









REVIEW ARTICLE

Molecular allergology and its impact in specific allergy diagnosis and therapy

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Abstract

Progressive knowledge of allergenic structures resulted in a broad availability of allergenic molecules for diagnosis. Component-resolved diagnosis allowed a better understanding of patient sensitization patterns, facilitating allergen immunotherapy decisions. In parallel to the discovery of allergenic molecules, there was a progressive development of a regulation framework that affected both in vitro diagnostics and Allergen Immunotherapy products. With a progressive understanding of underlying mechanisms associated to Allergen immunotherapy and an increasing experience of application of molecular diagnosis in daily life, we focus in analyzing the evidences of the value provided by molecular allergology in daily clinical practice, with a focus on Allergen Immunotherapy decisions.

KEYWORDS

allergen immunotherapy, component-resolved diagnosis, food allergy, major allergen, respiratory allergy

Abbreviations: AIT, Allergen Immunotherapy; CRD, Component-resolved diagnosis; EDQM, European Directorate for the Quality of Medicines; EMA, European Medicine Agency; HBV, Honeybee venom; LTP, Lipid transfer protein; PDV, Polistes Dominula venom; POIT, Peanut Oral Immunotherapy; YJV, Yellow Jacket venom.

All the authors contributed to the writing of the manuscript. D.B coordinated the work.

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1 | INTRODUCTION

The progressive advance in the knowledge and characterization of allergenic molecules responsible for allergic sensitization to most sources has had a profound impact in the etiological management of allergic disease. This impact can be summarized in three main aspects. Firstly, it has profoundly transformed the way allergenic extracts are characterized and standardized which is legally reflected in the regulatory framework for allergens, especially on the Note for Guidance on Allergenic Extracts, issued by the European Medicine Agency (EMA) and on the allergen monograph of the European Pharmacopoeia, issued by EDQM (Council of Europe), that is adopted by European Directives for Medicinal products.¹⁻³ Secondly, it improved the accuracy of allergy diagnosis. Single or multiplexed, allergenic molecules are routinely used and have changed the way we diagnose.⁴ Lastly, but not least, some sensitization profiles are linked to different clinical phenotypes and can be used to stratify allergic patients, to predict intervention outcomes, and to perform system biology studies that open new avenues for allergy disease management.⁵⁻¹¹

The implementation of a new regulatory framework led to the commercialization of allergen immunotherapy products with registration clinical trials performed during 5 years. Based on these studies, a better understanding of AIT mechanisms is now possible. From allergen-specific effector cell desensitization^{5,12,13,14} to a progressive onset of T- and B-cell-mediated regulatory mechanisms¹⁵⁻¹⁹ responsible for the disease-modifying effect, their temporal alignment is critical to establish best AIT practices. In this context, the quality of the extract used—and its standardization—the knowledge of patient's sensitization profiles, and the link to the AIT product used is pivotal to maximize the odds of AIT success. In this review, we discuss the different aspects of molecular allergology, from an overview of allergenic molecules and diagnostic methods to the quality of allergen preparation and the clinical approaches to AIT. We aim to offer a practical document to support the allergy specialist in the daily clinical management of allergic patients.

1.1 | Overview of allergenic molecules

Currently, more than one thousand allergens from various allergenic sources have been described. Most of these molecules, that are relevant for AIT decisions, belong to a limited number of protein families and have been extensively reviewed.^{4,20,21} More than 40% of the relevant plant allergens belong to six protein families: 2S albumins, non-specific lipid transfer proteins (nsLTP), legumins, vicilins, profilins, and pathogenesis-related (PR)-10 proteins.²² In contrast, there are other families with only a few but very relevant members, as they include major pollen allergens such as expansins, polcalcins, pectate lyases, and defensin-like proteins. Concerning animal allergen families, four of them (cysteine proteases, lipocalins, tropomyosins, and parvalbumins) account for over 70% of the relevant allergens (Table 1).²³ A separate category is formed by the allergens from hymenoptera venom (Table 2).

Major milestones

- IgE Discovery (1968)
- First commercial reagents for specific IgE (1973)
- Knowledge and Characterization of the most relevant allergens (1985–2005)
- Development of multiplexed allergens: 1999
- Handbook of Molecular Allergology: 2016
- Progressive use in clinical practice of CRD: 2010–2021
- Progressive understanding of AIT mechanisms: 2010–2021
- First AIT product registered following Pharmaceutical development guidelines: 2007
- First OIT for peanut allergy registered: 2020

Future research perspectives

- Affinity and avidity need to be explored in IgE response to allergens
- T-cell reactivity to allergens is a critical parameter
- Combination of new biologics and AIT and associated diagnosis will open new intervention strategies
- New regulation of in vitro diagnostics will increase the quality of CRD, but might limit innovation and available molecules

1.2 | Diagnosis methods and European regulation

There are different methods available for sIgE determinations of single allergenic components. These components might be available as individual diagnostics or multiplexed in arrays. These technologies are commercialized by Thermo Fisher, MADx, Hycor, Euroline, and Siemens. The list of available allergens in each platform has recently been described in detail.²⁰ Most of the existing methods for CRD (Component-resolved diagnosis) and multiplexed platforms have been commercialized for research purposes and will need an adaptation and upgrade to comply with new regulatory EU framework as required by the regulation affecting in vitro diagnostics and medical devices, with a transition implementation phase ending by 2025 (REGULATION (EU) 2017/746). This regulation implementation will secure the adequate performance of commercialized diagnostics.

The present document uses as a starting point the handbook of molecular diagnosis by Matricardi and cols that extensively describe best diagnosis strategies combining different approaches.⁴

1.3 | AIT regulations, biological potency, and link to clinical effect

The characterization of the main allergenic molecules led to the development of quantification methods and their progressive incorporation into regulations of extract preparation of AIT. In parallel, Create

TABLE 1 Characteristics and significance of pollen, vegetables, mites, and epithelia allergens available for CRD and relevant for AIT decisions

Source		Allergen	Sensitization rate	Available for routine CRD	Significance
Pollen	Grasses	Phl p 1	>90%	S*, M*	Expansin. Present in different plant parts. Major allergen and usually initiates sensitization
		Phl p 5	>60%	S, M	Unknown function. Most abundant pollen protein
		Phl p 7	<10%	S, M	Pan-allergen in pollens. Calcium-binding
		Phl p 12	5%–50%	S, M	Pan-allergen (food/pollen). Structural protein
	Birch	Bet v 1	>90%	S, M	Pan-allergen (food/pollen) Regulatory protein. Highly expressed in case of biotic stress.
	Olive tree	Ole e 1	>90%	S, M	Unknown function
	Olive	Ole e 7	0%–50%	S, M	nsLTP, minor allergen. Disease severity marker
	Olive	Ole e 9	0%–50%	S, M	Glucanase, minor allergen. Highly variable in different olive cultivars
	Russian thistle	Sal k 1	>90%	S, M	Pectin methylesterase
	Plain tree	Pla a 1	>90	S, M	Invertase inhibitor
	Arizona cedar	Cup a 1	>90%	S, M	Pectate lyase. Homologous and cross-reactive to Cry j 1 from Japanese cedar and Jun a 1 from mountain cedar
	English plantain	Pla l 1	>70	S, M	Ole e 1 like. Non-cross-reactive
	Artemisia	Art v 1	>70	S, M	Defensin like
	Ragweed	Amb a 1			Pectate lyase
	Parietaria	Parj 2	>90%	S, M	ns LTP
Foods	Peach	Pru p 3		S, M	ns LTP. Very stable. Frequent initiator in LTP syndrome
	Peanut	Ara h 1			11S Albumin. Storage protein. Highly abundant
		Ara h 2			2S albumin. Storage protein. >Highly abundant. Risk marker for severe reactions
		Ara h 3			11S Albumin. Storage protein. Highly abundant
		Ara h 5			Profilin
		Ara h 8			PR10. Bet v 1 like
	Ara h 9			nsLTP (Pru p 3 related)	
Mites	<i>Dermatophagoides pteronyssinus</i>	Der p 1	>90%	S, M	Cysteine protease. Associated to mite fecal particles
		Der p 2	>90%	S, M	NCP2. Lipid-binding protein. MD2 link. Associated to mite bodies
		Der p 10	<10%	S, M	Pan-allergen in arthropods.
		Der p 23			Peritrophin-like protein
	<i>Lepidoglyphus destructor</i>	Lep d 2	>90%	M	Related to Der p 2, but without IgE cross-reactivity. Cross-reactive with Group 2 proteins from other minor/storage mites.
Epithelia	Cat	Fel d 1	>90%	S, M	Secretoglobulin.
		Fel d 4,7	<50%	S, M	Lipocalins. Cross-reactivity with Can f 1
	Dog	Can f 1	>50%	S, M	Lipocalin
		Can f 5	>50%	S, M	Kalikrein. Serine protease. Regulating semen liquefaction. Present in male dogs

Note: Prevalence on food allergens depends on geographical locations and age. In general, storage protein allergy is frequent in pediatric cohorts, while LTP and PR10-linked allergy are of a later onset.

*Estimated from publications. S: Available in singleplex assays. M: Available in Multiplex assays.

TABLE 2 Characteristics and significance of Hymenoptera venom allergens available for routine CRD (modified according to¹³²).

Allergen	Sensitization rate*	Available for routine CRD	Significance
Honeybee venom (<i>Apis mellifera</i>)			
Api m 1 Phospholipase A2	57%–97%	S ¹⁻³ , M ⁴⁻⁶	Marker allergen for HBV sensitization; Allows discrimination between HBV and YJV/PDV sensitization
Api m 2 Hyaluronidase	28%–60%	S ¹⁻³ , M ⁴	Due to limited cross-reactivity with Ves v 2 and Pol d 2 in the absence of CCDs, potential marker for HBV sensitization**
Api m 3 Acid phosphatase	28%–63%	S ¹	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 ⁶	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 ¹	High cross-reactivity with Ves v 3 and Pol d 3 prevents its use as marker allergen***
Api m 10 Icarapin	35%–73%	S ^{1,3} , M ^{4,5}	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>Vespula vulgaris</i>)			
Ves v 1 Phospholipase A1	39%–66%	S ¹ , M ^{4,5}	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 ¹⁻³ , M ^{4,5}	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 ⁴	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 ¹ , M ^{4,5}	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

Abbreviations: CCDs, cross-reactive carbohydrate determinants; CRD, component-resolved diagnostics; HBV, honeybee venom; M, multiplex slgE assay system; PDV, *Polistes dominula* venom; S, singleplex slgE assay system; YJV, yellow jacket venom.

¹Thermo Fisher Scientific.

²Siemens Healthcare Diagnostics.

³Dr. Fooke Laboratories.

⁴Euroimmun.

⁵Macro Array Diagnostics.

⁶Faber test (different suppliers).

*Defined by different immunoassays and in different patient populations. Sensitization rates are referenced in the main text.; **Cross-reactivity with Ves v 2 and Pol d 2 is possible.; ***Api m 5 monosensitization may occur in HBV-allergic patients.

International project resulted in the first available allergen standard csndi Dates and corresponding methods for major allergen content determination.^{3,24,25,26} Today, for any AIT product intended for human use, a proper measurement of biological potency is required and information on major allergen content will become mandatory (a. Bet v 1, b. Phl p 5a). Major allergens use to be the most abundant proteins of allergenic sources, and their content in AIT products is closely related to total biological potency. Moreover, most of the patients included in clinical trials are sensitized to major allergens and in a significant percentage monosensitized to them.²⁷ In consequence, as a general criterium, patients elected for AIT should be sensitized to major allergens as there is no evidence of the clinical effect of AIT products in patients sensitized only to minor allergens. Unfortunately, the content for minor allergens in AIT preparations is, in the vast majority of cases, unknown and variable. In areas where pollen exposure is very intense, and sensitization to minor allergens is quite frequent, adverse events to AIT preparations containing variable amounts of minor allergens variability have been described.^{28,29}

Main AIT mechanisms involve early effector cells desensitization and progressive onset of tolerance through a regulatory response that needs at least 3 years to be consolidated.^{5,13} Dose-finding studies are usually performed in short-term studies, and thus effective dose is adjusted to target preferentially early immunological mechanism. Again, major allergen doses tolerated by patients sensitized to major allergens might serve as basis for dose definition in AIT.

1.4 | CRD: Monitoring of allergy progression

Birth cohort studies have shown a sequential broadening of the IgE response to complex allergen sources. Preliminary studies performed so far suggest that IgE response starts with a monomolecular stage and then, through an oligomolecular sensitization pattern (2–4 specificities) becomes polymolecular (>4).³⁰ This “molecular spreading” has been observed in many participants in the MAS birth cohort allergic to Timothy grass. In this case, the “initiator molecule” was Phl p 1. However, only a few patients develop an extremely polymolecular response, producing IgE antibodies to all diagnostically available allergenic molecules of *Phleum pratense* (Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, and Phl p 12).³⁰ Similarly, molecular spreading has been observed in the MAS cohort among children developing IgE sensitization against *Dermatophagoides pteronyssinus*.³¹ In this case, multiple “initiator molecules” have been observed (Der p 1, Der p 2, and Der p 23). In these children, sensitization started with Der p 1 and/or Der p 2 and/or Der p 23 (defined as group “A” molecules); then involved Der p 4, Der p 5, Der p 7, and Der p 21 (group “B” molecules); and was completed with IgE to Der p 11, Der p 14, Der p 15, Der p 18, and clone 16 (group “C” molecules). This expansion of the IgE response has also been defined as “the ABC march” of mite allergy.³¹ Children with a broader polymolecular IgE sensitization pattern were also more frequently affected by asthma when compared with those who remained in the “A” stage of IgE sensitization.³¹ A similar trend was observed among grass pollen allergic

children in the Manchester Allergy and Asthma Study (MAAS).³² In this case, children with broader molecular patterns of IgE sensitization had also a significantly increased risk for asthma, eczema, and rhinitis.³² It has also been proposed that earlier administration of AIT, that is, at a mono- or oligomolecular stage of sensitization, may be more effective than at later stages.³³

2 | CRD AND SELECTION OF PREPARATIONS FOR AIT

2.1 | Grass pollen allergy

Grasspollen allergy is probably the most studied allergic condition. There is a broad allergen panel for CRD of grass allergy, and a SLIT AIT product that is the only one with a complete clinical development program, including several 5-year studies reviewed by Barber et al,¹² that provides the best available information to understand the link between sensitization profiles and AIT.

Allergen composition studies of a *Phleum* pollen extract³⁴ allowed to establish that Phl p 5 and its related allergen Phl p 6 are the most abundant proteins, accounting for more of 50% of protein content. Accumulated content of Phl p 1 represents less than 10%. The rest of allergen components are in a range of 1%–10% of total protein. Despite its relatively low abundance, Phl p 1 is the most relevant single allergen sensitizer of grass allergic patients. Epidemiological studies performed in Spain^{35,36} and CRD analysis of 1905 patients included in AIT clinical trials of grass pollen allergy in North America²⁷ demonstrated the preponderance of Phl p1 as primary sensitizer to grass pollen. In fact, a significant proportion of patients were mono-sensitized to this allergen. Similar results in pediatric grass pollen allergic patients have been reported.³⁷

Recently, a potential explanation for this has been published.³⁸ Group 1 grass allergens, belonging to the beta expansins family, are present in other plant parts. In Autumn, upon plant death, plant particles are aerosolized and might be presented together with *Alternaria* spores, initiating sensitization process. No loss of effect in AIT is observed in patients mono-sensitized to either Phl p 1 or Phl p 5²⁷; however, patients with low (first tertile) sIgE to any of the two major allergens presented no clinical benefit in the first pollen treatment season. Presumably, early AIT effects are governed by desensitization and initial immunological events in a dose-dependent manner. Patients with low sIgE levels might need a higher allergen dose to get desensitized; however, in a 5-year mechanistic study,⁹ an impaired regulatory response for this type of patients was not observed, and thus, they should not be excluded for AIT if they meet clinical inclusion criteria.

2.2 | The role of pan-allergens

An interesting subgroup of grass pollen allergic patients are sensitized to profilin, a pan-allergen present in all vegetable tissues, with

a highly conserved structure. Usually, profilin is a confounding diagnostic factor, which is minimized by CRD diagnosis either “in vitro” or even “in vivo”.³⁹ Profilin sensitization strongly associates with grass pollen allergy³⁵ probably due to a relatively high profilin content in grass pollen compared to other pollens.⁴⁰ Usually, sensitized profilin patients present no food-mediated reaction or only local (oral) ones to, that is, sweet melon species. However, in areas of very high grass pollen exposure, some allergic patients do not respond to AIT and present severe food profilin-mediated reactions.^{41,42} In fact, T-cell reactivity to profilin increases along with grass pollen intensity exposure. Moreover, in patients from central Spain, T-cell proliferation induced by profilin is similar to that induced by the major allergen Phl p 1.³⁴ These peculiar patients, as well as others exposed to very high pollen levels, can be identified by combining CRD with clinical evaluation and provide invaluable models to understand allergic inflammation and AIT mechanisms and its limitations.

Finally, patients sensitized simultaneously to the pollen pan-allergens polcalcin and profilin have been shown to have twice as many primary sensitizations and to duplicate the duration of allergic disease,³⁶ suggesting that AIT might not be effective. Similar results supporting clinical value for Phl p 7 and Phl p 12 have been described in pediatric cohorts.³⁷ Moreover, this specific double sensitization profile to pan-allergens is not linked to any particular pollen.³⁶ At this

moment, other grass allergens present a limited diagnostic value for AIT decisions and some of them provide confounding information due to lack of specificity.⁴³

Figure 1 represents an algorithm to support decision-making in the selection of AIT for grass pollen allergy.

2.3 | Olive pollen allergy and nsLTP food allergy

One of the best models to understand the link between pollen exposure, sensitization profiles, and clinical impact is the olive pollen allergy model. Minor allergen sensitization is frequent in areas of very high exposure. Interestingly, sensitization to Ole e 7—the nsLTP from olive pollen—is associated with a unique clinical phenotype.^{29,35} Patients living in areas with high olive pollen exposure and sensitized to Ole e 7 develop a severe respiratory allergic phenotype. These patients do not respond to AIT and constitute another model to understand evolution to severe allergic phenotypes. In Figure 2, an algorithm to support the selection of olive pollen AIT is shown.

There are other pollens where nsLTPs play a preponderant role, as *Parietaria judaica*, whose main allergen is a LTPs, or *Artemisia artemisiifolia* and *Platanus orientalis*, whose Art v 3 or Plaa 3 is LTPs. Art

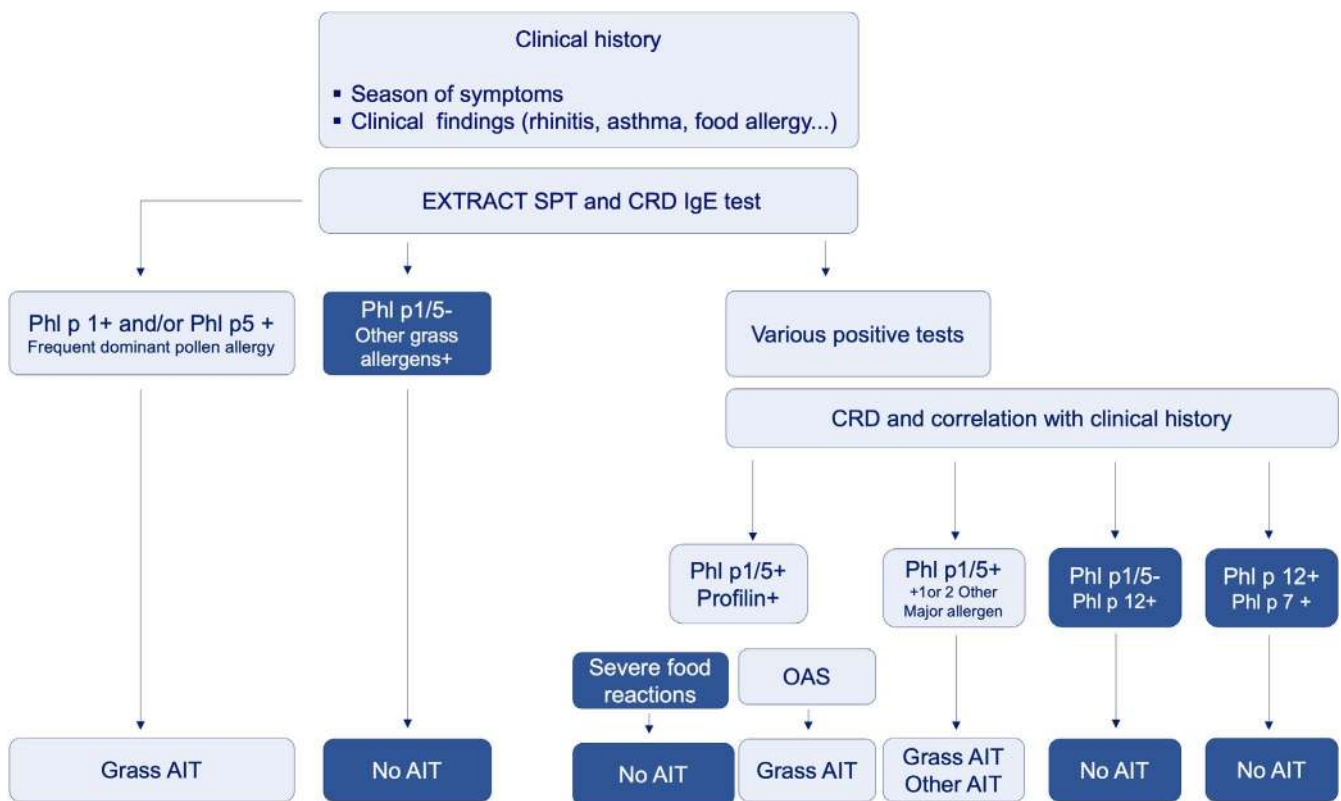


FIGURE 1 Diagnostic algorithm and decision tree for AIT using CRD in Grass pollen allergy. Grass pollen allergy is one of the most studied allergy models. Major allergen sensitization is required before considering AIT. The combination of major allergens and pan-allergens provides the necessary tools for AIT decisions. Profilin allergy might be a contraindication only in severe food allergic patients, while double sensitization to both pan-allergens is associated to many years of disease evolution, poly-sensitization, and poor intervention outcome

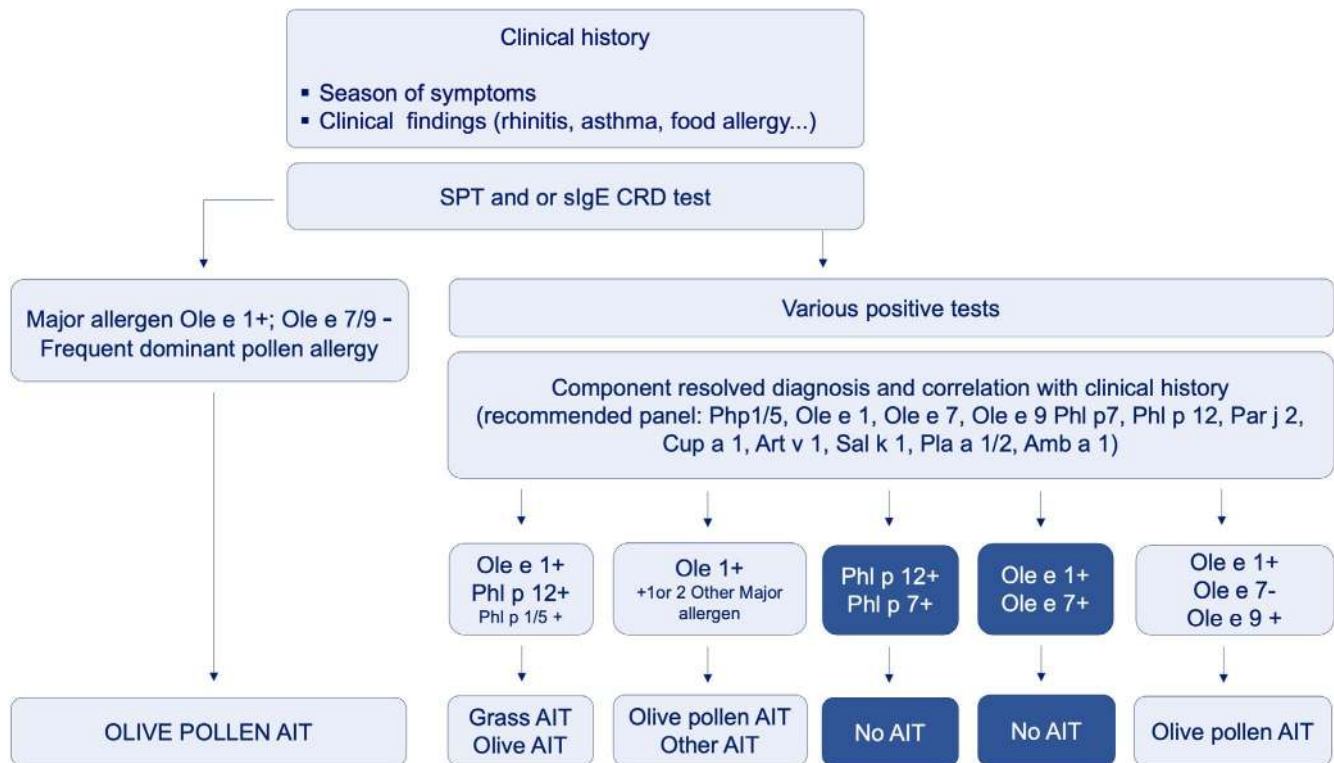


FIGURE 2 Diagnostic algorithm and decision tree for AIT using CRD in Olive pollen allergy. Olive pollen allergies are one of the most complex allergy models. Usually olive cultivars cover homogeneous areas and present acute differences in allergen pollen content. In dense pollen areas, patients are exposed to the highest pollen counts recorded. The existing of complex profiles, marked by minor allergens sensitization, makes CRD a fundamental tool for patient management and AIT decisions

v 3 and Pla a 3 cross-react with Pru p 3 complicating the differential diagnosis of the primary sensitization (to pollen or peach).

LTP-mediated allergy is the predominant food allergy in adults in the Mediterranean Area and Southern European countries.^{44,45} Pru p 3—the peach LTP—is the best marker for LTP allergy. Sublingual AIT has proved to be effective for the treatment of LTP allergy, with clinical effect not only against allergy to closely related food species such as *Rosaceae* fruits,⁴⁶ but also against allergy to species with about only 60% sequence identity, such as the Peanut LTP, Ara h 9.^{47,48} Evaluating the spectrum of recognition to multiple LTPs is helpful to make a potential AIT therapeutic decision. Patients sensitized to LTPs distant to Pru p 3 such as wheat Tri a 14 might not benefit completely from AIT based on Pru p 3. Unfortunately, SLIT AIT for LTP allergy is only available in a limited number of Countries. Figure 3 summarizes decision trees for LTP-mediated AIT.

2.4 | Birch and Oak tree pollen allergy and PR10 proteins

PR10 allergens are present in multiple species of *Fagales* tree pollen. Among them, birch in Europe⁴⁹ and oak in North America are regarded as primary sensitizers. There are products registered worldwide for the treatment of birch pollen allergy.⁵⁰ Recent data suggest that a birch-based AIT Product within defined Bet

v 1 content has clinical benefit during oak pollen season in North America.⁵¹ Interestingly, a study analyzed the effect of birch-based AIT in cross-reactive T-cell response to homologous PR10 allergens from other trees.⁵² This study demonstrated that down-regulation of T-cell reactivity can be achieved against multiple, T-cell cross-reactive PR10 molecules. The control of T-cell proliferation based on T-cell cross-reactivity might be the most relevant endpoint for successful AIT. As in the case of profilins, PR10 proteins are also present in multiple fruit, nut, legume, and vegetables species, leading to PR10-associated allergy symptoms. There is limited evidence on the clinical benefit of PR10-based pollen AIT in the amelioration of pollen-related food allergy syndrome (FPAS).^{53,54} However, immunotherapy with Mal d 1 has been associated with clinical improvement.⁵⁵ More studies will be needed to clarify potential use of PR10 pollen AIT in this particular type of secondary food allergy

2.5 | Cypress/cedar allergy

Japanese cedar pollen is the main cause for seasonal respiratory allergy in Japan, and Cupressaceae are relevant allergens in the United States and southern Europe as well. Major allergens belonging to the pectate lyase family (Cup a 1, Cup s 1, Jun a 1 and Cry j1) show a very high cross-reactivity.

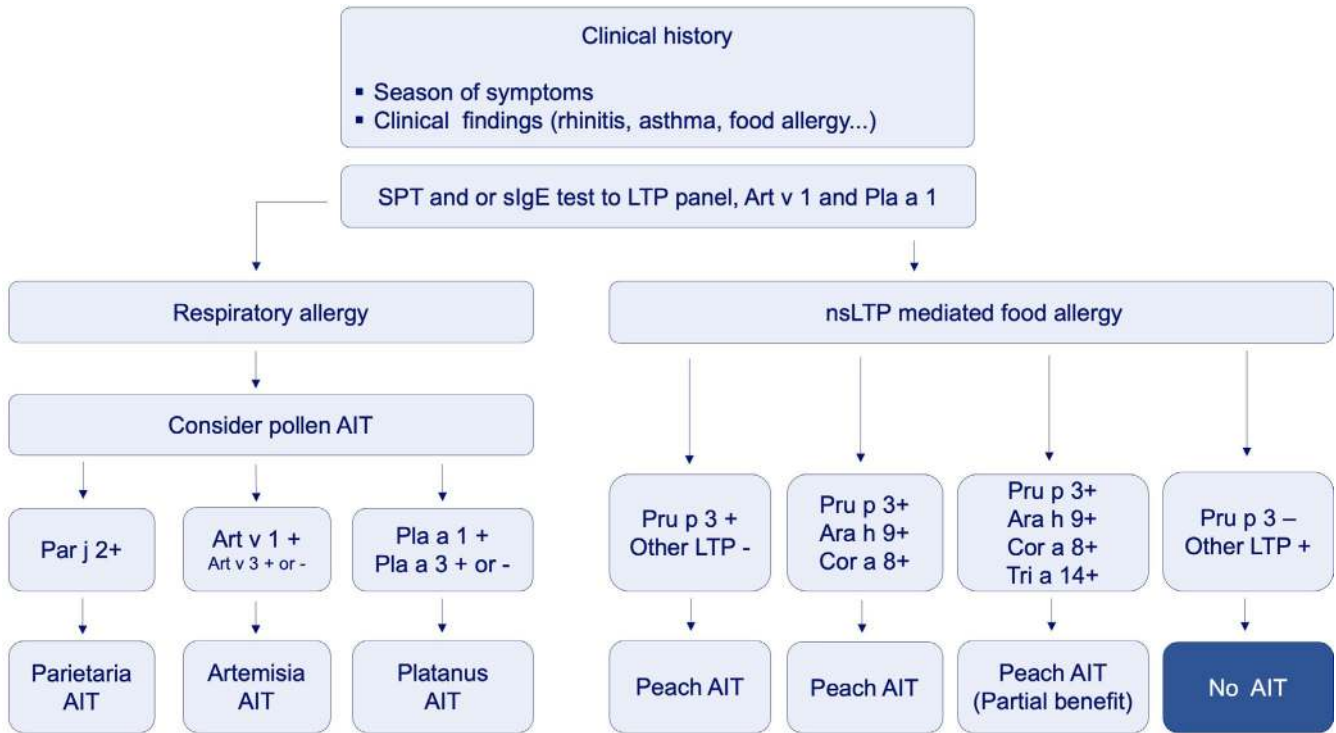


FIGURE 3 Diagnostic algorithm and decision tree for AIT using CRD in nsLTPs-mediated allergies. The existence of cross-reactivity between nsLTPs from *Artemisia* and *Platanus* pollens makes CRD necessary. LTP immunotherapy with an enriched Pru p 3 can be considered in Countries with availability of this type of therapy

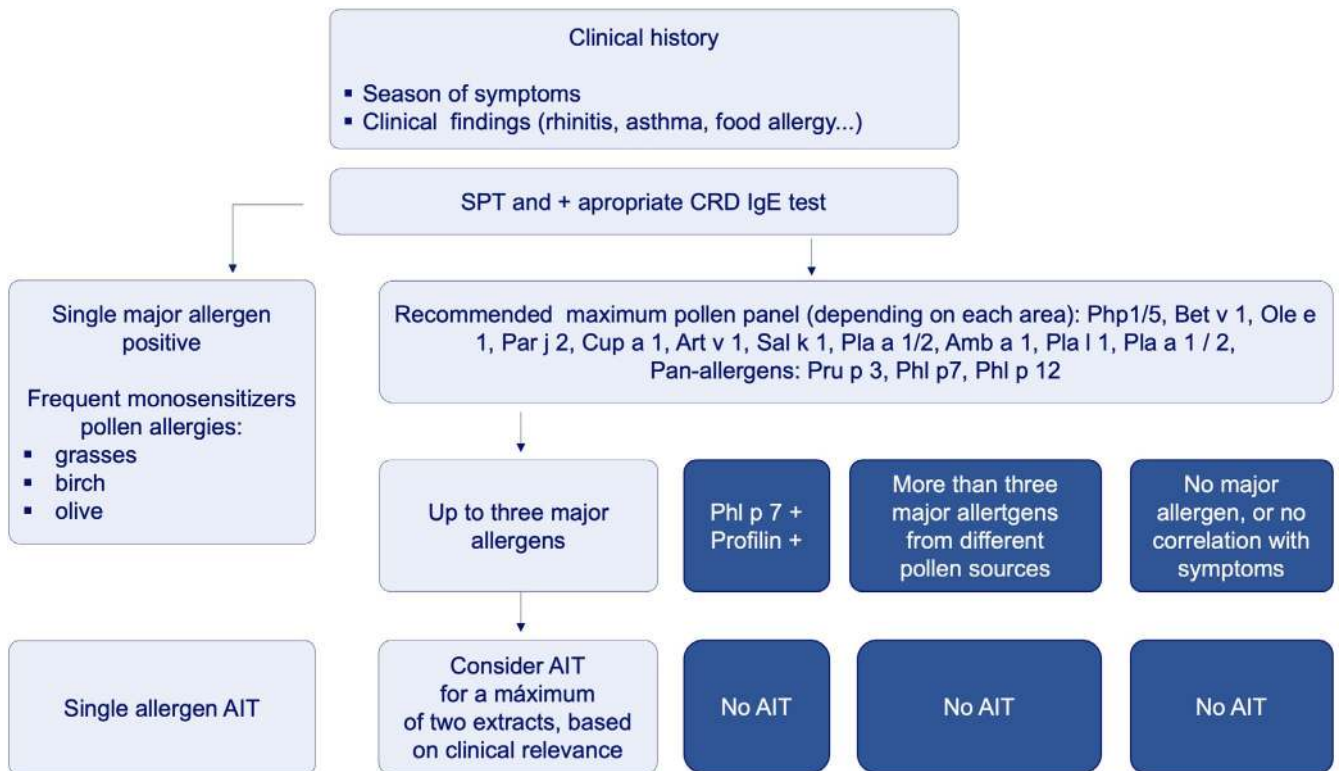


FIGURE 4 Diagnostic algorithm for in vitro diagnostics and Decision tree for AIT using CRD in pollen allergies. Discrimination of primary sensitizers and pan-allergens positivity is needed. AIT guidelines do not recommend AIT for poly-sensitized patients

There is only one AIT product^{56,57} with clinically documented efficacy. The recent registration of a Japanese cedar AIT product in Japan opens the possibility—as in the case of birch/oak AIT—to explore its value for treating cedar/cypress allergy worldwide.

2.6 | Ragweed pollen allergy

Ragweed allergy dominates weed pollen allergy in North America and, after the accidental introduction of ragweed in Europe about 100 years ago, is in continuous progression on the continent.

Major allergen Amb a 1, a pectate lyase, is a true marker of ragweed allergy and presents low cross-reactivity with related proteins from other pollen sources.⁵⁸ However, there is an extensive cross-reactivity among different *Ambrosia* species.⁵⁹ Currently, there are AIT products extensively documented in adults⁶⁰ both in American and European patients as well as in children⁶¹ and these products are available worldwide for AIT practice.

2.7 | Other pollen allergies

There is a limited clinical evidence of AIT benefit in the treatment of other pollen allergies. However, data on major allergens can be extrapolated from studies on existing registered pollen AIT products. Major specific allergens available for diagnosis include the following: Art v 1 (*Artemisia artemisifolia*), Salk k 1 (*Salsola Kali*), Pla l 1 (*Plantago lanceolata*), and Pla a 1 (*Platanus acerifolia*).⁵⁸

Figure 4 represents an algorithm to support decision-making in the selection of AIT in multiple pollen allergies.

2.8 | Cat and dog allergies

In general, the severity and progression of cat and dog allergies involve IgE recognition of a progressively increasing number of components from the sensitizing allergen source (molecular spreading).^{30,62,63,64,65}

Despite the fact that AIT with animal-derived extracts is clinically rarely indicated particularly due to risks in poorly controlled asthmatic patients, the availability of CRD for different cat and dog allergens has also raised the possibility of more precisely targeted interventions. CRD may distinguish primary sensitization from cross-sensitization, thereby enabling selection of the primary sensitizing allergen source for therapy.

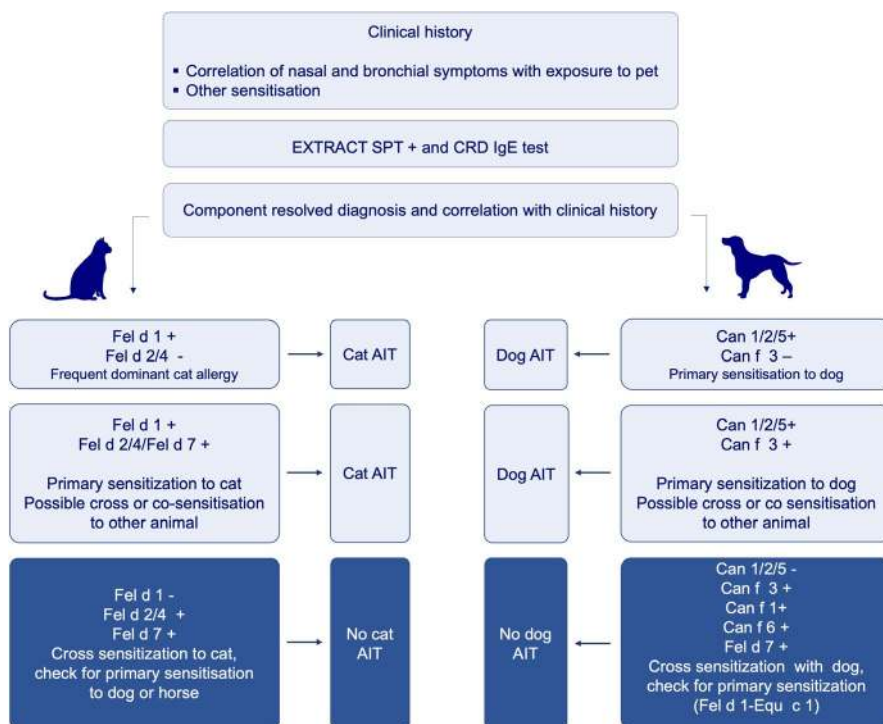
In a recent, comprehensive study,⁶⁶ including most of the components available, the pattern of IgE sensitization to cat allergens showed that 92% of cat-allergic patients had positive IgE antibodies results to Fel d 1. Other allergens also seem important, such as Fel d 4 and Fel d 7. Previous studies reported similar results.⁶⁷ The content of Fel d 1 in allergenic extracts varies substantially. Nevertheless, it has been demonstrated that the maintenance efficacy dose of Fel d 1 is 15 µg/ml.⁶⁸

In the same study, in patients with dog allergy, 52.4% were positive to Can f 1, and 57.2% to Can f 5. The most frequent monomolecular sensitization was to Can f 5.

The content of different dog allergen molecules in European AIT extracts has been recently studied.⁶⁹ These authors demonstrated great variability in extracts from five companies and scarce content of major allergens.⁷⁰

Based on limited evidence, AIT with cat extracts yield better clinical results than with dog ones. The higher complexity of dog allergy

FIGURE 5 Diagnostic algorithm for in vitro diagnostics in epithelia allergy and associated decision tree for AIT using CRD. The proposed algorithm discriminates between primary sensitization and cross-reactive sIgE response. Only patients sensitized to major allergens should be eligible for AIT



sensitization patterns, the lack of preparations with an adequate balance of major allergens is likely to explain this divergence.^{66,71,72}

Further studies are needed to determine whether CRD could be used to identify patients who are most likely to respond to AIT specially in dog allergy.

Figure 5 summarizes decision-making algorithms in cat and dog allergy AIT selection.

2.9 | Mite allergy

Mite major allergens Der p 1/Der f 1 and Der p 2/Der f 2 sensitize the vast majority of mite-allergic patients, being the double sensitization to groups 1 and 2 strongly associated with asthma.³² Group 1 sensitization is more prevalent in children than in adults, suggesting an initiation role probably associated with its proteolytic properties.⁷³ Recently, Der p 23 has been described to be associated with increased asthma risk.⁷⁴ Other mite species, known as minor or storage mites, have a limited allergenic cross-reactivity with major mites. Interestingly, they display a significant cross-reactivity between them. Lep d 2 could be a good marker for minor storage allergy sensitization.⁷⁵ Some patients exposed to high mite allergen levels, and sensitized to storage mites, develop anaphylactic reactions when exposed to foods contaminated with mites.⁷⁶ Interestingly, these patients present NSAIDs intolerance, and recently, they have been described to present extensive oral mucosa barrier damage.⁸

A post hoc analysis of a study with AIT with *D. pteronysinus* and *D. farinae* found no association between the clinical efficacy and sensitization to different mite allergens.⁷⁷

Recently, the antibody response to a SCIT preparation for mite allergy has been examined at the molecular level in 24 mite-allergic patients.⁷⁸ A protective IgG response has been observed for Der p 1 and Der p 2 and, to a lesser extent, Der p 23. By contrast, patients did not develop a strong response to other mite allergens. Interestingly, a better clinical response to the AIT was observed in patients sensitized only to Der p 1 and/or Der p 2, when compared to patients with a broader IgE response.⁷⁸ This suggests that the molecular profile of IgE sensitization may be useful in selecting patients that may benefit from subcutaneous AIT,⁷⁸ as previously hypothesized.⁷⁹ However, no studies are available so far to ascertain whether this conclusion can be applied also to sublingual immunotherapy.

2.10 | Hymenoptera venom allergy

Apis mellifera or honeybee venom (HBV) is the best-characterized Hymenoptera venom due to the outstanding importance of this species as elicitor of venom allergy worldwide. Detailed genomic information and proteomic data of the pure venom are available.⁸⁰⁻⁸² So far, 12 allergens of HBV are identified.⁸³ Only two of them present substantial amounts in the venom, the major allergen phospholipase A2 (Api m 1)(12%) and the minor peptidic allergen melittin (Api m

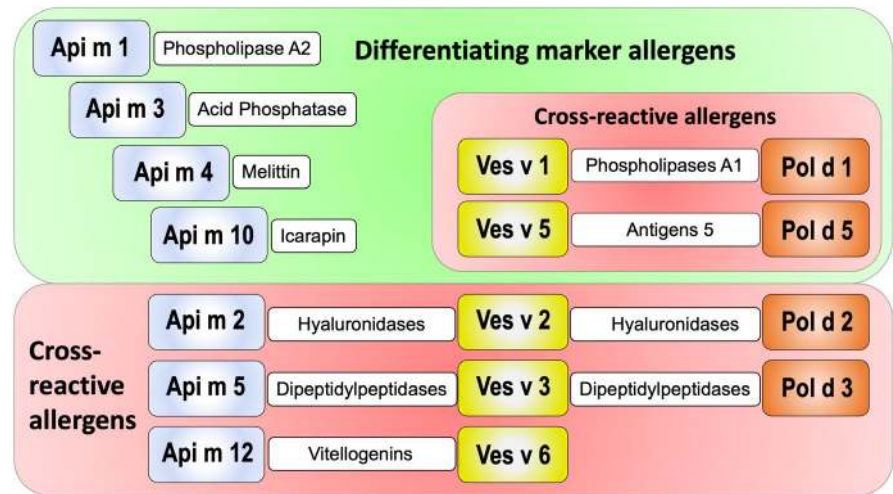
4)(50%).⁸⁴ Despite their lower abundance in HBV compared to Api m 1, a role as relevant major allergen was also confirmed for Api m 2 (hyaluronidase), Api m 3 (acid phosphatase), Api m 5 (dipeptidylpeptidase IV), and Api m 10 (icarapin) with sIgE reactivity in the range of 47.9%–52.2%, 49.6%–50%, 58.3%–61.7%, and 61.8%–72.2% of allergic patients' sera, respectively.^{85,86} For the other HBV allergens, less information about sensitization rates is available.⁸⁷⁻⁸⁹ Some HBV allergens have been identified as risk markers of more severe clinical phenotypes such as Api m 4 or of venom immunotherapy (VIT) failure such as Api m 10.^{85,90}

Prominent *Vesputa* spp. (known as yellow jackets in the USA) allergens include phospholipase A1 (Ves v 1), hyaluronidase (Ves v 2.0101), and antigen 5 (Ves v 5).^{91,92} A second hyaluronidase (Ves v 2.0201), carrying an inactivation mutation in the active site of the enzyme, was identified, which seems to be the predominant isoform.^{93,94} Yellow jacket venom (YJV) also contains a dipeptidylpeptidase IV (Ves v 3) which shows high homology to HBV Api m 5.⁹⁵ The sensitization rates of YJV-allergic patients to Ves v 1, Ves v 3, and Ves v 5 are 33.3%–54%,⁹⁶⁻¹⁰¹ 50%–62.8%,^{95,96} and 84.5%–100%, respectively.^{96,98,99,101,102,103} 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.¹⁰⁴

The allergen composition of *Polistes dominula* venom (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%,¹⁰⁵ 66.7%,¹⁰⁶ and 69%–72%,¹⁰⁵ respectively.

CRD is helpful to discriminate between genuine double sensitization and cross-reactivity, allowing physicians to optimize patient selection for VIT. The potential of CRD becomes evident by the fact that HBV and YJV in addition to homologous allergens also contain differentiating marker allergens that are present in either HBV or YJV. This is not the case when differential diagnosis between *Vesputa* spp. and *Polistes* spp. is required (Figure 6). CRD is also helpful in identifying patients with Hymenoptera venom-induced anaphylaxis having negative test results to whole venom extracts, as it can be the case in hymenoptera venom-allergic patients with mast cell disorders.¹⁰⁷ Currently available allergens of honeybee and vespid venoms (see Table 2) allow a molecular diagnosis in the vast majority of patients, but not in 100% of them. 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 *Vesputa* spp. venom, in order to prevent unnecessary double VIT (Figure 7A). 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 Figure 7B. Of note, the same algorithm using the corresponding PDV allergens can also be applied to discriminate between HBV and PDV allergy.

FIGURE 6 Established discriminating 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



2.11 | Peanut allergy

A product for oral immunotherapy (OIT) to peanut has been recently licensed by FDA and EMA and will soon become widely available.^{108,109} Other products and routes of administration have been explored.¹¹⁰

Peanut allergic reactions can be triggered by storage proteins, such as Ara h 1, Ara h 2, Ara h 3, and Ara h 6,¹¹¹ which have been associated to life-threatening symptoms, as well as by cross-reactive allergens, such as Ara h 5,¹¹² a profilin associated to grass pollen allergy, Ara h 8, belonging to PR10 family and associated to birch pollinosis and Ara h 9, a nsLTP, associated to the LTP syndrome discussed earlier (Table 1). Symptoms elicited by Ara h 5 and Ara h 8 are usually mild and limited to the oral cavity, the oropharynx, known as oral allergy syndrome. Ara h 2 sIgE levels are associated to both severity and threshold of allergic reactions during oral provocation challenges, thus being a good biomarker for severity.^{113,114} In fact, Ara h 2 is the dominant 2S globulin allergen.¹¹⁵ As storage proteins can trigger systemic food allergic reactions and anaphylaxis, peanut allergy with confirmed sensitization to seed storage proteins like Ara h 1, Ara h 2 and Ara h 6 can constitute an indication for POIT to peanut. A sIgE determination to a combination of the three allergens might constitute an economic diagnostic tool to assess anaphylaxis risk.

A summary of the decision process to select POIT is shown in Figure 8.

3 | CRD AND AIT SAFETY

In general, there are limited and heterogeneous evidences associating risk factors for adverse events during AIT and particular IgE sensitization profiles. The most studied model is grass pollen allergy. Adverse reactions with subcutaneous immunotherapy, both local and systemic, correlate with sensitization progression (Phl p 1 + 5 + 12 > Phl p 1 + 5 > Phl p 1/5).¹¹⁶ In sublingual AIT, adverse events were also related with highest sIgE levels for Phl p 5 or Phl p 1²⁷ and with allergen sensitivity.¹¹⁷ Under extreme exposure to grass pollen, patients are frequently sensitized to profilin and present

severe adverse reactions to foods caused by profilin. Interestingly, this type of these reactions to profilin-containing foods is similar to the infrequent reactions observed after application of SLIT. This rare phenotype constitutes therefore a unique model to understand disease severity and limits for AIT. Profilin severe reactors undergo extensive oral mucosa damage and present a unique systemic metabolic status that points to T-cell proliferation, sustained inflammation, and altered repair function. The fact that profilin-sensitized patients in Spain present an enhanced T-cell proliferation compared with similar patients from Denmark suggests that progression to severe phenotypes might be linked to an uncontrolled inflammation and T-cell proliferation. Interestingly, in the other severity models previously mentioned systemic barrier damage, uncontrolled effector cell response, and altered repair-associated biomarkers have been described. All cases might be explained by T-cell cross-reactive allergens: Profilin, Ole e 7 (a nsLTP from pollen) and Lep d 2 (a frequent food contaminant inducing reactions in patients with storage mite sensitization).

4 | FUTURE DIRECTIONS

Accurate diagnosis is essential before AIT can be considered. The routine use of molecular diagnosis for allergic diseases and AIT, theorized in the late 1990s by Rudolf Valenta and colleagues,¹¹⁸ is relatively recent and is still evolving. Constraints include the perceived complexity of this diagnostic approach and the slightly higher costs of test execution.¹¹⁹⁻¹²¹ The recent development of a novel multiplex test containing both extracts and molecules relevant for pollen allergy (a sort of "molecular pollen test") may help doctors in the prescription of the appropriate AIT products.¹²² The development of algorithms and clinical decision support systems integrated into apps for smartphones¹²³ will facilitate the clinical interpretation of the outcome of IgE molecular assays, as shown in a recent pilot experience (Arasi, S et al. Clin Exp Allergy, in press). Different estimates confirm that CRD has a significant impact on AIT formula selection.¹²⁴⁻¹²⁶

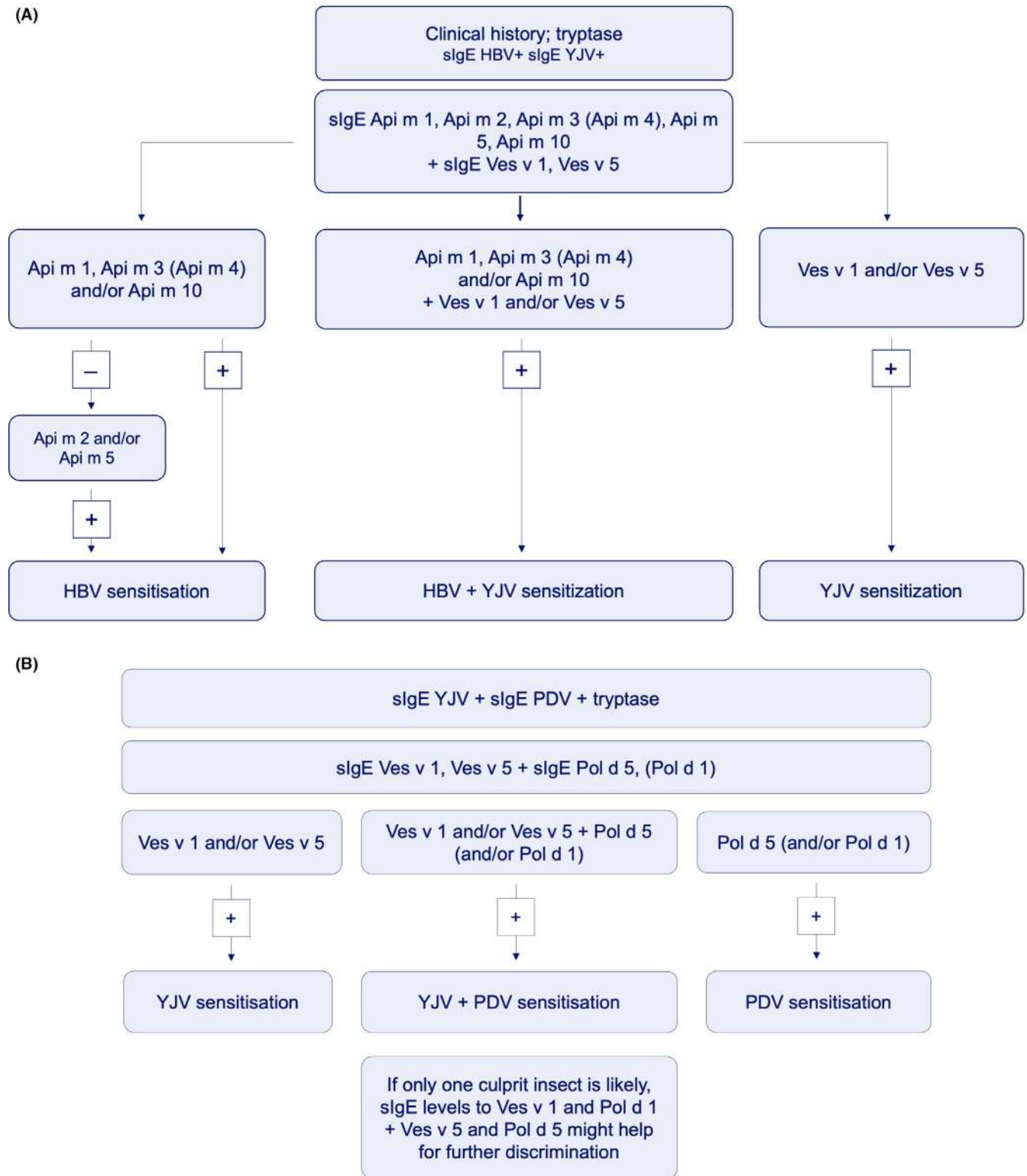


FIGURE 7 Diagnostic algorithm for in vitro diagnostics of (A) HBV and YJV allergy and (B) YJV and PDV allergy. The diagnostic algorithm shown in (A), with the corresponding PDV allergens Pol d 1 and Pol d 5, can also be used to discriminate between HBV and PDV allergy. In addition, to discriminate in 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 results do not necessarily exclude allergy to vespine venom

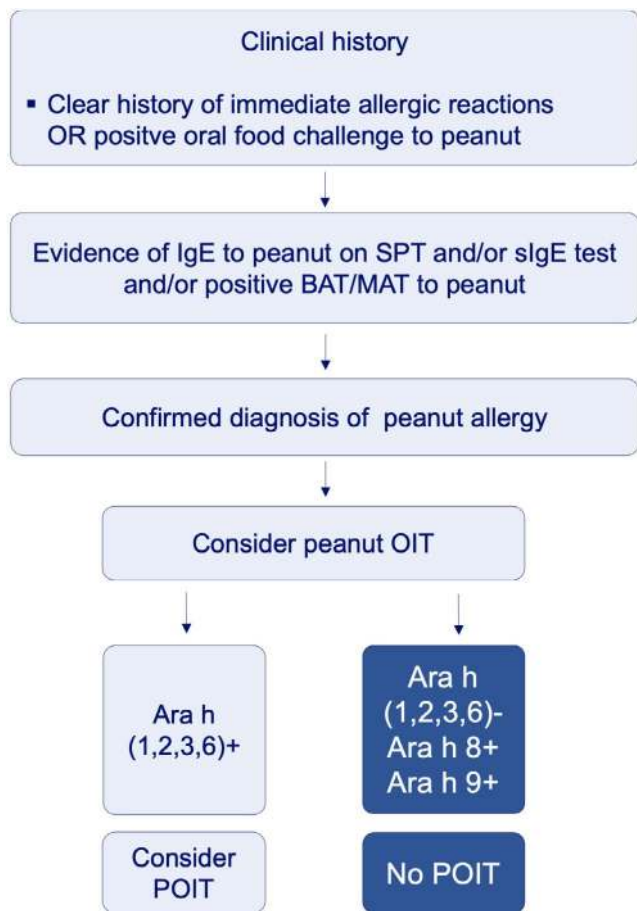


FIGURE 8 Diagnostic algorithm for in vitro diagnostics in peanut allergy oriented to make POIT decision. Discriminant analysis between storage allergens and cross-reactive to pollen and nsLTPs is proposed. Only patients with positive sIgE to any of the Ara h 1, 2, 3, 6 should be eligible for POIT

To rely on correctly standardized and clinically documented AIT products, as well as to understand their limitations, is as important as correctly diagnosing patients.

With an increasing number of new intervention possibilities, it is essential to optimize the use of AIT.^{58,127,128} Before considering AIT, patients should be sensitized to major allergens demonstrated by CRD and should be treated with one or two non-cross-reactive allergen preparations. In fact, even in complex exposure regions, most of the patients are sensitized to a limited number of allergens¹²⁹ and thus potentially eligible for AIT. An adequate dose of allergen as defined in dose-finding clinical trials will guarantee an adequate safety/efficacy balance. In the last years, an increasing number of allergen preparations developed with the highest pharmacological standards became available. This trend will continue and is expected to have a profound impact on AIT practice and to position it in the center of etiological management of allergic patients. In spite of correct diagnosis and the use of high-quality AIT products, a fraction of treated patients will not improve or will lose clinical benefit upon discontinuation after 3 years of intervention.¹³ The need to advance on

personalized medicine approaches to predict intervention outcome and safety—and to monitor AIT effect—is imperative.¹³⁰ System biology approaches are being explored to understand AIT response.⁵ These new approaches might help to understand biological effect kinetics and will provide new tools to evaluate new AIT product approaches. The desensitization of allergen-specific effector cells plays a determinant role for AIT effect during the first 2 years of intervention, while regulatory response, which is initiated shortly after AIT initiation, will only have a meaningful clinical benefit after at least 2/3 years of administration. We need better formulas, including adjuvants and tolerogenic signaling molecules to reduce AIT duration.⁶ At the same time, understanding severe phenotypes is needed. Recent data obtained by system biology approaches identify T-cell proliferation, inadequate regulatory function, and collapse of repair homeostasis as main causes for non-response to intervention and evolution to severe phenotypes.^{7,8,9,131} There are new possibilities of using these systemic signatures to explore the value of new biologics to stabilize severe phenotypes allowing ulterior AIT intervention.







While CRD has proved the value for correct patient inclusion and AIT standardization, we cannot expect that it will be of great value to predict intervention outcome or safety.⁷⁷ AIT is the only treatment in allergy able to modify the course of the disease, but for achieving this goal, the correct patients, with the correct product, with adequate treatment duration and compliance are needed. Even so, about 30% of patients will not benefit from this disease-modifying effect. This 70% effect,¹³ however, ranks at the top of successful pharmacological interventions. In 2003, Dr. Allen Roses, by that time vice president of the genetics division for GSK, commented that “The vast majority of medicines, more than 90 percent, only work in 30 or 50 percent of people.” Since then, massive efforts on personalized medicine are progressively changing the landscape. AIT has the potential of being the reference intervention in allergy, but for that, we need a commitment and upgrade of its clinical practice. The use of molecular allergology for diagnostic purposes will rapidly evolve in the next years and will be more linked to the identification of patients phenotypes and endotypes for an improved therapeutic approach, which implies the opening of the era of “precision allergology”.^{120,121} CRD, today, provides a good starting point.

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