Component IgE testing offers food for thought

Amy Carpenter Aquino

November 2018—Food component testing offers improved specificity for distinguishing IgE sensitized from truly allergic patients, and the menu for allergen components may soon expand. “The future will include a lot more component testing,” says Andre Valcour, PhD, MBA, DABCC, vice president and laboratory director of the Center for Esoteric Testing at LabCorp.

“IgE tests are extremely sensitive,” says Anthony A. Horner, MD, associate medical director of immunology, Quest Diagnostics, Nichols Institute. However, “they’re not very specific in that you can have a positive allergy test and not have a true clinical allergy to the food of concern.”

Oral food challenge, the gold standard, is not ideal from a patient perspective and can be deadly. “It takes a long time and it puts patients at risk for anaphylaxis,” Dr. Horner said in a presentation on advances in food allergy testing at this year’s AACC annual meeting.

The true prevalence of food allergy based on oral food challenges is about five percent in children and three to four percent in adults, Dr. Horner said. “[IgE] sensitization rates based on positive allergy tests are anywhere from three to 10 times higher than that, depending on which study you look at.”

Often overlooked is that “this testing is an adjunct to clinical history,” Dr. Valcour said in a recent interview. “In the absence of a positive history of reaction to exposure to a given allergen, a positive IgE test is clinically not relevant. I think a lot of people make that mistake.” Too many doctors, he says, are changing a patient’s lifestyle based on testing with no compelling clinical history. “That’s an unfortunate aspect of this and has to be avoided.”

While component testing is “very much becoming standard of care in Europe,” there is a limited menu—generally confined to peanut, tree nut, milk, and egg components—available through reference laboratories in the U.S., says Dr. Valcour, who is also discipline director of allergy, coagulation, and endocrinology at LabCorp. “We’ll be expanding the current menu of allergen components.”

Component testing for sensitization to red meat, in particular, will become more available in the next year or two, he says. “It’s a very interesting allergy. These patients tend to develop their allergic reaction later, several hours after ingestion of red meat, so people don’t always associate the allergic reaction as being allergy.”

Red meat is an uncommon allergen; it is tolerated even by children who are allergic to milk, egg, or chicken, Dr. Horner said in his presentation. Recent research by Thomas Platts-Mills, MD, PhD, of the University of Virginia, and others found a clustering of patients who had a delayed form of anaphylaxis after ingesting beef or pork in areas with high tick populations. Clinical histories revealed that the patients had had a tick bite in the weeks or months before developing the meat allergy.

“The molecular basis for this is a polysaccharide linkage, galactose-α-1,3-galactose [α-gal],” Dr. Horner said. “Most mammals have the enzymatic machinery to create these linked carbohydrate structures on their proteins, but humans and higher order primates do not.” Alpha-gal is expressed on non-human blood group B antigen and is a major transplantation barrier between primates and other mammals.

“Most of us have IgG to this molecular structure in our blood, but when we eat red meat, we do not have any symptoms,” he said. However, when people produce IgE antibody to carbohydrates containing alpha-gal, there is a risk that the ingestion of red meat will cause an allergic reaction. One theory is that ticks—the Lone Star tick in particular—cause red meat allergy by ingesting blood from a cow or other mammal and then biting a human, which results in skin exposure to the alpha-gal allergen. “The route of exposure, skin versus intestines, is thought to be the reason why anti-alpha-gal IgE is produced instead of anti-alpha-gal IgG,” he said.

Dr. Valcour

Commercial assays to detect IgE to alpha-gal are available, and Dr. Valcour believes physicians will begin to use more component testing for alpha-gal in the next year or so. He also predicts an increase in component testing for venoms, such as wasp or bee venom, and for furry animals, such as cats, dogs, and horses. “One of the dog components, Can f 5, is associated with a prekallikrein, a PSA-like protein, that is secreted by male dogs,” he says. “It seems to be correlated with exposure to male dogs as opposed to female dogs. The clinical utility has yet to be defined, but it may be that some patients are allergic to male dogs and not to female dogs, which is a very interesting story.”

“The bottom line is that there are so many components,” Dr. Valcour says. “Clinical stories are not fully developed for all of them, but over time I’m confident they will be.”

Component testing “helps fill the void” in the search for a better allergen test, though there is room for improvement, Dr. Horner said at the AACC meeting. “Component IgE testing is better than whole allergy testing, at least for some of the foods we are concerned with.”

While food component IgE testing is more specific than whole allergen testing, “there are limitations, such as the fact that most foods are a complex array of proteins,” Dr. Horner said. “More than 130 proteins have been identified in a peanut, for example. About 30 will bind to IgE if you put them on an electrophoresis gel and add patient serum.”

“Not all of the component proteins of a given food are contained in these component panels,” he added. This reduces their sensitivity with respect to IgE detection when compared with the whole food allergen. “Nonetheless, most allergenic proteins are represented in these food component panels.”

If there are 130 proteins in a food item, only some are likely to cause clinical allergy, and there are several reasons. “The relevant abundance of each of these proteins is a consideration,” as is the nature of the binding interactions between IgE and some of the proteins. Many foods contain highly cross-reactive proteins. “But IgE affinities to these cross-reactive proteins are often weak. Therefore, IgE reactivity to select food component proteins is strongly associated with having an allergic reaction, while IgE reactivity to others is not,” Dr. Horner said.

Then there are the component’s physical characteristics. Proteins that are resistant to heat and/or enzymatic digestion in the intestines are far more likely to be allergens and cause clinical symptoms than foods that are readily digested.

“There’s good data supporting the use of component testing for at least some of the foods,” Dr. Horner said, adding that the data most strongly support component testing for allergy to peanut, hazelnut, milk, egg, and wheat.

Although a peanut is a legume and more closely related to soy and peas than to tree nuts, it shares features with tree nuts: All contain profilins and storage proteins. Peanuts and hazelnuts contain the pathogen-related protein 10 (PR-10 protein), and peanuts, hazelnuts, and walnuts contain transfer proteins.

“Peanut and tree nut storage proteins are generally the most highly allergenic because they are resistant to heat denaturation and enzymatic digestion,” Dr. Horner said. “The good news is there’s not a lot of cross-reactivity between these storage proteins. Although if someone’s allergic to walnut, they’re likely to be allergic to pecan because these storage proteins do have a fair amount of cross-reactivity in terms of IgE.” The same can be said for

cashew and pistachio.

The lipid transfer proteins are expressed by a variety of plant foods as well as tree and wheat pollens, and they're found in fruits, nuts, and vegetables. "The degree of cross-reactivity in this family of proteins is varied," he said, "but they are fairly stable to heat in enzymatic digestion, so they have been associated with allergic reactions in some patients."

PR-10 proteins are more cross-reactive and less likely to be associated with severe allergic reactions. These proteins, particularly in the case of peanut, cross-react with birch pollen, which explains why some people who are allergic to trees will develop oral allergy symptoms when they ingest tree nuts or peanut. They're rarely associated with systemic reactions.

The profilins "are probably the most promiscuous when it comes to cross-reactivity," Dr. Horner said, and the least likely to cause severe allergic reactions.

Peanut is the best example of the utility of nut component testing. "Approximately 10 percent of American children have a positive IgE response to peanut if tested with whole anti-allergen, but they don't necessarily develop symptoms," he said. For children who do not develop allergic symptoms, the recommendation is to continue eating peanuts because it can help maintain tolerance.

"There are concerns that if the child begins avoiding these foods, the chances of becoming clinically allergic over time actually increase."

In contrast to these children who are sensitized, he said, about two percent of children have true peanut allergies. "These are patients who have the positive allergy IgE test results for whole allergen, and they develop immediate clinical symptoms after ingestion." Unlike for sensitized children, the recommendation for these children is to avoid peanut ingestion and to carry EpiPens.

"It's important to get it right and to be able to distinguish these two patient groups," Dr. Horner said. Component testing can be helpful but does not have the diagnostic accuracy of an oral food challenge.

A 2013 study published online in *Clinical and Translational Allergy* (van Veen WJ, et al., 3[1]:34) looked at 427 pediatric patients who had tested positive for peanut IgE in a Netherlands laboratory between 2003 and 2010. The investigators sent questionnaires to the patients' parents to obtain information on exposure to peanut and associated symptoms, which was used to categorize patients as allergic, non-allergic, or possibly allergic. Of those 427 patients, 280 were assessed further for peanut-specific IgE levels.

"Even though there are significant differences between the groups, there is a lot of overlap" in peanut IgE levels of the allergic and non-allergic patients, Dr. Horner said. "For any one patient, it's hard to know what a peanut IgE result really means unless it is completely negative. That's why it's so important to use these tests in the context of a good clinical history."

He presented the case of two pediatric patients diagnosed with peanut allergy to illustrate the advantages of component testing in distinguishing true peanut allergy from peanut sensitivity.

"Caroline and Emma both come into the allergist with the same set of symptoms. They have respiratory allergies and positive skin test results for peanut that are equivalent in size," he said. "They had ImmunoCAP testing for whole peanut, and their whole peanut IgE results are similar"—26 for Caroline, 28 for Emma.

Component testing for PR-10 protein, lipid transfer protein, and storage protein revealed a significant difference between the two patients. "Caroline has a high level of Ara h 8 while very little reactivity to the storage proteins, while Emma's IgE reactivity is almost all to the storage proteins," Dr. Horner said. Since storage proteins are much more strongly linked to true peanut allergy, the clinician would conclude that Emma has a high likelihood of having a real peanut allergy and should avoid peanuts, while Caroline is most likely only peanut IgE sensitized and should be encouraged to ingest peanuts regularly.

Dr. Horner

“This is where allergy component testing can be very helpful,” Dr. Horner said, “though this is an ideal case because it’s unusual to see no reactivity to select food component proteins with a high level of reactivity to the other component proteins. For most food-sensitized patients, the results of component IgE tests are less clear cut. This is the kind of information you get with component testing, and you can use that to make clinical decisions.”

A study published in 2017 in *Annals of Allergy, Asthma, and Immunology* (Valcour A, et al., 119[3]:262–266.e1) looked at IgE antibody measurements to five peanut allergen components (Ara h 1, 2, 3, 8, and 9) from 12,155 peanut-sensitized patients across the United States. “LabCorp’s patient database and access to demographic data make it possible to observe patterns that smaller institutions and local physicians just don’t see,” Dr. Valcour says. “For example, we were able to see that sensitization to Ara h 8 was markedly higher in the northeastern United States relative to other regions of the country.” This finding, he says, correlates with the fact that birch pollen sIgE sensitization was higher in the Northeast than in other regions of the U.S.

“What’s particularly interesting about the peanut study,” he says, “is the age dependency of component sensitization patterns. Young infants with detectable peanut extract IgE tend to be sensitized or positive for components Ara h 1, 2, and 3, which are the seed storage proteins and generally associated with a higher risk of systemic allergy.”

Infants and very young children who are positive for peanut extract IgE are often sensitized to the seed storage proteins, he says. “Older children, adolescents, and adults who are peanut extract positive are more often sensitized to pollen-related proteins. People with a pollen-related protein sensitivity tend to have a less dangerous clinical profile, often limited to oral allergy syndrome, which is similar to seasonal allergy-type symptoms.”

For a nine year old who tests positive for peanut extract specific IgE, for example, “you really are missing important information if you don’t have the component data. Peanut component results can help determine whether the sensitization is seed storage protein-related, which may put the patient more clinically at risk for anaphylaxis, as opposed to pollen-based sensitization, which may be less dangerous.”

A study of hazelnut component sensitization is in press, Dr. Valcour says. “We’re finding a very similar pattern. The two major hazelnut storage proteins are predominant in young children. In older populations we see the Cor a 1—the birch pollen-related protein—as predominant, and we see a drop in the predominance of the seed storage proteins.” He expects comparable results for walnut, cashew, and Brazil nut.

Component testing also can be useful to determine reaction risk to milk and egg components. Some of the proteins are labile to heat denaturation; others are not. “If you were truly allergic to milk and can’t tolerate it in any form, it’s likely you have reactivity to casein or, in the case of egg, ovomucoid,” Dr. Horner said. There is a subset of children who can tolerate milk or egg in baked goods but not the whole food.

He presented another case in his AACC session: A child had a positive skin prick test for egg allergy and had been following an egg avoidance diet. The allergist ordered component testing and discovered that almost all of the patient’s IgE bound to ovalbumin, which is a labile protein susceptible to heat denaturation. The child then tolerated an oral food challenge to baked egg, and the allergist instructed the child to continue to ingest egg in baked goods. One year later, the child returned with reports of physical reactions after accidentally eating scrambled eggs.

“Generally, in that setting there is no reason to do another allergy test. You wait a bit because these IgE levels fluctuate with time,” Dr. Horner said. Between 50 and 80 percent of children with allergies to egg or milk will

outgrow the allergy by the time they are adolescents. “With kids who are food allergic, the general practice is to serially test them every few years to see if they potentially have outgrown their allergy.” Since the child in this case was primarily reactive to ovalbumin, he was allowed to continue eating baked egg.

Wheat, which is a grass, presents special testing challenges. “Many people have IgE to grasses and they cross-react with wheat, so wheat is a particularly poor allergen for whole allergen testing,” Dr. Horner explained, noting a 2014 meta-analysis that found a specificity of 43 percent. “With component testing, you can get much better specificity.”

“It looks like the gliadins are the primary allergens of importance in terms of identifying people who are clinically allergic to wheat,” Dr. Horner continued. Omega-5-gliadin (Tri a 19) IgE is associated with more severe reactions to wheat ingestion and is linked to a disorder called exercise-induced anaphylaxis. “It’s an interesting phenomenon because these patients do not react to the food of concern unless they exercise within a few hours of ingesting it,” he said. “This syndrome is often associated with wheat ingestion, and omega-5-gliadin IgE has been found to be a very good marker for identifying associated exercise-induced anaphylaxis triggered by this grain.”

Dr. Horner calls the basophil activation test “an oral food challenge in a test tube.” He describes it as a functional, rather than a molecular, allergy test. “This is a test that allows us to understand what happens when the effector cells of IgE mediated allergies—basophils and mast cells—get exposed to the allergen of interest.”

BAT is a simple test to perform, he said. “You take peripheral blood basophils and mix them with the allergen of interest at various concentrations to find out whether the basophils granulate. You can identify these cells by flow cytometry.”

The markers CCR3, FceR1, and CD203c have been used to identify basophils in this assay. “These cells don’t express CD63 on their surface unless they degranulate,” he said. “But when you expose them to a clinically relevant allergen they degranulate, and as a result begin expressing CD63 on their surface. CD63 is found within basophil granules, and when the granules fuse to the cell membrane, they can be detected by flow cytometry. This phenomenon occurs quickly after exposure if the cells degranulate.”

A 2014 study published in the *Journal of Allergy and Clinical Immunology* (Santos AF, et al., 134[3]:645–652) found that BAT, when used as a secondary test for peanut component Ara h 2 IgE positive patients, was able to distinguish peanut sensitized from truly peanut allergic patients with almost 100 percent accuracy, Dr. Horner said. It also had better diagnostic accuracy than peanut skin and serum IgE tests, when used as a standalone test. “It does quite well with most of the foods in which it’s been studied, and it may also have clinical utility in the setting of idiopathic urticaria, which is autoimmune in nature,” he said.

Dr. Horner predicts that the use of BAT could reduce the need for oral food challenges. BAT can also be used to determine whether a patient will pass an oral food challenge. Among the test’s greatest drawbacks is the fact that patient basophils need to be alive for them to degranulate after exposure to peanut. “This limited sample stability will be a major challenge for laboratories interested in offering this test commercially,” he says.

Dr. Valcour acknowledges that BAT use is growing in Europe and the data are clinically interesting, but he does not envision U.S. availability anytime soon, largely because of the test’s drawbacks. “The logistics are very difficult. It has to be tested very quickly. Most clinicians don’t know how to use it. It may well have a future,” Dr. Valcour says, “but it’s not here yet.”

Laboratory leaders who consider adding component testing to their menus need to give equal attention to whether they can provide appropriate clinical support, Dr. Valcour says.

“There is a learning curve for the clinicians on how to properly use and interpret component test results. It’s not as intuitive as traditional allergy testing from a nomenclature perspective,” he says. “If I were to tell a clinician that a patient was peanut positive, they would readily recognize the possible cause of the patient’s sensitization. However, if I were then to go on and tell that clinician that this patient is only positive for one component, Ara h 8,

most clinicians don’t understand intuitively what that means—why a person who is mono-sensitized to Ara h 8 might have a different risk profile from a patient who is sensitized to Ara h 2. This can be pretty complicated for a busy clinician to understand, and this will only get more difficult as more and more allergen components are introduced.”

LabCorp plans to increase its clinical diagnostics support by including interpretations of components in its reports, with a launch planned for 2019. Adding more information to the report about component results, Dr. Valcour says, will help clinicians have more-informed treatment discussions with their patients.

Amy Carpenter Aquino is CAP TODAY senior editor.