

Rostrum

A Pitfall to Avoid When Using an Allergen Microarray: The Incidental Detection of IgE to Unexpected Allergens

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The introduction of new laboratory techniques to detect specific IgE antibodies against single allergen molecules rather than whole extracts represents a significant advance in allergy diagnostics. The advantages of such component-resolved diagnosis can be summarized as follows: (1) the ability to identify the truly responsible allergens in polysensitized patients, whether they be genuine (causing specific sensitization to their corresponding allergen source) or primary (the original sensitizing molecule); (2) distinguishing these allergens from simply cross-reactive components; (3) improving the appropriateness of the prescribed specific immunotherapy; and (4) identifying a risk profile for food allergens. Component-resolved diagnosis is performed using either a singleplex (1 assay per sample) platform or a multiplex (multiple assays per sample) platform. Using an immuno solid-phase allergen chip microarray that falls into the latter category—it currently tests sensitivity to 112 allergens—may lead to a pitfall: detecting IgE to unexpected allergens, such as Hymenoptera venom. In fact, testing insect venom sensitivity in individuals with no history of reactions to stings is contrary to current guidelines and presents the physician with the dilemma of how to manage this information; moreover, this may become a legal issue. Based on what is currently known about venom allergy, it remains likely that a positive sensitization test result will have no clinical significance, but the possibility of reacting to a future sting cannot be completely ruled out. Because this problem has not been previously encountered using the more common allergy tests, no indications are currently available on how to effectively manage these cases. © 2014 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2014;■:■-■)

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The availability and use of single allergen molecules in place of whole raw extracts in laboratory techniques represents a significant advance in allergy diagnosis.¹ This technology is currently defined as component-resolved diagnosis (CRD) or molecular-based allergy (MA) diagnostics.²⁻⁶ One major rationale supporting the use of CRD is that most allergic patients are polysensitized (sensitized to >1 allergen),⁷ but standard testing regimens using allergen extracts are unable to identify the molecular mechanisms underlying polysensitization. Instead, CRD enables clinicians to generate an IgE sensitization profile for each patient by identifying the specificities of the primary (the original sensitizing molecule) or genuine (causing specific sensitization to their corresponding allergen source) sensitizing allergens and distinguishing these allergens from cross-reactive specificities. Thus, CRD makes it possible for clinicians to readily identify the specificities of the relevant causative allergens.^{4,8}

MA technology can be used on either a singleplex (1 assay per sample) platform or a multiplex (multiple assays per sample) platform. In the singleplex platform, clinicians must select the allergen molecules to test on the basis of the clinically suspected allergen, which is indicated by the history of the patient. This approach has provided invaluable information to aid in diagnosis because detection of the specific IgE against the genuine sensitizing allergen molecule is strictly related to the clinical symptoms from the allergen source containing this molecule. In fact, in patients testing positive for multiple common allergens, the presence of specific IgE to given allergens pinpointed the actual allergy in 3 different types of allergy (pollens, foods, and Hymenoptera venom); as an example, sensitization to Api m 1 identified the culprit insect for Hymenoptera allergy as the honeybee, whereas sensitization to Ves v 1 and/or Ves v 5 identified the culprit insect as the yellow jacket wasp.⁹ In contrast to the singleplex platform, multiplex technology detects IgE responses against a large array of preselected allergens assembled on a chip and is mainly useful in patients with numerous positive allergy test results. The commercially available multiplex assay is the immuno solid-phase allergen chip (ISAC), which currently contains 112 allergens.

CLINICAL UTILITY AND LIMITATIONS OF MICROARRAY TESTING

There is abundant literature on ISAC performance, and findings related to detecting IgE against a large panel of allergens are especially worthy of attention. In particular, IgE reactivity profiles generated by ISAC have made IgE-based epidemiology studies feasible and allowed clinicians to geographically map aeroallergen exposure, which has improved our understanding of sensitization patterns within the general population.^{10,11} Data collected from ISAC have also been useful in improving the

*Abbreviations used**CRD- component-resolved diagnosis**HVA- Hymenoptera venom allergy**ISAC- immuno solid-phase allergen chip**LLR- large local reaction**MA- molecular-based allergy**SR- systemic reaction*

appropriateness of prescribed allergen immunotherapy. In fact, Sastre et al¹² found that in patients with pollen-induced rhinitis and asthma who were living in complex pollen areas, the choice of allergens to use for immunotherapy differed before and after the ISAC results were taken into account, with an agreement in only 46% of the prescribed immunotherapy regimens. Hence, more correct indications for treatment were given to patients after more accurate information was provided by MA diagnosis.¹² This has also been observed with venom immunotherapy because measuring IgE to single venom allergens can distinguish between cross-sensitization and dual sensitization to honeybee and vespid wasp venoms, thus helping to limit the prescribed venom immunotherapy to a single venom instead of multiple venoms in many patients.^{13,14}

The above observations suggest that MA, including the microarray technique, has a clinically relevant and significant role to play in assessing allergic patients. A 2013 consensus report on MA diagnostics put together by the World Allergy Organization, the Allergic Rhinitis and its Impact on Asthma initiative, and the Global Allergy and Asthma European Network concluded that "Although MA diagnostics is a complex area, it provides novel and relevant information for the allergist and will soon become a standard tool in the allergist's armamentarium."⁴ However, this document also highlighted that

a disadvantage is that patients may be at risk of revealing unanticipated sensitivities, possibly to potentially harmful molecules. Although this could also be considered as an advantage, the interpretation of such sensitization in clinically unresponsive patients is difficult or even impossible.⁴

This last statement in the consensus report raises a pivotal issue in the allergy field of symptomatic (ie, clinical allergy) versus asymptomatic sensitization. It is common knowledge in the field that sensitization occurs much more frequently than clinical allergy in individuals (rates have been shown to be up to 50% higher), and this phenomenon has been recently reconfirmed.¹⁵⁻¹⁷ Thus far, however, the mechanisms underlying the development of symptoms in sensitized individuals remain unclear. A Global Allergy and Asthma European Network project suggested that the following factors might be associated with asymptomatic sensitization: the lack of a family history of atopy, monosensitization, a reduction in T_H2 cytokine production, quantitative or qualitative differences in allergen-specific IgE with respect to functional activity and differential expression of IgE isoforms, the fine specificity of the IgE response, and others.¹⁸ Thus, because most of the sensitizations revealed by allergy tests (including MA techniques) are not clinically expressed, consulting patient history becomes essential to identify the causal allergens associated with the clinical symptoms. However, sensitization to allergens that elicit positive test results

but do not induce symptoms has a very different impact on clinical management than does sensitization to allergens that do induce clinical symptoms. Although allergen specificities that induce IgE antibodies but no apparent clinical symptoms in respiratory allergy are not of general concern because they typically induce only mild symptoms, detectable IgE antibodies against select allergen specificities in foods (eg, peanut, egg, and milk) and Hymenoptera venoms that have not induced clinical symptoms are a cause of concern because of their potential to induce severe allergic reactions on allergen reexposure.

POSITIVE ALLERGEN SENSITIVITY BY MICROARRAY ANALYSIS INFLUENCES THE MANAGEMENT OF PATIENTS WITH ANAPHYLAXIS

Similar to other allergies, in Hymenoptera venom allergy (HVA) asymptomatic sensitization to venom is much more frequent than are allergic reactions to stings; in a study by Golden et al,¹⁹ the prevalence of individuals with allergy in each group was 15% and 3.3%, respectively. More recently, Sturm et al²⁰ performed a sting challenge on a population of individuals with a previous negative history but positive sensitization test results to bee and wasp venom: only 5.3% exhibited a systemic reaction (SR) after the sting challenge, but 43.6% exhibited a large local reaction (LLR). Compared with the general population, this translates to a 9.5-fold higher risk for LLR but not SR. Of interest, this incidence of 5.3% observed by Sturm et al²⁰ is approximately double compared with the approximately 2% (range, 1%-3%) risk of SR in the general adult population based on reported incidence. Taken together, these data indicate that a positive test result in an individual with no history of SRs to stings has little clinical significance but the risk of LLR should be considered. Indeed, LLRs are particularly important in occupationally exposed subjects (such as farmworkers and beekeepers), who are candidates for immunotherapy.²¹ In addition, patients with LLR have a 4% to 10% risk of experiencing SR to subsequent stings.^{22,23}

In any case, consensus reports recommend that diagnostic tests for HVA must be performed only in patients with allergic reactions to stings.^{24,25} On the basis of this recommendation, clinicians are not likely to confront the problematic situation in which an individual tests positive for insect venom in the absence of an allergic reaction. However, because the ISAC test includes venom allergens, incidental detection of specific IgE to venom allergens is possible when testing patient sera for respiratory or food allergy; indeed, this may be a relatively common occurrence. Although this possibility has been the subject of informal discussion among allergists, the recent article by Passalacqua et al²⁶ has formally introduced this topic into the literature.²⁶ In their study, a population of 318 polysensitized patients was evaluated by microarray. Sensitization to at least 1 additional allergen was identified by microarray analysis in approximately 60% of the patients, and this information resulted in therapeutic adjustments. Six patients in this study exhibited a positive sensitization test result to venom allergens even though they had no history of reaction. Although this approximately 2% frequency of asymptomatic sensitization to venom is much lower than previously reported, it is important to consider that the current microarray evaluates specific IgE against only the major venom allergens Api m 1, Ves v 5, and Pol d 5; moreover, specific IgE to cross-reactive

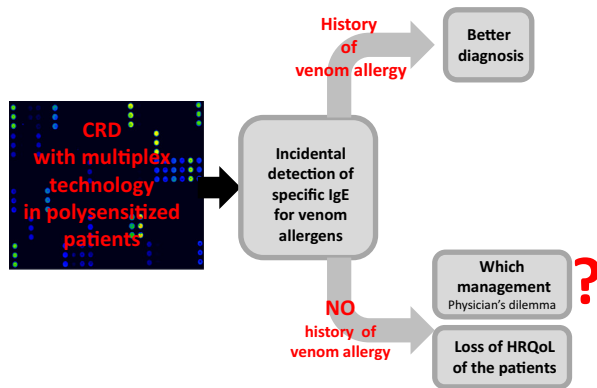


FIGURE 1. The consequences faced by physicians and patients after the incidental detection of specific IgE against insect venom. *HRQOL*, Health-related quality of life.

carbohydrate determinants, which are a major cause of cross-reactions because they usually occur in many allergen sources including pollens and foods, were not taken into account by this microarray.²⁷ Even allowing for this limited allergen repertoire or a possible role for cross-reactive carbohydrate determinants, however, it remains difficult to explain this low 2% rate described by the Passalacqua et al²⁶ study.

Notwithstanding the results by Passalacqua et al,²⁶ even a 2% positive rate of previously unknown sensitization to venom allergens from these increasingly used multiplex tests presents the physician with the dilemma of how to manage this information (Figure 1). Although it is very likely that a positive sensitization test result against a venom allergen often has no clinical significance, the possibility of a reaction to a future sting cannot be completely ruled out,²⁸ and this knowledge may also impair the health-related quality of life of a patient. Moreover, legal consequences can potentially occur if the allergist has not provided adequate or sufficient advice to the patient. Because this problem has not been previously encountered using the more common allergy tests, no indications are currently available regarding the most effective way to prevent future reactions, such as venom immunotherapy or the administration of drugs (antihistamines, corticosteroids, and epinephrine), in case a reaction does occur.

This issue is not restricted to HVA because many other allergen specificities are present on the ISAC chip, especially for foods such as peanut, milk, and egg, which are all known to induce severe reactions in some patients. In particular, sensitization to components such as lipid-transfer proteins or storage proteins from hazelnuts, walnuts, and peanuts is associated with SR, which can be severe.^{10,29-32} Indeed, the much higher frequency of sensitization than actual clinical allergy is also a concern for food allergy. For example, in a population-based birth cohort of 933 children, 110 (11.8%) were peanut-sensitized; of these, children with a convincing history of reactions on exposure, specific IgE level of 15 kUa/L or more, and/or a wheal diameter of 8 mm or more after a skin prick test were considered allergic without challenge, while 79 children underwent the challenge. Only 7 of these children exhibited objective signs of allergic reaction after the challenge and were thus designated as having peanut allergy.³³ Regarding the predictive value of *in vitro* IgE tests, Johanssen et al³⁴ reported different findings in peanut-allergic children: patients with a specific IgE

cutoff of 2 kUa/L or more displayed a sensitivity and a negative predictive value of approximately 80%, whereas those with a skin test result of 7 mm or more (similar to the aforementioned study) displayed a sensitivity of 83% and a negative predictive value of 84%. Although the usual recommendation after detecting the presence of specific IgE to food allergen molecules is to avoid foods containing the allergen, the insufficient specificity of the IgE tests for food allergy limits the predictive capacity of these findings, as recently reviewed.³⁵ In fact, when managing a patient with an anaphylactic reaction to a food, excluding all the other foods containing homologous allergens from the patient's diet is unjustified unless supported by the clinical history of the patient.

CONCLUSIONS

For allergists, detecting specific IgE antibodies has long represented a diagnostic tool to confirm a suspected allergy on the basis of the clinical history of the patient. Currently, the availability of singleplex-based techniques that can detect IgE antibodies may allow for more precise diagnosis. The ISAC multiplex format challenges this belief and suggests that detection of any sensitization can be useful. However, the consequences on patient management when HVA or particular food allergens are incidentally detected using multiplex technology highlight a dilemma that needs to be taken into account, especially in light of the increasing use of this multiplex format. The current ISAC is an excellent research tool, but it is likely to raise unwarranted concerns when used as a clinical tool and also may present potential legal concerns for the clinician as well as unnecessary risks for the patient. Perhaps, a "clinical" ISAC (without venoms) should be available, with a separate chip (or the original ISAC) reserved for the clinical evaluation of patients allergic to insect stings. This challenge that comes with the use of panel-based multiplex systems serves to highlight and reinforce the unique and vital role that the allergist plays in diagnosing allergy through linking the clinical history of a patient with the patient's own particular sensitization patterns.

REFERENCES

- Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Grönlund H. The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896-904.
- Melioli G, Passalacqua G, Canonica GW, Baena-Cagnani CE, Matricardi P. Component-resolved diagnosis in pediatric allergic rhinoconjunctivitis and asthma. *Curr Opin Allergy Clin Immunol* 2013;13:446-51.
- Skamstrup Hansen K, Poulsen LK. Component resolved testing for allergic sensitization. *Curr Allergy Asthma Rep* 2010;10:340-8.
- Canonica GW, Ansotegui IJ, Pawankar R, Schmid-Grendelmeier P, van Hage M, Baena-Cagnani CE, et al. A WAO-ARIA-GA²LEN consensus document on molecular-based allergy diagnostics. *World Allergy Organ J* 2013; 6:17.
- Eller E, Bindslev-Jensen C. Clinical value of component-resolved diagnostics in peanut-allergic patients. *Allergy* 2013;68:190-4.
- Incorvaia C, Rapetti A, Aliani M, Castagneto C, Corso N, Landi M, et al. Food allergy as defined by component resolved diagnosis. *Recent Pat Inflamm Allergy Drug Discov* 2014;8:59-73.
- Ciprandi G, Incorvaia C, Puccinelli P, Soffia S, Scurati S, Frati F. Polysensitization as a challenge for the allergist: the suggestions provided by the Polysensitization Impact on Allergen Immunotherapy studies. *Expert Opin Biol Ther* 2011;11:715-22.
- Stumvoll S, Westritschnig K, Lidholm J, Spitzauer S, Colombo P, Duro G, et al. Identification of cross-reactive and genuine *Parietaria judaica* pollen allergens. *J Allergy Clin Immunol* 2003;111:974-9.
- Müller U, Schmid-Grendelmeier P, Hausmann O, Helbling A. IgE to recombinant allergens Api m 1, Ves v 1, and Ves v 5 distinguish double sensitization from crossreaction in venom allergy. *Allergy* 2012;67:1069-73.

10. Scala E, Alessandri C, Bernardi ML, Ferrara R, Palazzo P, Pomponi D, et al. Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. *Clin Exp Allergy* 2010;40:911-21.
11. Cecchi L, Dell'Albani I, Frati F. Towards a global vision of molecular allergology: a map of exposure to airborne molecular allergens. *Eur Ann Allergy Clin Immunol* 2013;45:17-23.
12. Sastre J, Landivar ME, Ruiz-Garcia M, Andregnette-Rosigno MV, Mahillo I. How molecular diagnosis can change allergen-specific immunotherapy prescription in a complex pollen area. *Allergy* 2012;67:709-11.
13. Ebo DG, van Vaerenbergh M, de Graaf DC, Bridts CH, De Clerck LS, Sabato V. In vitro diagnosis of Hymenoptera venom allergy and further development of component resolved diagnostics. *Expert Rev Clin Immunol* 2014;10:375-84.
14. Golden DB. New directions in diagnostic evaluation of insect allergy. *Curr Opin Allergy Clin Immunol* 2014;14:334-9.
15. Barbee RA, Lebowitz MD, Thompson HC, Burrows B. Immediate skin-test reactivity in a general population sample. *Ann Intern Med* 1976;84:129-33.
16. Baldacci S, Modena P, Carrozzì L, Pedreschi M, Vellutini M, Biavati P, et al. Skin prick test reactivity to common aeroallergens in relation to total IgE, respiratory symptoms, and smoking in a general population sample of northern Italy. *Allergy* 1996;51:149-56.
17. Pallasaho P, Rönmark E, Haahtela T, Sovijärvi AR, Lundbäck B. Degree and clinical relevance of sensitization to common allergens among adults: a population study in Helsinki, Finland. *Clin Exp Allergy* 2006;36:503-9.
18. Bousquet J, Anto JM, Bachert C, Bousquet PJ, Colombo P, Cramer R, et al. Factors responsible for differences between asymptomatic subjects and patients presenting an IgE sensitization to allergens: a GA2LEN project. *Allergy* 2006;61:671-80.
19. Golden DB, Marsh DG, Kagey-Sobotka A, Freidhoff L, Szklo M, Valentine MD, et al. Epidemiology of insect venom sensitivity. *JAMA* 1989;262:240-4.
20. Sturm GJ, Kranzelbinder B, Schuster C, Sturm EM, Bokanovic D, Vollmann J, et al. Sensitization to Hymenoptera venom is common, but systemic sting reactions are rare. *J Allergy Clin Immunol* 2013;133:1635-43.
21. Hamilton RG, Golden DB, Kagey-Sobotka A, Lichtenstein LM. Case report of venom immunotherapy for a patient with large local reactions. *Ann Allergy Asthma Immunol* 2001;87:134-7.
22. Mauriello PM, Barde SH, Georgitis JW, Reisman RE. Natural history of large local reactions from stinging insects. *J Allergy Clin Immunol* 1984;74:494-8.
23. Golden DB. Stinging insect allergy. *Am Fam Physician* 2003;67:2541-6.
24. Portnoy JM, Moffitt JE, Golden DB, Bernstein WE, Dykewicz MS, Fineman SM, et al. Stinging insect hypersensitivity: a practice parameter. *J Allergy Clin Immunol* 1999;103:963-80.
25. Bilò BM, Rueff F, Mosbech H, Bonifazi F, Oude-Elberink JN. EAACI Interest Group on Insect Venom Hypersensitivity. Diagnosis of Hymenoptera venom allergy. *Allergy* 2005;60:1339-49.
26. Passalacqua G, Melioli G, Bonifazi F, Bonini S, Maggi E, Senna G, et al. The additional value of microarray allergen assay in the management of polysensitized patients with respiratory allergy. *Allergy* 2013;68:1029-33.
27. Brehler R, Grundmann S, Stöcker B. Cross-reacting carbohydrate determinants and hymenoptera venom allergy. *Curr Opin Allergy Clin Immunol* 2013;13:360-4.
28. Golden DB. Insect sting anaphylaxis. *Immunol Allergy Clin North Am* 2007;27:261-72.
29. Glaumann S, Nopp A, Johansson SG, Rudengren M, Borres MP, Nilsson C. Basophil allergen threshold sensitivity, CD-sens, IgE-sensitization and DBPCFC in peanut-sensitized children. *Allergy* 2012;67:242-7.
30. Flinterman AE, Akkerdaas JH, Knulst AC, van Ree R, Pasmans SG. Hazelnut allergy: from pollen-associated mild allergy to severe anaphylactic reactions. *Curr Opin Allergy Clin Immunol* 2008;8:261-5.
31. Pascal M, Munoz-Cano R, Reina Z, Palacin A, Vilella R, Picado C, et al. Lipid transfer protein syndrome: clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. *Clin Exp Allergy* 2012;42:1529-39.
32. Romano A, Scala E, Rumi G, Gaeta F, Caruso C, Alonzi C, et al. Lipid transfer proteins: the most frequent sensitizer in Italian subjects with food-dependent exercise-induced anaphylaxis. *Clin Exp Allergy* 2012;42:1643-53.
33. Nicolaou N, Poorafshar M, Murray C, Simpson A, Winell H, Kerry G, et al. Allergy or tolerance in children sensitized to peanut: prevalence and differentiation using component-resolved diagnostics. *J Allergy Clin Immunol* 2010;125:191-7.
34. Johanssen H, Nolan R, Pascoe EM, Cuthbert P, Noble V, Corderoy T, et al. Skin prick testing and peanut-specific IgE can predict peanut challenge outcomes in preschoolchildren with peanut sensitization. *Clin Exp Allergy* 2011;41:994-1000.
35. Soares-Weiser K, Takwoingi Y, Panesar SS, Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. The diagnosis of food allergy: a systematic review and meta-analysis. *Allergy* 2014;69:76-86.