIgE levels in one platform [21].

As sIgE sensitization rates depend on various factors, the definition of minor and major allergens is difficult in many cases and should perhaps be handled flexible, depending on the assessed patient popula-

tion and the methods used. Additionally, sIgE sensitization per se implies no information about its clinical relevance [22] and the ability of allergens to activate effector cells [23].

Phospholipases A1 and A2 (Ves v 1, Pol d 1 and Api m 1)

Phospholipases are hydrolases that catalyze the cleavage of fatty acids from phospholipids in lipid bilayers of cell membranes. Phospholipase A1 (PLA1) and phospholipase A2 (PLA2) catalyze the cleavage at the sn-1 and sn-2 position, respectively. Therefore, the enzymatic activity leads to direct toxic effects such

as cell lysis, pore formation, hemolysis, platelet aggregation and the release of proinflammatory mediators (e.g. histamine, prostaglandins and leukotrienes) [24]. Moreover, catalytic-independent neurotoxicity of PLA2 is mediated by binding to N(neuronal)-type receptors [25].

PLA2 (Api m 1) is the most prominent allergen of HBV and accounts for up to 16% of its dry weight [4]. The rate of sensitization to Api m 1 in different cohorts of HBV-allergic patients ranges between 57 and 97% [13–16, 18–20, 26–32]. While some studies report higher sensitization rates in HBV/YJV-DS patients compared to HBV-MS patients [15, 18], other studies reported equal values in both groups [16] or

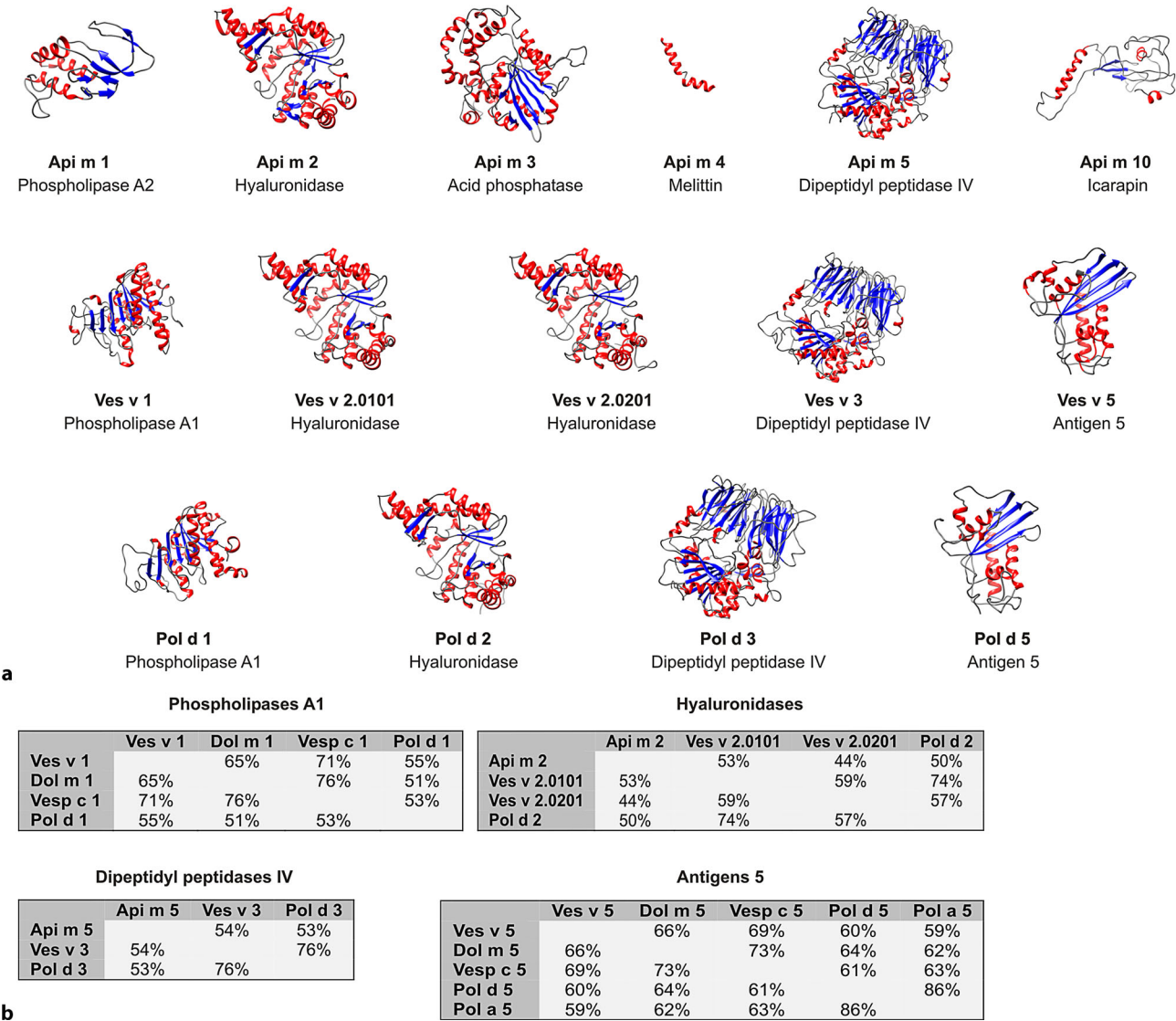


Fig. 1 Structural features of selected Hymenoptera venom allergens. **a** Three-dimensional structures of important HBV, YJV and PDV allergens. α -helices, β -strands and coiled regions are shown in red, blue and grey, respectively. The structures of Api m 1 (PDB: 1POC), Api m 2 (PDB: 1FCU), Api m 4 (PDB: 2MW6), Ves v 2.0101 (PDB: 2ATM) and Ves v 5 (PDB:

1QNX) were either solved by crystallography or NMR. All other structures were generated by structural modeling (PHYRE² server [83]). **b** Protein sequence identity of phospholipases A1, hyaluronidases, dipeptidyl peptidases IV and antigens 5 of selected Hymenoptera venoms shown in percentage. PDB Protein Data Bank

even higher values in MS patients [28]. As mentioned above, this diversity might be attributed to different inclusion criteria or analyzed patient numbers. One study compared rates of sensitization to Api m 1 in different regions of Europe and found that sensitization decreases from northern to southern Europe [17]. Annotated PLA2 allergens of other *Apis* spp. (*A. cerana*, *A. dorsata*) show 91–93% sequence identity with Api m 1 and are most likely completely cross-reactive [33].

Although catalyzing a related enzymatic reaction, PLA2s of Apidae venoms share neither noteworthy sequence identity nor extended structural similarity with PLA1s of vespid or ant venoms (Fig. 1a). The resulting lack of cross-reactivity renders phospholipases ideal marker allergens for the discrimination of bee and vespid venom allergy [15, 31].

PLA1 has been described as relevant venom allergen in a variety of species of the Vespoidea superfamily including yellow jackets, paper wasps, hornets, *Polybia* wasps and stinging ants [34]. The PLA1 of different *Vespula* species share sequence identity of approximately 95% (around 70% with the American species *V. squamosa* and *V. vidua* which belong to a different subgenus) and are thought to be almost completely cross-reactive [35]. Sequence identity between YJV PLA1 Ves v 1 and hornet venom Vesp c 1, PDV Pol d 1 and fire ant Sol i 1 is around 71, 55 and 35%, respectively (Fig. 1b). Despite the sometimes low sequence identity, all PLA1s are structurally similar ([36]; Fig. 1a) and cross-reactivity can be observed between PLA1s of most Vespoidea species [37, 38].

IgE sensitization to YJV Ves v 1 ranges between 39 and 66% in different populations of YJV-allergic patients [15, 39–42] and is higher in YJV/HBV-DS compared to YJV-MS patients [15]. The addition of Ves v 1 to Ves v 5 (see below) increased sensitivity of CRD of YJV allergy in the range of 4 to 11% depending on the study populations [17, 19, 20, 40–43]. The sensitization rate to Pol d 1 was found to be 87% in a population of PDV/YJV-DS patients [38]. Although PLA1s are valuable marker allergens for the discrimination between bee and vespid venom allergy, the pronounced cross-reactivity between PLA1s within in the Vespoidea superfamily impedes their use for discrimination between allergies to these species [38]. While Api m 1 and Ves v 1 are available for routine diagnostics on the most commonly used sIgE singleplex assay platform, Pol d 1 is exclusively available for multiplex testing (Table 1).

Hyaluronidases (Ves v 2, Pol d 2 and Api m 2)

Hyaluronidases cleave hyaluronan, the most abundant glycosaminoglycan in vertebrates' extracellular matrix and thereby promote the spread of venom at the site of injection [44]. Hyaluronidases are common components of Hymenoptera venoms and have been annotated as allergens for eight species, includ-

ing honeybee (Api m 2) and different vespids (e.g. Ves v 2 and Pol d 2) [34]. In YJV an enzymatically active (Ves v 2.0101) and an inactive (Ves v 2.0201) hyaluronidase were identified that share sequence identity of 59%, whereby the latter represents the major isoform [45]. Sequence identity between Api m 2 and the YJV and PDV hyaluronidases ranges between 44 and 53% while Pol d 2 exhibits identity of 74 and 57% with Ves v 2.0101 and Ves v 2.0201, respectively (Fig. 1b).

Api m 2 represents a major allergen of HBV with sensitization rates ranging from 28 to 60% in different study populations (28–55% and 45–60% in HBV-MS and HBV/YJV-DS patients, respectively) [14–16, 18–20, 26, 27]. Interestingly, while one study demonstrated clearly lower levels of sensitization in HBV-MS compared to HBV/YJV-DS patients [15], the other studies found comparable rates in both groups. Due to sequence identity, hyaluronidases were thought to be one major cause for cross-reactivity between HBV and YJV. However, recent studies applying CCD-free allergens and inhibition experiments demonstrated that this cross-reactivity is mainly attributed to IgE directed against CCDs and that both Ves v 2 isoforms are only minor allergens of YJV [46, 47]. Approximately 10–15% of patients with YJV allergy are estimated to have IgE against protein epitopes of Ves v 2 and peptide-specific cross-reactivity with Api m 2 occurs in half of these patients [46]. This lack of cross-reactivity, despite the given sequence identity and similar folding (Fig. 1), may be explained by significant differences in surface topology and charge distribution and, hence, most likely surface epitopes [48, 49]. Additionally, the low degree of cross-reactivity between Api m 2 and Ves v 2 may be further reflected by identical sensitization rates to Api m 2 in patients allergic to HBV and YJV and patients MS to HBV which is in stark contrast to what is found for Api m 5 (see below) [18]. Less is known about sIgE sensitization to Pol d 2. Preliminary unpublished data suggests a sensitization rate of approximately 40% in PDV-allergic patients. Primary sensitization to Pol d 2 may induce cross-reactivity with Api m 2 and Ves v 2.0201. However, only very few Api m 2-reactive patients show sIgE to Pol d 2.

Due to the limited cross-reactivity of Api m 2 and Ves v 2/Pol d 2, CCD-free Api m 2 may contribute as marker allergen to detect primary sensitization to HBV (Table 1). However, as cross-reactivity and primary sensitization to Ves v 2/Pol d 2 cannot be excluded with absolute certainty, Api m 2-sIgE has to be interpreted carefully and seen in the context of clinical history. A possible solution is the addition of Ves v 2/Pol d 2 to routine diagnostics which allows a comparison of sIgE results.

Acid phosphatase (Api m 3)

So far, acid phosphatase was exclusively annotated as allergen for HBV (Api m 3), although bumblebee venom comprises an allergenic acid phosphatase that shows moderate cross-reactivity with Api m 3 [50]. Acid phosphatases cleave phosphoryl groups from their substrates; however, their function in Hymenoptera venoms remains unknown. It was suggested for snake venoms that acid phosphatases play a role in liberating purines (mainly adenosine) which act as multitoxin (for instance, they cause vasodilation, edema and pain) [51].

The rates of sensitization to Api m 3 range between 28 and 63% in different cohorts of HBV-allergic patients (28–31% in HBV MS and 42–63% in HBV/YJV DS patients) [15, 16, 18, 26]. The lack of cross-reactivity of sera from YJV-allergic patients renders Api m 3 a marker allergen of HBV. Moreover, Api m 3, together with Api m 10, plays an important role in accurate diagnostics of HBV-allergic patients who are DS to HBV and YJV extract and were not able to identify the culprit insect. In this patient population the combination of Api m 3 and Api m 10 is able to confirm primary HBV allergy in 65% of patients that exhibit negative sIgE to Api m 1, as 26 and 47% of the Api m 1-negative patients exhibited sIgE to Api m 3 and Api m 10, respectively [13]. Hence, both allergens can act as useful marker allergens for primary HBV sensitization in YJV- and HBV-DS patients.

Melittin (Api m 4)

Melittin (Api m 4) is the main component of HBV and accounts for approximately 50% of its dry weight [4]. Melittin is a cytotoxic 26 amino acid peptide that—as a tetramer—can integrate into cell membranes and forms pores that are permeable for ions [52]. This leads to cell death, destruction of mast cells and vascular dilation. By activating nociceptors, it further acts as the main pain-producing substance of HBV [53].

Api m 4 is described as minor allergen of HBV and sIgE sensitization to native and synthetic Api m 4 is found in 17–43% (higher in HBV/YJV-DS patients) [14, 15, 27] and in 54% [54] of HBV-allergic patients, respectively. However, in a population of 144 HBV-allergic patients, it was demonstrated that sIgE to Api m 4 contributes only a small percentage (median 2%) to sIgE to whole HBV, underlining its role as a minor allergen [15]. In the same study, applying 6 HBV allergens, 1.4% of patients (2/144) were MS to Api m 4. Another study showed that 2/28 (7%) of HBV-allergic patients could be diagnosed using Api m 4 but not with the marker allergens Api m 1, Api m 3 and Api m 10 [13]. Although Api m 4 is a marker allergen of HBV [15], its value for increased sensitivity of CRD is limited. Additionally, Api m 4 is currently only available for multiplex testing (Table 1).

Interestingly, a recent study demonstrated a high prevalence of Api m 4 sensitization among HBV-allergic patients who experienced systemic reactions during the build-up phase of VIT [54]. A subsequent prospective study confirmed higher rates of systemic reactions during the VIT induction phase in patients that had sIgE to Api m 4 >0.98 kU_A/L [55]. Additionally, this patient group was characterized by more severe systemic reactions after honeybee stings, increased baseline skin reactivity and HBV-sIgE as well as by more persistent responses in intradermal testing during VIT, suggesting a more complex or advanced form of the disease in this patient group. However, the number of included patients was low, and no other known risk factors for side-effects were considered. Still, this data supports the concept that CRD might help to define different phenotypes of the disease and that marker allergens such as Api m 4 might contribute to a personalized risk stratification and optimization of treatment protocols.

Antigens 5 (Ves v 5 and Pol d 5)

Antigen 5 (Ag5) proteins are listed as important major venom allergens for most allergy-relevant Vespoidea species [34, 56]. Although Ag5 allergens are one of the most abundant proteins in most Vespoidea venoms, their function within the venoms remains largely unclear. They belong to the CAP (cysteine-rich secretory proteins, antigen 5, and pathogenesis-related 1 proteins) superfamily, whose members are found in a wide range of organisms [57]. In blood-feeding ticks, flies and mosquitoes, Ag5 proteins are part of a mixture of salivary proteins that are thought to act either in suppression of the host immune system or in preventing platelet aggregation [58].

Studies, addressing sensitization rates in large, well-defined patient populations on commercial sIgE assay platforms, are currently only available for Ves v 5 from YJV. Sensitization to Ves v 5 can be found in 82–98% of patients with a history of YJV allergy [15, 17, 19, 20, 28, 40–43, 59]. Sensitization to the second commercially available Ag5, Pol d 5 from PDV, in primary PDV-sensitized patients is difficult to assess since a substantial percentage of the respective patient populations is DS to PDV and YJV with unknown primary sensitizer. Nevertheless, the available studies suggest that Ag5 proteins represent the most potent allergens in almost all studies allergy-eliciting Vespoidea species [56].

Ag5 allergens are valuable marker allergens to discriminate between primary HBV and vespid venom allergy. Although an Ag5-like protein was also identified at the transcriptomic level in the venom glands of winter bees (but not of summer bees), the coded protein product shows no cross-reactivity with YJV Ves v 5 [60]. Thus, Ag5 sensitization represents a clear marker for vespid venom allergy. In contrast, the Ag5 allergens of various Vespoidea species display pronounced

sequence identity, structural similarity (Fig. 1) and cross-reactivity, both, in sIgE measurements and BAT [59]. Therefore, Ag5 proteins are no reliable marker allergens to differentiate between allergies to these species. To date, only the Ag5 allergens of YJV (Ves v 5) and PDV (Pol d 5) are available for routine diagnostics (Table 1).

Dipeptidyl peptidases IV (Api m 5, Ves v 3 and Pol d 3)

Dipeptidyl peptidases IV (DPP IV) are aminopeptidases that cleave (pro)peptides at the N-terminus of proteins and separate dipeptides from the main chain, thereby, activating or inactivating the substrate [61]. DPP IV allergens are annotated for HBV (Api m 5), YJV (Ves v 3) and PDV (Pol d 3) (Fig. 1a) and all of them were demonstrated to be major allergens [62, 63]. In HBV and YJV, DPP IV catalyzes the reaction from promelittin to melittin and promastoparan to mastoparan, respectively [64, 65]. The substrate of PDV DPP IV remains elusive. With the activity-triggering enzyme being part of the venom, the insects probably protect themselves against toxic effects of the peptide substrates.

Sensitization to Api m 5 was found in 16–70% of HBV-allergic patients (16–39% and 41–70% in HBV-MS and HBV/YJV-DS patients, respectively) [15, 16, 18, 26]. Sensitization to Ves v 3 and Pol d 3 is less investigated but was found in 57% of YJV- and 66% of PDV-allergic patients, respectively [62, 63]. Api m 5 shares sequence identity of 53–54% to Ves v 3 and Pol d 3, while Ves v 3 and Pol d 3 are to 76% identical (Fig. 1b), resulting in extensive cross-reactivity between all three allergens. For instance, 32% of HBV-allergic patients and 63% of YJV-allergic patients are also reactive with Pol d 3 (in each case also reactive with the respective homologue of HBV or YJV) [63]. In the same study, cross-reactivity between all three allergens was additionally observed in BAT. Moreover, cross-reactivity may also be reflected by the more than doubled rate of sensitization to Api m 5 in patients allergic to HBV and YJV compared to HBV-MS patients [18].

Taken together, due to the pronounced cross-reactivity, DPP IV allergens cannot be considered reliable marker allergens to discriminate between HBV and vespid venom allergy. Moreover, to date, only Api m 5 is available for routine diagnostics (Table 1) and comparative sIgE measurements with its homologues from vespid venoms are not possible. Hence, an unambiguous identification of primary sensitization, particularly in patients that did not identify the culprit insect, should not be based on sIgE to Api m 5 alone. However, in case of a convincing history of HBV allergy, sIgE detection to Api m 5 may be helpful as Api m 5-MS occurs in rare cases [15, 18].

Icarapin (Api m 10)

Icarapin (Api m 10) is a major allergen of unknown function and low abundance in HBV [15, 66]. Although icarapin-like proteins are predicted for several insect species [67], proteomic evidence for the presence in another Hymenoptera venom exists so far only for PDV [68].

Despite its low abundance in the venom, sensitization to Api m 10 is found in 35–73% of HBV-allergic patients (35–47% and 35–73% in HBV-MS and HBV/YJV DS-patients, respectively) [15, 16, 18, 26, 66]. Api m 10 is a marker allergen for primary sensitization to HBV since YJV-allergic patients lack sIgE reactivity to this allergen [15, 66]. Despite the presence of a homologous protein in PDV (48% amino acid sequence identity), preliminary unpublished data hint to missing cross-reactivity between Api m 10 and its homologue in PDV and/or a negligible role of PDV icarapin as the sensitizing component of PDV. Hence, the marker allergen concept of Api m 10 most likely also holds true for the discrimination of HBV and PDV allergy. Interestingly, Api m 10 contains one major IgE epitope (Api m 10_{160–174}) that is recognized by 100% of Api m 10-reactive patients [69] and that is not present in its PDV homologue. Due to the high rate of sensitization, this Api m 10 peptide might be an interesting and easy-to-produce alternative in diagnostics to the recombinant allergen. Moreover, as discussed in the section “Acid phosphatase”, Api m 10 together with Api m 3 is able to confirm primary HBV allergy in 65% of HBV and YJV DS patients that exhibit negative sIgE to Api m 1 and were not able to identify the culprit insect [13]. Hence, Api m 10 represents an important tool in diagnostics of HBV allergy.

Additionally, Api m 10 might be an interesting marker for personalized risk stratification in VIT. Recently, a retrospective multicenter study of VIT-treated HBV-allergic patients demonstrated that a predominant sensitization to Api m 10 (defined as >50% of sIgE to HBV) represents a relevant risk factor for treatment failure (according to sting challenge tests) with an odds ratio of 8.44 [26]. Dominant sensitization to Api m 10 is found in 6–12% of HBV-allergic patients [15, 18, 26]. Such an association was not found for dominant sensitization to other tested allergens such as Api m 1, Api m 2, Api m 3 and Api m 5. Interestingly, all patients who exhibited sIgE to Api m 10 higher than 60% of HBV sIgE were therapy nonresponders [26]. Current research addresses the question whether this is due to the lack of native Api m 10 in therapeutic preparations commonly used for VIT [70]. Although the role of Api m 10 in tolerance induction during VIT is not finally understood, the knowledge of patients' sensitization profiles allows a better risk stratification in VIT and personalized treatment. For instance, by choosing a therapeutic venom preparation that contains high amounts of Api m 10 for VIT of Api m 10-sensitized patients [71].

Other allergens

Other less investigated allergens such as major royal jelly proteins (Api m 11) [72] or protease inhibitor (Api m 6) [73] of HBV or serine protease (Pol d 4) [74] of PDV might serve as additional marker allergens. However, preliminary, partially unpublished data suggest rather a role as minor allergens. Nevertheless, such allergens may be of particular relevance for selected patients and be able to close diagnostic gaps of CRD in the future.

Marker allergens to discriminate between HBV and vespid venom allergy

Positive sIgE test results to two or even more Hymenoptera venoms, which are frequently observed in clinical routine [28, 31, 32], may either reflect true primary sensitization to different venoms or may be caused by IgE directed against CCDs or homologous allergens present in the venoms (Fig. 2). In the first case, VIT with both venoms is recommended, while in the second scenario VIT with the primary sensitizing venom is sufficient. As venom extract-based sIgE

testing does not allow discrimination between cross-reactivity and primary sensitization, multiple positive results strongly complicate the choice of the correct venom for VIT and might lead to unnecessary treatment with more than one venom, particularly in patients who were not able to correctly identify the culprit insect.

The current CRD has particularly contributed to the discrimination between cross-reactivity and primary allergy to HBV and YJV venom. This is due to the number of allergens available for routine diagnostics (Table 1) and to the identification of several allergens that are exclusive for bee or vespid venom (Fig. 2).

The use of the commercially available allergens Ves v 1 and Ves v 5 results in a sensitivity of 92–100% for the diagnosis of YJV allergy [17, 19, 20, 31, 40–43]. In HBV allergy, the situation is even more complex. In the first study that applied an experimental allergen panel for the detection of HBV sensitization ($n=144$; 54 HBV-MS and 90 HBV/YJV-DS), the combination of 6 allergens (Api m 1–5 and 10) resulted in a sensitivity of 94% [15]. In another study, using the same assay platform, combining the allergens Api m 1–3, 5 and 10 lead to a diagnostic sensitivity of only 79%

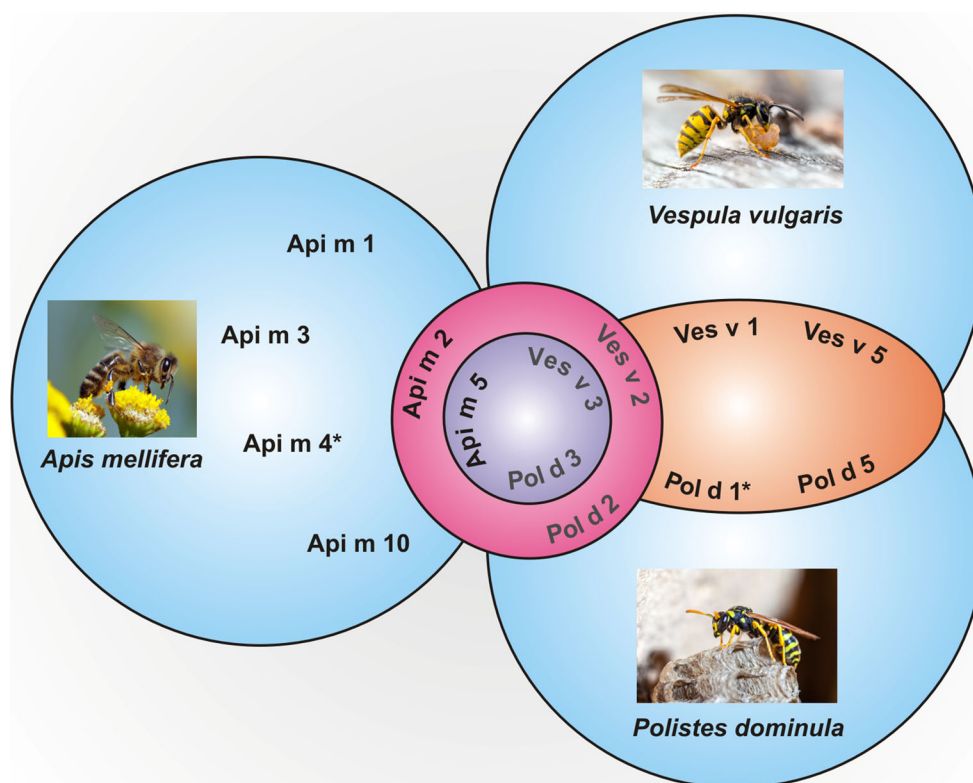


Fig. 2 Marker and cross-reactive allergens of HBV, YJV and PDV. While allergens are known that enable the differentiation between cross-reactivity and primary sensitization to HBV (Api m 1, Api m 3, Api m 4 and Api m 10) and YJV/PDV (Ves v 1/Pol d 1 and Ves v 5/Pol d 5), the major allergens of YJV and PDV identified so far exhibit cross-reactivity. The hyaluronidases (Api m 2, Ves v 2 and Pol d 2) and dipep-

tidylpeptidases IV (Api m 5, Ves v 3 and Pol d 3) are shared between all three venoms and exhibit a varying degree of cross-reactivity. Allergens shown in *black* are commercially available for diagnostics. An *asterisk* indicates allergens that are exclusively available at selected multiplex sIgE platforms. Allergens shown in *grey* are currently not available for routine diagnostics

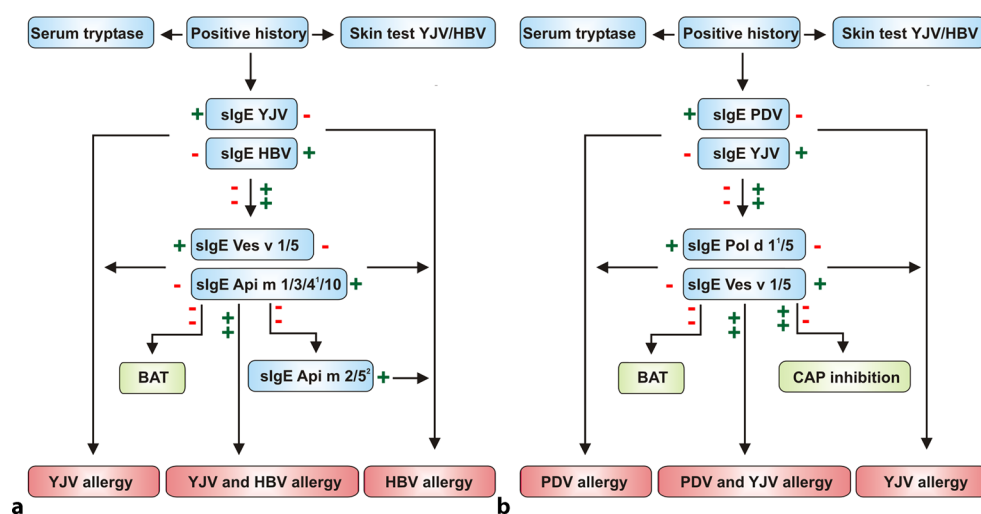


Fig. 3 Diagnostic algorithm for component-resolved diagnostics of **a** HBV and YJV allergy and **b** YJV and PDV allergy. The diagnostic algorithm presented in **a** can also be used to discriminate between HBV and PDV allergy using the PDV homologues of Ves v 1 and Ves v 5, Pol d 1 (only available for multiplex testing; Table 1) and Pol d 5. A *plus* indicates a positive and a *minus* a negative test result. ¹These allergens are only available for selected multiplex sIgE platforms. ²The HBV allergens Api m 2 and Api m 5 show potential cross-reactivity to not commercially available homologous allergens of YJV

and PDV so that a positive test result does not necessarily exclude YJV or PDV allergy. Despite the potential of component-resolved diagnostics, clinical history, skin tests and the measurement of venom-sIgE and serum tryptase build an indispensable basis for accurate diagnosis in Hymenoptera venom allergy. Moreover, BAT and CAP inhibition assays may be helpful tools in dissecting double-positive or double-negative test results. *BAT* basophil activation test, *HBV* honeybee venom, *PDV* *Polistes dominula* venom

[18], most likely due to a different composition of the patient population, in particular the number of HBV-MS ($n=134$; diagnostic sensitivity 72%) and HBV/YJV-DS ($n=55$; diagnostic sensitivity 93%) patients. For the same allergen panel another study reported a diagnostic sensitivity of 92% in the whole population of HBV-allergic patients and of 90 and 94% in HBV-MS and HBV/YJV-DS patients, respectively [16].

Taking into consideration, that CRD is particularly important for the elucidation of DS, the commercially available allergen panel can be considered highly valuable for adequate diagnosis. This particularly holds true for the challenging group of patients who are HBV/YJV-DS and were not able to identify the culprit insect. As described above, in this patient population, primary sensitization to HBV could be confirmed in 54% of cases using Api m 1. In the remaining Api m 1-negative patients, sIgE to the marker allergens Api m 3 and Api m 10 confirmed primary sensitization to HBV in 65% of cases [13]. This is of particular relevance, as without the additional sIgE measurements, those patients would have been regarded as having a sensitization only to YJV and not to HBV. Interestingly, a recent study demonstrated that the panel of Ves v 1, Ves v 5, Api m 1 and Api m 10 allowed the identification of the culprit venom in 98% of patients sensitized to YJV and/or HBV with good agreement to skin testing [75]. Contrary, another study questioned the ability of the available allergen panel to resolve double-sensitization, as 70% of the patients DS to venom extracts were also DS with at least one allergen of YJV and HBV. A possible expla-

nation was found in the unavailability of potentially cross-reactive allergens from both venoms for CRD [76]. However, this study included the highly cross-reactive Api m 5 as marker allergen for HBV sensitization. Thus, it is not clear to which extent this phenomenon might be caused by cross-reactivity or true primary sensitization to both venoms.

Although diagnostic sensitivity of the currently available allergen panel, particularly of HBV, is not 100%, CRD has clearly improved discrimination of primary allergy and cross-reactivity in YJV and HBV allergy, thus, facilitating correct prescription of VIT. A suggested diagnostic algorithm to discriminate between HBV and YJV allergy using CRD is given in Fig. 3a. Of note, the same algorithm using the corresponding PDV allergens can also be applied to discriminate between HBV and PDV allergy.

Marker allergens to discriminate between PDV and YJV allergy

In Southern Europe double-sensitization to YJV and PDV is more frequently observed than that to vespidae venom and HBV [77–79]. Here, a definite resolution of cross-reactivity and true primary allergy to both venoms is rarely possible due to a high degree of cross-reactivity between the major allergens of the venoms (Fig. 2). Considering the increasing spread of *Polistes dominula* on several continents, associated diagnostic problems are likely to gain importance in other areas of the world.

Closely related wasp species such as *Vespula* spp. and *P. dominula* share similar venom composition. The respective venomes were recently studied and elucidated using a mass spectrometric approach. Despite the identification of previously unknown components and, thus, potential new allergens for CRD, the high degree of venom and protein similarity lead to the authors' conclusion that marker allergens to discriminate YJV and PDV allergy are rather unlikely [68]. An approach based on cross-reactive allergens as proposed by Monsalve et al. [38] seems more promising to solve this persisting problem. Here, comparing levels of sIgE to Ag5 and PLA1 allergens of PDV and YJV allowed a reliable identification of the culprit venom in 67% of DS patients. However, only Pol d 5 is currently available for routine diagnosis of PDV allergy on the most common sIgE singleplex platform (Table 1).

To date, the gold standard to resolve DS in PDV and YJV allergy are CAP-inhibition assays with the venoms [2, 80, 81]. Current limitation of the commercially available homologous allergens Pol d 5 and Ves v 5 to distinguish between YJV and PDV allergy in DS patients by CRD is reflected by the fact that, in contrast to former reports [81, 82], a recent multicenter study did not find any association between CAP-inhibition test results and double sIgE values of Ves v 5 over Pol d 5 or vice versa [2].

The available data demonstrates that the use of Ag5 allergens in CRD has extensive limitations in resolving DS in PDV and YJV allergy. Hence, the commercial availability of additional cross-reactive major allergen pairs (at least an addition of Pol d 1) for routine diagnosis might be beneficial to uncover primary sensitization in PDV and YJV DS patients. A proposed diagnostic algorithm to distinguish PDV and YJV allergy using CRD is given in Fig. 3b.

Conclusions and future needs

Although clinical history, extract-based sIgE testing and skin testing build an indispensable basis for accurate diagnosis in HVA, CRD using recombinant CCD-free marker allergens has substantially improved discrimination of cross-reactivity and primary allergy, particularly in HBV and YJV allergy.

A clear limitation of the currently available CRD is that it is not able to reliably differentiate between cross-reactivity and primary allergy to the venoms of different vespid species such as PDV and YJV due to the high degree of cross-reactivity between all major allergens. The availability of cross-reactive allergens and a comparison of sIgE levels to several of these pairs may contribute to an increased diagnostic resolution in the future.

Furthermore, there is a need for additional allergens to accurately diagnose allergy to other species such as *Polybia* species or to discriminate allergy to European and American Polistes species.

There is first evidence that some allergens and patients' sensitization profiles may act as biomarkers to diagnose particular phenotypes of HVA. However, further prospective studies are needed to verify whether allergens such as Api m 4 and Api m 10 are useful markers to predict severe VIT side-effects and an elevated risk for therapeutic failure, respectively.

Despite the remaining limitations, the ongoing identification and characterization of Hymenoptera venom allergens and the growing availability of diagnostic tools have opened new options for the classification of HVA and, hence, for personalized medical approaches and precision medicine in HVA.

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