



Pro/Con Debates

Component resolved diagnostic testing adds clinical utility over existing testing for food allergy-PRO



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Allergies are increasing in prevalence and have become a global concern. The traditional diagnosis of an allergic disorder is based on a positive clinical history and a combination of positive skin testing or specific immunoglobulin E (IgE) to a commercial whole allergen extract; these testing modalities, however, present certain limitations. Allergen extracts are aqueous extracts of the crude allergen source containing many components, most of which are irrelevant for clinical allergy diagnosis. Therefore, a positive result to the crude allergenic extract confirms allergic sensitization, rather than presence of clinical symptoms or clinical allergy. Crude commercial extracts also present an inherent variability in composition, containing varying amounts of different allergenic and non-allergenic components.¹ In addition, despite their high sensitivity, both skin prick testing (SPT) and specific IgE traditional testing exhibit low specificity, and false positive results often occur. There is a clear need for newer diagnostic tools with improved specificity, which has led to the development of molecular diagnostics.

Molecular diagnostics or component resolved diagnostics (CRDs) involve the use of single allergenic molecules (components) instead of the crude extract. Allergen components can be classified as belonging to different protein families based on their structure and function, and IgE antibodies directed at allergenic proteins in the same family often show cross-reactivity.² Different protein families include storage proteins, pathogenesis-related protein family 10 proteins (PR-10), profilins, nonspecific lipid transfer proteins (nsLTP), cross-reactive carbohydrate determinants (CCDs), calcium binding proteins, serum albumins, parvalbumins, tropomyosins, and lipocalins.^{1,2} The examination of allergen sensitization at a molecular level offers certain advantages over traditional skin

prick and specific IgE testing. Generally, CRDs offer a higher diagnostic precision (high specificity and positive predictive value) and allow for better patient management in clinical practice. The benefits of CRDs are seen in the diagnosis of a wide range of allergic disorders, as discussed in the following sections.

For suspected food allergy, in situations where a) the patient is sensitized without a history of clinical reaction, b) skin and serum allergen tests are discrepant, or c) skin and serum tests are positive, but the patient may be tolerant, the only available option would be an oral food challenge. However, oral food challenges are time-consuming, labor-intensive, and costly, and they may put the patient at risk of a severe allergic reaction, including anaphylaxis. Component resolved diagnostics can help differentiate between cross-reactive sensitization and true food allergy (Table 1). For example, specific IgE to Ara h 2 (a seed storage protein and major peanut allergen) appears to be the best predictor of clinical peanut allergy, reducing the need for oral peanut challenges by at least 50%.³ A recent systematic review concluded that, compared with the currently used SPT and IgE to peanut extract, specific IgE to Ara h 2 was superior in diagnosing peanut allergy, especially in children.⁴ For soy, specific immunoglobulin E to Gly m 2S albumin was shown to have the best accuracy in diagnosing soy allergy in adults.⁵ In wheat allergy, a positive result to wheat flour extract often does not correlate with clinical allergy but is merely an indication of cross-reactivity with grass. In contrast, specific IgE to Tri a gliadin (a seed storage protein and member of the cereal prolamin family) indicates primary wheat allergy with low risk of pollen cross-reactivity. In children, specific IgE to Tri a 19 (omega 5 gliadin) is also associated with primary wheat allergy and immediate allergic reactions.² When considering the diagnosis of shrimp allergy, significant cross-reactivity is reported between dust mites and crustaceans, but sensitization to tropomyosin (Pen a 1, a major heat-stable allergen present in all shellfish species) is a good marker for clinical allergy to crustaceans rather than mites. A recent study revealed that in terms of diagnostic efficiency, measurement of IgE to shrimp tropomyosin was

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Table 1
Components and Their Clinical Relevance in Food Allergy, Venom Allergy, Allergic Rhinitis, and Pet Allergy

Components		Clinical relevance for diagnosis	Clinical relevance for severity of allergic reactions, persistent allergy, or other clinical associations		
Food allergy	Peanut	Ara h 2	Major allergen Best predictor of clinical allergy Clinical peanut allergy (low risk of cross-reactivity)	Higher risk of severe allergic reactions	
		Ara h 1, Ara h 3 Ara h 8		Higher risk of severe allergic reactions Mild allergic reactions (pollen food syndrome)	
	Hazelnut	Cor a 9, Cor a 14	Clinical hazelnut allergy (low risk of cross-reactivity)	Higher risk of severe allergic reactions	
		Soy	Gly m 2S Gly m 5, Gly m 6	Best predictor of clinical allergy in adults Clinical soy allergy (low risk of cross-reactivity)	Higher risk of severe allergic reactions
	Wheat	Tri a gliadin	Primary wheat allergy (low risk of pollen cross-reactivity)		
		Tri a 19 (ω - 5 -gliadin)	Primary wheat allergy (low risk of pollen cross-reactivity)	Higher risk of severe allergic reactions	
	Milk	Bos d 8 (casein) Bos d 5 (β -lactoglobulin)		Persistent milk allergy Decreased probability of heated milk tolerance Higher risk of severe allergic reactions	
		Egg	Gal d 1 (ovomucoid)	Persistent egg allergy Decreased probability of heated egg tolerance	
	Shrimp	Pen a 1 (Tropomyosin)	Clinical shrimp allergy (low risk of cross-reactivity with mites)		
	Venom allergy	Bee	Api m 1 (bee venom phospholipase)	Major allergen (low risk of cross-reactivity)	Higher risk of systemic reactions during bee venom IT
			Api m 4 (melittin)	Major allergen (low risk of cross-reactivity)	Higher risk of systemic reactions during bee venom IT
Allergic rhinitis	Wasp	Ves v 5 (antigen 5 for vespula venom)	Major allergen (low risk of cross-reactivity)		
	Grass	Phl p 1, Phl 5 & Phl 12		Higher risk of local and systemic reactions during IT	
		Phl p 1, Phl 5	Major allergens (low risk of cross-reactivity)		
Pet allergy		Can f 1 Can f 2 Can f 3 Can f 5		Persistent rhinitis Asthma Moderate/severe rhinitis Asthma	
			Major allergen (low risk of cross-reactivity)	Persistent and moderate/severe rhinitis	

superior to measurement of IgE to shrimp and skin prick testing (88.5%, 74.2%, and 65.7%, respectively).⁶

Component-resolved diagnostics also can be used to stratify the risk of severe food-allergic reactions (Table 1). Seed storage proteins for peanut, hazelnut, and soya (Ara h 1, Ara h 2, and Ara h 3 for peanut, Cor a 9 and Cor a 14 for hazelnut, Gly m 5 and Gly m 6 for soya) have been associated with a higher risk of severe allergic reactions.¹ Sensitization to multiple components (compared with sensitization to a single component) has also been associated with a higher risk of severe allergic reactions. In contrast, Bet v 1 homologues are considered markers of mild allergic reactions to fruits and vegetables because of cross-reactivity with birch pollen. The PR-10 and profilins are labile allergens, also associated with mild oral allergic reactions (also known as “oral allergy syndrome” or “pollen food syndrome”), for example, Ara h 8 for peanut.¹

Another important contribution of CRDs is the assessment of the natural course of food allergy, that is, providing information on whether the allergy is likely to be outgrown or remain persistent (Table 1). In milk- and egg-allergic children, studies have shown that most may exhibit tolerance to heated milk and egg, which is considered the first step toward tolerance development. Specifically, for milk allergy, casein (Bos d 8), β -lactoglobulin (Bos d 5), and α -lactalbumin (Bos d 4) have been identified as major allergens. Children reacting to heated milk (with decreased likelihood of tolerance development) have been reported to have higher levels of casein and β -lactoglobulin IgE. These children are also shown to be at higher risk of systemic reactions.² In egg allergy, Gal d 1 (ovomucoid) has been reported as a dominant allergen, stable against heat and digestion by proteases, despite representing only 10% of the total egg white protein. High levels of IgE antibodies to Gal d 1

have been associated with persistent egg allergy and a decreased probability of cooked/heated egg tolerance.² The ability to identify different phenotypes (transient vs persistent) of milk and egg allergy is an important contribution of CRDs, because it guides the decision-making on whether an oral food challenge to heated milk or egg would be beneficial.

In clinical practice, CRD for certain food allergens may be used instead of testing with whole allergens. Interpretation of results would require knowledge of cutoff diagnostic values that have been proposed for these components. For example, in children with peanut allergy, a cutoff point of equal or >5 kU/L for specific IgE to Ara h 2 demonstrated a PPV of 96% and an NPV of 71% and an AUC of 0.90.³ For hazelnut allergy, IgE sensitization to Cor a 14 was shown to be the best predictor of clinical allergy in children; the optimal cutoff level was identified as 0.72 kU/L, resulting in a sensitivity of 0.80 and a specificity of 0.92 (AUC was 0.85).⁷ In sesame allergy, the optimal cutoff for rSes I 1 was reported as 3.96 kUA/L, with a sensitivity and specificity of 86.1% and 85.7%, respectively.⁸ For other food allergens such as egg white, CRD may be used in addition to whole allergen testing to provide more information. Research has shown that specific IgE to egg white performed better in the diagnosis of raw egg allergy, but for reactions to baked egg, levels of specific IgE antibodies to ovomucoid were superior (positive decision point for an OFC was reported as 10.8 kUA/L and negative decision point as 1.2 kUA/L). Therefore, the combination of these testing modalities would allow for better decision making in patients with egg allergy.⁹ The cutoff values for CRD in food allergy may vary in different populations.

Idiopathic anaphylaxis is another important area of practice that can be improved with the use of molecular diagnostics. A recent study

performed in the United Kingdom included patients with episodes of anaphylaxis with no identifiable cause and reported new allergenic sensitizations in 50% of patients studied. In 20% of these cases, a cause of anaphylaxis was identified, with omega-5-gliadin and shrimp allergens accounting for 45% of the previously unrecognized sensitizations.¹⁰ In clinical practice, significant benefit is found in identifying the triggering allergen; it allows the consulting physician to implement avoidance measures and provide personalized education and a management plan. Such diagnoses may be missed with traditional testing, because certain molecules are poorly represented in crude allergen extracts. The use of immuno solid-phase allergen chip can be considered in cases of idiopathic anaphylaxis to provide additional information on potentially unrecognized sensitizations. However, any detected new sensitizations would require further evaluation, including food allergen challenges, to confirm that the identified allergen is clinically relevant.

In venom allergy, false-positive SPT and specific IgE results can occur because of cross-reactive carbohydrates (CCDs) contained in traditional commercial extracts. Cross-reactive carbohydrates are carbohydrate molecules attached to proteins, present in all plants and some insects and mites. Several major allergens (mostly glycoproteins) have been identified in the venom of bees and vespids. With the use of molecular diagnostics, one may differentiate between true venom allergy and cross-reactivity resulting from presence of CCDs (Table 1). For example, Api m 1 (bee venom phospholipase) and Ves v 5 (antigen 5 for vesperula venom) are both free from CCDs; therefore, sensitization to these likely represents true venom allergy.¹¹ Double positivity to yellow jacket and honey bee when using whole venoms for testing may be addressed with the use of recombinant Ves v 1 and Ves v 5. Components can therefore clarify situations in which traditional testing cannot discriminate between clinical venom allergy and cross-reactivity. An additional benefit of component testing in venom allergy includes guidance in appropriate selection of patients and extracts for allergen immunotherapy (Table 1). In case of patients with selected recognition of cross-reactive allergens only, the role of allergen immunotherapy is arguable.¹¹ Safety of immunotherapy has been reported to be improved with the use of components, because certain sensitization patterns are associated with systemic reactions during allergen immunotherapy. Patients with levels of specific IgE to Api m 4 greater than 0.1 kU/L and Api m 1 greater than 20 kU/L were shown to have a higher risk of systemic reactions during bee venom IT. Additionally, the risk of a grade III systemic reaction was 7.5 times higher in patients with an nApi m 1 level greater than 50 kU/L and 8 times higher for patients with an rApi m 2 level greater than 10 kU/L.¹²

For inhalant immunotherapy, components add significant value to clinical practice by offering a “personalized” immunotherapy treatment plan, especially in polysensitized patients. Most commercial extracts for inhalant immunotherapy are standardized for major allergens only. The SPT or specific IgE positivity to crude extract is unable to differentiate between sensitization to major or minor allergens. As a result, patients sensitized to minor allergens will be unlikely to reap any benefit from immunotherapy if the minor allergen(s) to which they are reactive are not represented in adequate quantities in the extract used.¹¹ In polysensitized patients, research studies have shown that the use of molecular diagnostics improves selection of IT mixes. Specifically, more than 40% of IT prescriptions were changed after considering CRD results in a study of 651 Italian children.¹³ The investigators reported that extracts from pollen sources contain panallergens, such as profilins, polcalcins, and lipid transfer proteins, which are highly cross-reactive and responsible for skin test reactions to many pollen sources with widely overlapping seasons. Therefore, clinical history and SPT

responses with extracts are not sufficient to discriminate the pollens responsible for eliciting symptoms.¹³ When measuring levels of IgE to major allergens in clinical practice, the clinician can decide which allergenic molecules are relevant for use in the IT mix for each individual patient. Additionally, sensitization to multiple inhalant allergen components or high levels of specific IgE to certain components may predict adverse reactions to pollen immunotherapy (Table 1). For instance, sensitization to a higher number of grass allergens and sensitization to Phl p 1, Phl 5, and Phl 12 were associated with both local and systemic reactions during the build-up phase of SCIT in a recent trial.¹⁴ In clinical practice, when selecting patients for SCIT, a slower up-dosing protocol may be preferable in those at higher risk of systemic reactions, as identified by component testing.

For pet allergy, polysensitization to different animals (cat, dog, and horse) can occur without clinical significance and may be attributable to cross-reactive lipocalins and albumins in the animal dander (Table 1). High variability is also seen between commercial dog extracts with good representation of the major dog allergen (Can f 5), but little and rather heterogeneous representation of the minor allergens (Can f 1, Can f 2, Can f 3).¹¹ When considering immunotherapy for dog allergy, ascertaining that the patient is sensitized to the major allergen is important. Additionally, molecular diagnostics may predict clinical symptoms and severity of reactions in pet-allergic patients. A recent study of over 150 patients with rhinitis/asthma and sensitization to dog, cat, and horse revealed that Can f 1 was significantly associated with persistent rhinitis, Can f 2 with asthma, Can f 3 with moderate/severe rhinitis and asthma, and Can f 5 with persistent and moderate/severe rhinitis. Sensitization to ≥ 2 molecules or to pet albumins was associated with more severe respiratory symptoms¹⁵ (Table 1).

In conclusion, molecular allergology is a fast-growing area of intense research and clinical interest. Components offer significant benefits over traditional testing modalities and are fast becoming a promising diagnostic tool in clinical practice. They enable the clinician to differentiate between true clinical allergy and nonrelevant sensitization/cross-reactivity, limiting the number of OFCs that need to be performed. Information from molecular diagnostic testing facilitates identification of appropriate allergens for immunotherapy and also can contribute to better safety profiles of both venom and inhalant immunotherapy. Finally, components can predict persistence, clinical reaction phenotype, and severity of food allergy in selected populations and may act as biomarkers for both patient follow-up and treatment progress. Overall, CRDs have a prominent role in precision medicine and personalized patient treatment.

References

- Matricardi PM, Kleine-Tebbe J, Hoffmann HJ, et al. EAACI molecular allergology user's guide. *Pediatr Allergy Immunol*. 2016;27(Suppl 2):1–250.
- Sastre J. Molecular diagnosis in allergy. *Clin Exp Allergy*. 2010;40(10):1442–1460.
- Klemans RJB, Otte D, Knol M, et al. The diagnostic value of specific IgE to Ara h 2 to predict peanut allergy in children is comparable to a validated and updated diagnostic prediction model. *J Allergy Clin Immunol*. 2013;131(1):157–163.
- Klemans RJB, van Os-Medendorp H, Blankestijn M, Bruijnzeel-Koomen CAFM, Knol EF, Knulst AC. Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review. *Clin Exp Allergy*. 2015;45(4):720–730.
- Klemans RJB, Knol EF, Michelsen-Huisman A, et al. Components in soy allergy diagnostics: Gly m 2S albumin has the best diagnostic value in adults. *Allergy*. 2013;68(11):1396–1402.
- Yang AC, Arruda LK, Santos ABR, et al. Measurement of IgE antibodies to shrimp tropomyosin is superior to skin prick testing with commercial extract

- and measurement of IgE to shrimp for predicting clinically relevant allergic reactions after shrimp ingestion. *J Allergy Clin Immunol*. 2010;125(4):872–878.
7. Eller E, Mortz CG, Bindslev-Jensen C. Cor a 14 is the superior serological marker for hazelnut allergy in children, independent of concomitant peanut allergy. *Allergy Eur J Allergy Clin Immunol*. 2016;71(4):556–562.
 8. Maruyama N, Nakagawa T, Ito K, et al. Measurement of specific IgE antibodies to Ses i 1 improves the diagnosis of sesame allergy. *Clin Exp Allergy*. 2016;46(1):163–171.
 9. Ando H, Movérare R, Kondo Y, et al. Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. *J Allergy Clin Immunol*. 2008;122(3):583–588.
 10. Heaps A, Carter S, Selwood C, et al. The utility of the ISAC allergen array in the investigation of idiopathic anaphylaxis. *Clin Exp Immunol*. 2014;177(2):483–490.
 11. Luengo O, Cardona V. Component resolved diagnosis: When should it be used? *Clin Transl Allergy*. 2014;4(1):1–9.
 12. Ruiz B, Serrano P, Verdu M, Morreno C. Sensitization to Api m 1 , Api m 2 , and Api m 4: association with safety of bee venom immunotherapy. *Ann Allergy Asthma Immunol*. 2015;114(4):350–351.
 13. Stringari G, Tripodi S, Caffarelli C, et al. The effect of component-resolved diagnosis on specific immunotherapy prescription in children with hay fever. *J Allergy Clin Immunol*. 2014;134(1):75.
 14. Sastre J, Sastre-Ibañez M. Molecular diagnosis and immunotherapy. *Curr Opin Allergy Clin Immunol*. 2016;16(6):565–570.
 15. Uriarte SA, Sastre J. Clinical relevance of molecular diagnosis in pet allergy. *Allergy Eur J Allergy Clin Immunol*. 2016;71(7):1066–1068.