



Pro/Con Debates

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



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We live in a world with considerable diagnostic uncertainty at times. Diagnostic tests for food allergy have poor specificity and poor ability to discriminate allergic from nonallergic.¹ Sadly, the absence of food-specific immunoglobulin E (IgE) is infinitely more easy to interpret than its presence. Because these tests are so poorly specific, we often cannot explain why some people have elevated specific IgE to foods yet can eat them without developing symptoms. Such imprecision eliminates our present ability to effectively screen anyone.^{1,2} In a perfect world, there would be a singular diagnostic test with highly accurate sensitivity, specificity, and the ability to correctly discriminate an allergic individual from a nonallergic individual, precise enough to be used in someone who has never eaten the allergen before to answer the age-old question of “doctor, what is my child going to be allergic to?” before the food is ingested and this item avoided. A test with such properties could also potentially discriminate a mild vs severe reaction. Although component-resolved diagnostics (CRD) aims to represent a step toward this more perfect test, it falls way short of these noble goals given present data.

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The science behind CRD has been reviewed in great detail elsewhere.³ Briefly, CRD isolates the “parts of the whole” and allows for specific assessment of recognition of key molecular level amino-acid sequences within an allergen, called the allergenic components. Using peanut as an example, peanut has multiple distinct allergenic proteins, each of which can bind IgE, but not all parts that become bound necessarily have the same clinical symptomatology associated with such recognition. For example, components such as Ara h 1, 2, 3, and 6 represent distinct seed storage proteins (2S and 7S globulins), whereas Ara h 8 is a PR-10–related family member and Ara h 9 is a lipid transfer protein.^{3,4} Proponents of the value of peanut CRD cite reported associations between peanut CRD binding patterns and so-called reaction phenotypes, mainly that binding of Ara h 1, 2, 3, or 6 results in traditional symptoms of an IgE-mediated allergy, whereas binding Ara h 8 is associated with symptoms typical of pollen food allergy syndrome, which are felt to be “milder” and Ara h 9 associated with allergic rhinitis unless in lipid transfer protein-endemic areas, where this acts similar to Ara h 1-3,6.^{5–7} Indeed, multiple publications have made such claims that this clinical phenotyping can tell who has a so-called “anaphylactic” vs “oral allergy syndrome” phenotype. Although this would be a fantastic property to be able to use, it lacks both prospective validation and validation at a population level, given studies performed exclusively in enriched cohorts at referral centers. Worse, some studies have perpetuated a belief that Ara h 2 recognition is associated with “severe” reactivity, despite such studies having the aforementioned limitations.⁵

Unfortunately, we are far from such a reality. In fact, the more data from clustered, enriched, and atypical samples denoting properties of components in known allergic populations that are published, the further we actually remain from our understanding of how CRD could help enhance diagnostic specificity.^{5,8} It is easy to be fooled into thinking that such testing, based on the current data that supposedly justify its use, actually aids our clinical decision making. However, what is actually envisioned is using these tests in the situations for which they are not validated, and there is limited

Table 1
Current Weaknesses in the Application of Component Resolved Diagnostic Testing in Clinical Food Allergy Management

Weaknesses	Critical issue(s)	Importance
Patient selection bias	Use of referral centers, cross-sectional cohorts, inconsistency in age ranges studied	With limited exception, most CRD studies do not reflect a representative population, which limits generalizability of their use
Study design	Use of retrospective samples, or banked samples where not all the cohort has serum banked or were not enrolled consecutively	This increases the likelihood of selection bias, which can limit the generalizability of results
Questionable reference standard	The patient outcome is not determined by oral food challenge, and is based on history	This reduces the sensitivity and specificity of the test given that the outcome may not be compared with the standard criterion
Determination of positive and negative predictive values in studies, and derivation of optimal cutoff levels	Positive and negative predictive values are attributes of populations, and not samples. Cutoff levels are also highly dependent on the sample.	- Only 1 CRD study to date has involved a population-level sample. However, many studies cite PPV/NPV. - When a PPV and NPV are calculated from a study that was not sampled at a population level, those PPV/NPV are only applicable to the center or clinic population sampled, and not to the general population. - There is concern making clinical decisions based on cutoffs established in a population that is not representative.
Utility of high test specificity	It is unclear whether a highly specific test is an advantage in the current diagnostic paradigm	- CRD have not been tested as a screening measure, or to differentiate someone who is allergic but has not yet eaten the allergen in question and developed an allergic reaction. - Given the weaknesses of not all CRD studies having been tested against an adequate gold standard for diagnosis, there is uncertainty about how accurate the specificity of CRD actually are. - The utility of a highly specific test vs a moderately specific test when there is good pretest probability of a diagnosis has not yet been shown, though there are advantages if the higher specificity reduces the number of potentially false-positive results.
No ability to infer reaction severity	To date, there is no conclusive evidence that any specific IgE value or CRD value or threshold is clearly associated with a severe vs nonsevere reaction	There is potential danger in clinical decision-making to ascribe a particular reaction phenotype to a particular threshold of sensitization when this concept has not been robustly demonstrated

understanding of when such instruments would be optimally used.^{5,8} The best arguments supporting CRD use revolves around 2 central tenants: 1) a practitioner only truly needs a highly specific test (over a less specific one) when there is inadequate pretest probability of a reaction history that reliably indicates that someone is likely to be allergic to the allergen; and 2) the false-positive result, not the false-negative result, is the biggest problem in food allergy, given that the false positive often results in unnecessary avoidance, preventing discovery of a true outcome, whereas the false-negative result will sort itself out clinically. The issue at hand is that diagnostic tests of higher specificity by definition reduce this likelihood of false positivity and the resulting outcomes.^{9,10} Based on these central arguments, one could presume that CRDs may actually have high clinical relevance. In reality, the practitioner wants neither a false-positive nor a false-negative result. However, in the abstract hypothetical, the least preferred situation is one in which a true outcome is undiscovered. What is desired is a highly accurate test that can indicate whether someone is allergic, and could avert the use of a food challenge to determine an outcome in a less clear situation.¹¹ The issue is the nature of the data supporting their use, and the context of why a particular test is drawn, with the choices being a whole extract skin prick test (SPT), whole extract serum-specific IgE test (sIgE), and a CRD. Simply put, the CRD studies that have been done to date are inadequate to provide such

an answer, and potentially spurious conclusions about what the interpretations of CRDs can infer have been published.^{5,8}

In a typical patient with a relatively unambiguous history of symptomatic ingestion of a particular allergen, the simple SPT or sIgE can sufficiently document sensitization. In this situation, the history is highly suggestive, and the pre-test probability is very high (if not certain) for allergy. Although some test is needed to confirm sensitization, no particular advantage exists for a highly specific test vs a moderately specific test, and the likelihood ratio difference between the tests provides no leverage on the post-test odds.¹⁰ All that such a situation needs is confirmation of allergen-specific IgE, and it does not influence the clinical decision-making or management if that is Ara h 2, ovomucoid, casein, or the whole allergenic extract reflective of all the proteins. Contrast this to the situation in which the history is ambiguous or unclear that the food in question was ingested or that it actually led to symptomatic ingestion, and the pre-test probability is potentially low. Here, the SPT or sIgE test is poorly specific, and given an unclear situation under which this patient is tested, there is some likelihood of a potentially false-positive result.¹⁰ By definition, a highly specific test could significantly reduce the likelihood of a false positive, and for those with a proclivity toward risk aversiveness with respect to oral food challenges, may potentially eliminate the need to perform a confirmatory procedure (which would be the gold standard in this

situation).⁹ If the allergen is peanut, a tree nut, or a seed, being able to determine which specific allergenic protein is recognized (a primary allergen or an allergen reflecting cross-sensitization/reactivity with pollen) could also help clarify what the sensitization pattern may mean, in particular if this individual comes from a birch pollen endemic area and may have reacted to a plant-based food such as a peanut or tree nut.^{12,13} Although this is not to argue against the value of a test that could provide this information in this context, the issue is that such data simply do not exist at present.

Few, if any, CRD studies have been performed outside of already clinically well-characterized populations, where there was any lack of certainty of the known allergy.^{5,8} Thus, these CRD studies simply add additional information to a patient in whom the diagnosis is already clear and well characterized. The patient who develops diffuse urticaria after ingesting peanut is not additionally aided by knowing that they have moderate levels of Ara h 2. However, it is an error of deductive reasoning to suggest that because a group of patients known to develop symptoms after ingesting peanut have sensitization to moderate levels of Ara h 1 and 2 and do not have sensitization to Ara h 8, that Ara h 1 and 2 are therefore exclusively associated with peanut allergy and Ara h 8 is not.⁵ Nonetheless, this is exactly what such studies are attempting to conclude. When clustered samples (such as a single-center cross-sectional cohorts of individuals with known food allergy from large tertiary referral centers) are analyzed, often either retrospectively or selectively in a prospective study, this only tells half the story. The aforementioned litany of such studies has, as a whole, failed to assess for potential component sensitization in those less likely to be allergic, and exhausted any possibility that there is any sensitization to these proteins in such populations.¹⁴ Until this is exhaustively and comprehensively both demonstrated retrospectively and then validated prospectively, no claims or statements about any associations of particular component sensitization for any allergen can be made. These populations represent the “other” side of the equation that is overlooked in robustly assessing the specificity and sensitivity of diagnostic tests—establishing the true negative. Remembering that non-allergic individuals can and often do have elevated sIgE or positive SPTs, whereas CRD is supposed to have higher specificity and (by definition of higher specificity) eliminate the likelihood of a false-positive test; this must actually be proven in large non-allergic samples or at a population level. Therefore, one cannot simply assume that any component signal is going to be absent in those without disease, at least without proving this to be true. Until this is done, there is no reliability to statements that Ara h 2, Ara h 8, ovomucoid, casein, or any of the other CRDs touted to have superior “predictive” abilities can discern allergic from non-allergic or have better clinical utility than standard PST or sIgE.^{1,2}

Two recent systematic reviews using Grading of Recommendations Assessment, Development, and Evaluation methodology have explored CRD in detail. Although these do cast a more favorable light on the potential use of CRD, they highlight significant biases in the studies.^{5,8} Moreover, 1 of these reviews included studies that have not robustly demonstrated outcomes against the criterion standard of a food challenge, providing low-quality

GRADE assessment to their use.⁵ What can be taken away from these studies is that mounting evidence is accumulating that suggests Ara h 2 has higher sensitivity or specificity and the most optimal positive/negative likelihood ratio of any peanut test/component. No evidence suggests that any component denotes severity of a reaction, or that any component can discern a more vs less reactive phenotype, or is conclusively “predictive” of tolerance of extensively heated product. No study has been conducted across multiple ages at a population level including likely and unlikely allergic cases to establish proper sensitivity and specificity, show how these tests truly provide advantage in their use in a clinical management pathway, or show that such use of these tests as a primary or reflexive test is cost-effective.^{5,8} The limitations of CRD studies are summarized in Table 1. I would not argue that CRD testing is not the wave of the future, and potentially possesses properties that can evolve our understanding of food allergy. However, at present, these remain a tool for which the field have not defined a purpose or know how to use, and rigorous future study that is of distinctly different methodology from the current batch of CRD studies is necessary to define the clinical utility of these tests.

References

1. Oria MP, Stallings VA, eds. *Finding a Path to Safety in Food Allergy: Assessment of the Global Burden, Causes, Prevention, Management, and Public Policy*. Washington, DC: National Academies Press; 2016.
2. Sampson HA, Aceves S, Bock SA, et al. Food allergy: a practice parameter update—2014. *J Allergy Clin Immunol*. 2014;134:1016–1025.
3. van Erp FC, Klemans RJ, Meijer Y, van der Ent CK, Knulst AC. Using component-resolved diagnostics in the management of peanut-allergic patients. *Curr Treat Options Allergy*. 2016;3:169–180.
4. Beck SC, Huissoon AP, Baretto RL, Krishna MT. A critical analysis of the utility of component tests in the diagnosis of pollen-related peanut and hazelnut allergy in the context of the BSACI guideline. *Clin Exp Allergy*. 2017;47:1223–1224.
5. Klemans RJ, van Os-Medendorp H, Blankstijn M, Bruijnzeel-Koomen CA, Knol EF, Knulst AC. Diagnostic accuracy of specific IgE to components in diagnosing peanut allergy: a systematic review. *Clin Exp Allergy*. 2015;45:720–730.
6. Asarnoj A, Nilsson C, Lidholm J, et al. Peanut component Ara h 8 sensitization and tolerance to peanut. *J Allergy Clin Immunol*. 2012;130:468–472.
7. Vereda A, van Hage M, Ahlstedt S, et al. Peanut allergy: clinical and immunologic differences among patients from 3 different geographic regions. *J Allergy Clin Immunol*. 2011;127:603–607.
8. Flores Kim J. Diagnostic accuracy, risk assessment, and cost-effectiveness of component-resolved diagnostics for food allergy: a systematic review. *Allergy*. 2018;73:1609–1621.
9. Vetter TR, Schober P, Mascha EJ. Diagnostic testing and decision-making: beauty is not just in the eye of the beholder. *Anesth Analg*. 2018;127:1085–1091.
10. Caraguel CG, Vanderstichel R. The two-step Fagan's nomogram: ad hoc interpretation of a diagnostic test result without calculation. *Evid Based Med*. 2013;18:125–128.
11. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol*. 1997;100:444–451.
12. Uotila R. Component-resolved diagnostics demonstrates that most peanut-allergic individuals could potentially introduce tree nuts to their diet. *Clin Exp Allergy*. 2018;48:712–721.
13. Stiefel G, Anagnostou K, Boyle RJ, et al. BSACI guideline for the diagnosis and management of peanut and tree nut allergy. *Clin Exp Allergy*. 2017;47:719–739.
14. Beyer K, Grabenhenrich L, Hartl M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. *Allergy*. 2015;70:90–98.