A Laboratory Strategy to Identify Peanut Allergy Risk Factors

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Laboratory Assessment of Risk for Clinical Reaction to Peanut

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- About 20% of peanut IgE positive patients have "true" peanut allergy and may exhibit clinical reactions
- Peanut allergy effects about 1% of the US population and only 20% of those patients will outgrow the allergy
- Risk of true peanut allergy increases with the presence of elevated Ara h 1, Ara h 2, or Ara h 6 concentrations
- The level of Ara h 2 specific IgE tends to correlate with the severity of response in peanut allergic individuals
- Up to 50% of peanut allergic individuals develop clinical tree nut allergy
- Risk of peanut anaphylaxis increases at peanut specific IgE levels above 15kU/L
- Patients with either Ara h 2 or Ara h 6 specific activity of 2% or greater are likely to have a clinical reaction
- Clinical peach allergy increases risk of true peanut allergy
- True peanut allergic patients with positive tree nut allergy are at increased risk of sesame seed allergy
- **Laboratory results indicating a low number of risk factors do not preclude the possibility of the patient experiencing a reaction to peanuts (may be due to avoidance of peanuts)**

(> indicates equivocal, >> indicates elevated level)

	True Peanut Risk Factors (IgE)	
>>	1. Ara h 2	
>>	2. Ara h 2 specific activity	30.1%
	3. Ara h 1	
>>	4. Ara h 6	
>>	5. Ara h 6 specific activity	30.9%
>>	6. Peanut-HD	
>>	7. Peanut-HD =>15 kU/L	
>>	8. Peach (food)	
>>	9. Almond (Ara h 2 cross-reactive)	
	10. Brazil Nut (Ara h 2 cross-reactive)	

*-Specific activity (%) = specific IgE Ara h 2 (kU/L) / Total IgE (kU/L)
*-Specific activity (%) = specific IgE Ara h 6 (kU/L) / Total IgE (kU/L)

	Tree Nuts (IgE)
	Cashew
>>	Chestnut
	Hazelnut /Filbert
	Pecan
>>	Pistachio
	Walnut, English
>>	Sesame Seed

	Cross Reacting Pollen (IgE)
	Birch, River (tree)
	Mugwort (weed)
>>	Timothy (grass)

Laboratory Diagnosis of "True" Peanut Allergy

Peanut is as common as egg allergy and represents an extremely potent allergen that can result in severe life-threatening reactions. The awareness of peanut allergy and its potential danger has increased the demand for peanut testing. One of the most common tests used to help identify clinical peanut allergy is peanut specific IgE. This test has been shown to have utility as a tool to prevent severe reactions in children when given an oral peanut challenge. However, the use of peanut specific IgE does not perform well as a diagnostic tool for clinical peanut allergy[1]. Studies to differentiate peanut sensitized patients as defined by the presence of peanut specific IgE and clinically allergic peanut patients have shown that only about 20% of peanut sensitized patients have peanut allergy. Investigations in many countries have demonstrated that the blood test for the purified peanut allergens, Ara h 2 and Ara h 6, are highly specific and sensitive for clinical peanut allergy. To help identify "true" peanut allergy, a panel of specific IgE tests has been developed by Allermetrix for physicians as an aid to evaluate potential risk for clinical reactions.

Peanut Allergens

Peanuts when extracted contain many different proteins. At the time of writing there have been 13 allergens described. A peanut allergen is a protein that has been demonstrated to bind to IgE from a peanut allergic individual. Each allergen is named using the genus and species of the source material, *Arachis hyopgaea* for peanut, and by its order of discovery. Therefore, the peanut allergens are named Ara h 1 through Ara h 13, where Ara h 1 was discovered first and Ara h 13 is the most recently described. Ara h 4 was found after discovery to be the same as Ara h 3, and the Ara h 4 name is no longer used.

Some of the individual allergens like Ara h 1 also have variations due to post-translational modifications like glycosylation. Some of the proteins are glycosylated differently from each other and some not at all. From these differences in post-translational modifications arise isoforms. Some individual protein allergens may have several isoforms, and each may react differently to IgE.

Some of these peanut allergens are very similar to allergens in different source materials (e.g. other foods and plant pollens). Cross-reactivity occurs when IgE that binds a peanut allergen also binds a similar allergen in other sources. It is a characteristic of IgE antibodies that are formed in response to allergens that share similar structures with allergens from other sources. Cross-reactivity is important to recognize because the IgE that binds an allergen could have been formed to a cross-reactive source and not to the allergen being tested. In some instances, this cross-reactivity will result in clinical symptoms and not in others. Identifying cross-reactions can add to the clinical picture for a patient by ascertaining whether the presence or absence of symptoms can be linked to the cross-reactive source.

Laboratory Testing

Allergy laboratory testing measures circulating allergen specific IgE, which is formed after an initial priming exposure to an allergen. Not all allergen specific IgE laboratory methods are alike because allergen sources and technologies differ. Some methods use recombinant purified allergen because they are less expensive to produce. Allermetrix uses purified natural allergens because recombinant allergens are not identical to the natural allergens and may not react exactly like the natural peanut allergens[2]. The Allermetrix quantitative method uses liquid allergens which preserve the natural conformation of proteins unlike solid phase allergen systems that cause significant denaturation of the allergen proteins. Sensitivity of the liquid allergen method is much better than solid phase systems because of very low background binding that cannot be accomplished with solid phase systems. Allermetrix uses the WHO 3rd IRP 11/234 IgE primary calibrator and the lowest non-zero calibrator (0.04 kU/L), which corresponds to the concentration for an equivocal positive result. Solid phase systems use much higher positive calibrators (e.g. 0.35 kU/L) and extrapolate to zero.

When testing for peanut allergy using peanut specific IgE, positive results do not always correspond to clinical peanut allergy. Peanut has allergens that cross-react with a wide variety of other allergen sources that can result in a positive blood test when the patient has grass, tree, weed, or other food allergies. In a report from NHANES (National Health and Nutrition Examination Survey) 2005-2006 several allergen, specific IgE measurements were performed on a cross sectional sample of the US aged 6 and up[3]. Each person answered a standardized questionnaire and was categorized as non-allergic, having current allergies, or as having current hay fever. Individuals with high levels of peanut specific IgE were much more likely to have current hay fever, suggesting there is strong cross-reactivity between peanut and many pollen allergens.

Allermetrix investigated the in-vitro response of patients who presented with clinical signs of peanut allergy and compared them to samples that were from atopic and non-atopic individuals. Each sample was tested for specific IgE to Peanut, Ara h 1, Ara h 2, and a set of known and potential cross-reactants: timothy and alfalfa grass; acacia, mesquite and birch tree; ragweed and mugwort pollens; English walnut, black walnut, soybean, hazelnut, brazil nut, almond, and cashew nut; and peach. Each sample was also assayed for total IgE. As in the previous studies, allergen specific IgE to Ara h 2 was best correlated with clinical peanut allergy.

Laboratory testing to aid identification of “true” peanut allergy should include total IgE and specific IgE to the following allergens: peanut, Ara h 1, Ara h 2, Ara h 6, timothy grass, river birch, mugwort, brazil nut, walnut, cashew, pistachio, chestnut, pecan nut, sesame seed, hazelnut, and peach.

Ara h 1, Ara h 2, and Ara h 6 are associated with peanut allergy. In many studies, Ara h 2 has been demonstrated to be most closely associated with "true" peanut allergy[1,4,5,6,7]. More recently Specific IgE to Ara h 6 in the absence of Ara h 2 has been demonstrated to also be highly associated with clinical peanut allergy[8]. Ara h 1 also associates with "true" peanut allergy[1,4,6].

In Spain Ara h 9, also known as a lipid transfer protein (LTP), was associated with "true" peanut allergy in a cohort of mostly adults[9]. Twenty-two of the 26 peanut allergic individuals also reported symptoms after eating peaches. Peach, hazelnut, and mugwort all contain LTP allergens and are included in the test panel to help detect specific IgE to this protein that may be relevant to "true" peanut allergy, especially in people who are allergic to peaches.

Peanut allergens Ara h 8, hazelnut allergen Cor a 1 and birch allergen Bet v 1 are all cross-reactive and belong to a pathogenesis related protein family. Ara h 5, Cor a 2 and Bet v 2 are cross-reactive and are members of the profilin family which includes allergens from grasses such as timothy and other plants. In a recent study of children in Sweden, the presence of Ara h 8 specific IgE without detectable specific IgE to Ara h 1, Ara h 2 or Ara h 3, was associated with no or mild reactions to peanut challenges. This family of allergens tends to be labile to digestion and when relevant to clinical reactivity is most often associated with oral allergy syndrome (OAS). The profilins like Ara h 5, Cor a 2 and Bet v 2 are rarely associated with clinical allergic reactions, however, may be involved in OAS.

Patients with pollen specific IgE often have IgE antibodies directed against a carbohydrate antigen found in many pollens including timothy grass and birch pollen as well as peanut. The carbohydrate antigens have been designated cross-reactive carbohydrate determinants (CCD) and specific IgE to CCDs are found in 20 – 60 % of pollen sensitive people. The clinical relevance of CCD specific IgE is not totally understood, but it has generally been thought to have little clinical relevance to peanut allergy[10].

The diagnosis of "true" peanut allergy must include the patient clinical history, clinical findings, and symptoms as well as laboratory findings. The use of the Allermetrix peanut panel will help physicians identify patients who may be at higher risk of developing or having strong clinical reactions to peanuts. Several studies have demonstrated that a high level of peanut specific IgE (15- 57 kU/L) is highly predictive of anaphylaxis[11]. This cutoff was developed as a tool to identify patients in whom a peanut challenge could be life threatening. It is not a very sensitive cutoff as many patients who have peanut specific IgE at lower concentrations have clinical peanut allergy.

The Allermetrix peanut panel can help stratify the risk of patients reacting to peanuts. The presence of specific IgE to Ara h 2 appears to identify those at highest risk of being clinically sensitive to peanut. A negative Ara h 2 finding with elevated specific

IgE to Ara h 1, peach, hazelnut, or mugwort may represent an intermediate risk level for the patient to have clinical reactions to peanut. Patients with positive peanut specific IgE that are not reactive to Ara h 1, Ara h 2, Ara h 6, and peach but demonstrate reactivity to any of the pollen allergens in the peanut panel most likely have cross-reactive antibodies. These patients represent the lowest laboratory risk for a clinical peanut reaction and may be considered for further inhalant testing. All results and follow up testing must be considered with the history and other clinical findings.

Patients with demonstrated or suspected peach clinical allergy must be considered at risk for clinical peanut allergy. In one study[8], about two-thirds of patients with clinical sensitivity to both peanut and peach developed clinical reactions to peach before peanut. In another earlier study[12], 32% of patients, mono-sensitized to peach LTP were clinically allergic to peanut. The possible sensitizing effect of lipid transfer protein (LTP), Pru p 3, and cross-reactivity with peanut LTP, Ara h 9, is suspected to be involved.

Patients with more than one antibody to a purified allergen have a diverse response and are more likely to have strong clinical reactions[13]. Higher specific IgE concentrations to purified allergens correlate to more diverse antibody responses[14]. Also, a diverse antibody response in a peanut sensitive patient is correlated with the number of organ systems that have clinical reactions to peanut[15]. Therefore, the level of Ara h 2 and Ara h 6 specific IgE tends to correlate with the severity of response in peanut allergic individuals.

The total IgE level in each patient can be used with the specific IgE level to Ara h 2 or Ara h 6 to calculate the specific activity of Ara h 2 and Ara h 6:

$$\frac{\text{concentration of Ara h 2 specific IgE}}{\text{concentration of total IgE}} = \text{Specific Activity}$$

$$\frac{\text{concentration of Ara h 6 specific IgE}}{\text{concentration of total IgE}} = \text{Specific Activity}$$

In house studies have demonstrated that specific IgE to Ara h 2 may represent as much as 26% of the total serum IgE. One study evaluating the specific IgE/Total IgE ratio in atopic adults for 182 different allergens found that the average specific activity was about 2%[16]. High specific activity to Ara h 2 suggests that mast cells are more likely to have more Ara h 2 specific IgE molecules bound to their receptors. Natural Ara h 2 isoforms are closely associated with effector activity[5].

An in-house study using samples from a patients highly suspected of having clinical peanut allergy, samples that were positive for peanut specific IgE, and samples that had peanut specific IgE less than 0.05 kU/L were tested for specific Ara h 2 and Total IgE (Figure 1). The highly suspected peanut allergic patients had much

greater specific activity of Ara h 2 than the other populations. Although this is a laboratory study, the data agrees with the general finding that only 20% of peanut positive patients have “true” peanut allergy.

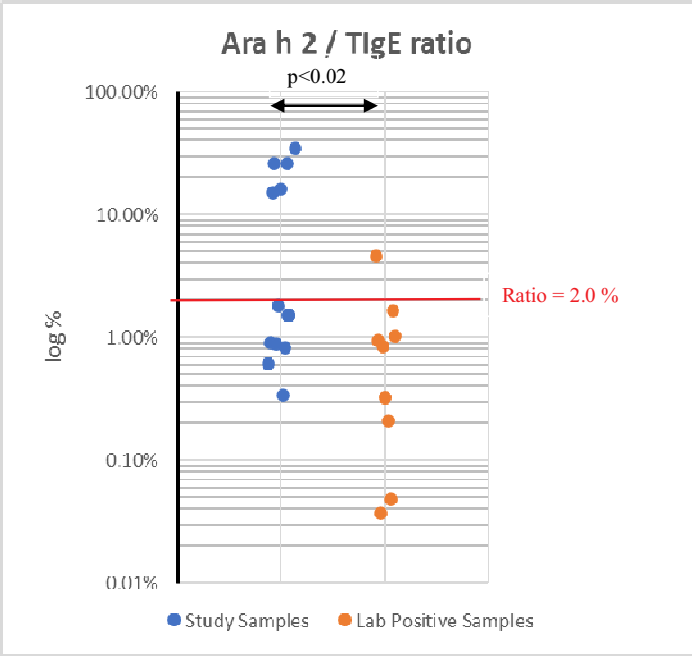

Figure 1 Twenty four serum samples were tested for specific activity to Ara h 2. Fourteen highly suspected peanut allergic patients, ●, ten sera with positive specific IgE to peanut, ●, and ratio of specific IgE to Ara h 2 to Total IgE determined.

The Allermetrix peanut panel uses natural allergens as well as natural purified allergens that include isoforms in their natural conformation. The liquid allergen technology ensures that allergens are presented to serum IgE in their natural non-denatured conformations. The peanut panel has been devised to help the physician understand how the array of peanut specific IgE antibodies present in a patient’s serum may indicate different levels of risk for “true” peanut allergy and the potential for clinical reactions.

When “true” peanut allergy is suspected, tree nuts and sesame seed need to be evaluated for cross-reactive clinical reactions. Up to 50% of peanut allergic patients have or develop allergy to tree nuts[17]. Ara h 2 has been shown to cross-react with both almond and brazil nut[18]. In peanut allergic individuals who also have tree nut allergy, the likelihood of sesame seed allergy is greatly increased[19].

The Allermetrix Peanut Panel helps to identify patients at risk for “true” peanut allergy and identifies potentially important clinical cross-reactants. The patient history and physician’s clinical findings must always guide the care for the patient. If strict peanut avoidance is maintained in the household, children may not be exposed and therefore

may not have had a priming exposure necessary to elicit antibody formation. It is important to consider the likelihood of exposure when evaluating negative findings.

Laboratory results indicating low risk patients do not preclude the possibility of these patients experiencing peanut reactions.

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