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Invited review article

Recent advances in component resolved diagnosis in food allergy

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CRD, component resolved diagnostics; DBPCFC, double-blind placebo-controlled food challenge; EoE, eosinophilic esophagitis; FDEIA, food-depend-exercise-induced allergic reaction/anaphylaxis; IgE, immunoglobulin E; IgE-ab, IgE-antibody; LTP, lipid transfer proteins; MA, molecular allergology; OIT, oral immunotherapy; OAS, oral allergy syndrome; OFC, oral food challenge; PFS, pollen-food allergy syndrome; PR, pathogenesis-related; SPT, skin prick test

ABSTRACT

Due to the high prevalence of food allergic diseases globally there are increasing demands in clinical practice for managing IgE-mediated conditions. During the last decade, component resolved diagnostics has been introduced into the field of clinical allergology, providing information that cannot be obtained from extract-based tests. Component resolved data facilitate more precise diagnosis of allergic diseases and identify sensitizations attributable to cross-reactivity. Furthermore it assists risk assessment in clinical practice as sensitization to some allergenic molecules is related to persistence of clinical symptoms and systemic rather than local reactions. The information may also aid the clinician in prescription of oral immunotherapy (OIT) in patients with severe symptoms, and in giving advice on food allergen avoidance or on the need to perform food challenges. The use of allergen components is rapidly evolving and increases our possibility to treat food allergic patients with a more individual approach. Using molecular allergology, we can already now better diagnose, prognose and grade the food allergy. In summary, daily routine molecular allergy diagnostics offers a number of benefits that give us a higher diagnostic precision and allow for better management of the patient.

Introduction

Component resolved diagnosis (CRD) provides a major step in improving the accuracy of diagnosing IgE-mediated food allergy. We are living in an era of exciting research and growth in the field of food allergy. With this CRD concept, allergology is experiencing a technological revolution, which is transforming into a rapid change in clinical practice. Our traditional way of diagnosing is challenged by this new concept. Our tools, based on sometimes poorly standardized and highly variable allergenic preparations become clearly defined and allow more analyses in depth.[1] The ability to identify and characterize single allergens at a molecular level is increasing our knowledge as to the mechanism of sensitization to foods. The increasing availability of food allergen components allow for a comprehensive review of the pattern of sensitization. Studies regarding structural similarity between food allergens help to explain cross-reactivity between allergens which may be clinically relevant. Certain pan allergen molecules can indicate broad cross-sensitization and underlie particular pollen-food or plant food syndrome.[2]

The relatively high prevalence of food allergy has led to increased diagnostic testing. CRD can be utilized both in the initial diagnostic workup and to follow specific IgE levels over time to determine when patients may be resolving their allergy and

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evaluated by food challenge. Sensitization to allergen components can either be measured by simplex or multiplex testing.[3]

This review is meant to be a general overview of IgE testing for food allergy with focus on recent advances of component testing.

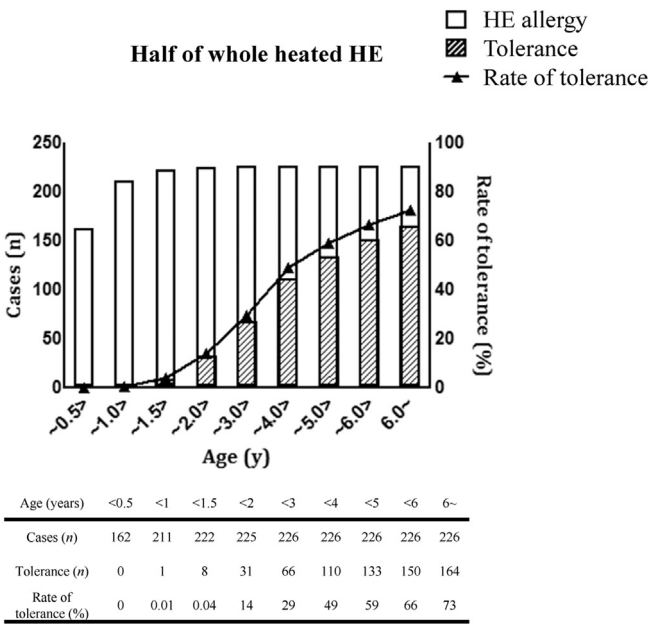
Egg (Table 1)

Egg white is the most important source of allergens in egg, and contains almost 80 non-allergenic and allergenic proteins.[4] Allergens that have been identified to be important and for which the clinician can test are ovomucoid (Gal d 1), ovalbumin (Gal d 2), ovotransferrin/conalbumin (Gal d 3), and lysozyme (Gal d 4).[5] Ovomucoid has been shown to be the dominant allergen in egg white. It has unique characteristics, such as stability to heating and cleavage by proteases and it appears to be allergenic in minute amounts. Ohtani *et al.* has recently shown that high levels of IgE to ovomucoid in egg allergic children is associated with delayed tolerance development (Fig. 1).[6] They investigated tolerance development in a group of 226 Japanese children allergic to hen's egg and found that those experiencing delayed tolerance developments had higher IgE levels of ovomucoid compared with children who developed tolerance early (Fig. 2).

Egg white IgE testing is in general mostly recommended for primary diagnosis of egg allergy because it combines the most common major allergens recognized in egg allergy (ovomucoid and ovalbumin). Molecular diagnosis has been shown to be helpful in a more fin-tuned diagnosis of egg allergy. Three different clinical situations can be distinguished. First scenario is when the individual is sensitized to egg white but is able to eat egg without symptoms. Second situation is when the patient is allergic to raw or partially raw egg only. Thirdly, when the patient is allergic to all forms of egg, which is the most severe form.

Ando *et al.* showed that a concentration of IgE antibodies against ovomucoid higher than 10.8 kU_A/l (positive decision point) indicated a high risk of reacting to heated (as well as raw) egg.[7] At the same time, a concentration below approximately 1 kU_A/l (negative decision point) means there is a low risk of reaction to heated egg, although the patient may well react to raw egg.

Benhamou Senouf *et al.* have recently shown in a similar study but with different patients' characteristics, a cut-off value for ovomucoid of 6.9 kU_A/L with a 95% specificity.[8] Also, they were able to show that a cut-off of 4.1 kU_A/L can be used for egg white in order to distinguish between allergy to all forms of egg, and sensitization in absence of allergy.



Age (years)	<0.5	<1	<1.5	<2	<3	<4	<5	<6	6~
Cases (*n*)	162	211	222	225	226	226	226	226	226
Tolerance (*n*)	0	1	8	31	66	110	133	150	164
Rate of tolerance (%)	0	0.01	0.04	14	29	49	59	66	73

Fig. 1. Tolerance acquisition of hen's egg allergy with aging. White bars represent number of HE allergy patients and black bars represent number of patients who tolerated half of whole heated HE. Filled triangles represent rate of tolerance.

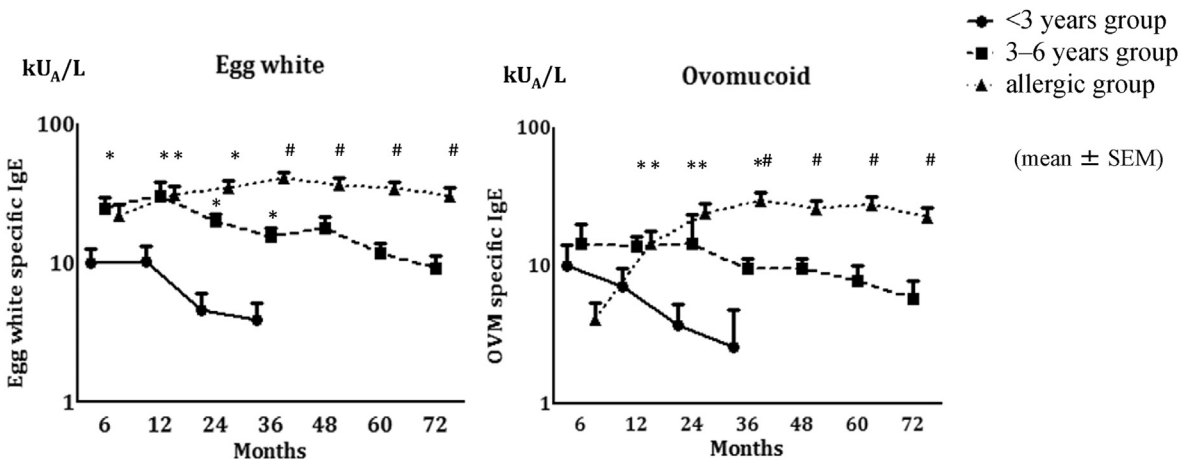
Similarly, the heat-labile egg white allergen ovalbumin can help distinguishing between various patterns of clinical reactivity to egg.[9]

Sequential IgE testing starting with egg white, followed by ovomucoid, will significantly increase the sensitivity of diagnostic testing compared to testing egg white only, although with a decrease in specificity.[8]

Wright *et al.* tried to identify the component as potential biomarkers of sustained unresponsiveness in oral immunotherapy and

Table 1
Clinical characteristics of food allergen components (egg, milk, and wheat).

Antigen	Component to food allergens	Author	Published year	Results	Ref #
Egg	Gal d 1 (ovomucoid)	Ando *et al.*	2008	OVM-sIgE was a good marker for reacting to heated egg.	7
		Ohtani *et al.*	2015	High levels of OVM-sIgE was associated with persistent egg allergy	6
		Benhamou *et al.*	2015	OVM was best to distinguish between allergy to raw only, and allergy to all forms of egg.	8
	Gal d 2 (ovalbumin)	Benhamou *et al.*	2015	OVA was the best test for the diagnosis of allergy to raw and cooked egg	8
Milk	Bos d 4 (alpha-lactoglobulin) Bos d 5 (beta-lactoglobulin)	Ahrens *et al.*	2012	Low levels of IgE to milk allergen components (casein, Bos d 4, Bos d 5) predicted outgrowth of milk allergy	16
	Bos d 8 (caseins)	Kuitinen *et al.*	2015	High baseline IgE levels to milk components (casein, Bos d 4, Bos d 5) predict less successful milk oral immunotherapy	20
	Bos d 8 (caseins)	Boyano-Martínez *et al.*	2009	High levels of casein-sIgE was associated with persistent milk allergy	14
		Caubet *et al.*	2013	Casein-sIgE predict clinical reactivity to baked milk	19
		Yanagida *et al.*	2015	Casein-sIgE were significantly reduced during low-dose-induction OIT	21
Wheat	Gliadin	Kotaniemi-Syrjänen *et al.*	2010	high levels of IgE to gliadins was correlated with persistent wheat allergy and the development of asthma in children	33
	Omega-5 gliadin	Ebisawa *et al.*	2011	Omega-5 gliadin was useful diagnostic marker in immediate type of wheat allergy	38
		Nilsson *et al.*	2015	High levels of omega-5 gliadin-sIgE was associated with severity of reaction during wheat challenge	31
	Omega-5 gliadin HMW-glutenin	Morita *et al.*	2009	Omega-5 gliadin and HMW-glutenin were causative antigens in WDEIA	34
	Lipid transfer protein (LTP)	Palacin *et al.*	2007	Wheat lipid transfer protein was assosiated with Baker's asthma	44
	Alpha-amylase inhibitors	Pastorello *et al.*	2007	Alpha-amylase inhibitors and lipid transfer protein were associated with immediate type of wheat allergy	46



Months	6	12	24	36	48	60	72
<3 years group (n = 66)	39	49	47	29			
3–6 years group (n = 98)	43	59	68	70	69	56	39
allergic group (n = 62)	35	43	46	55	54	49	39

Months	6	12	24	36	48	60	72
<3 years group (n = 66)	25	43	46	28			
3–6 years group (n = 98)	22	51	59	68	60	49	36
allergic group (n = 62)	19	37	42	52	51	45	39

Fig. 2. Changes in the levels of egg white- and ovomucoid-specific IgE among phenotypes of hen's egg allergy with aging. Left side is egg white-specific IgE (n = 226), right side is ovomucoid-specific IgE (n = 226). Solid circles represent <3 years group, solid squares represent 3–6 years group and filled triangles represent allergic group. Data are shown as the mean ± standard error of the mean (SEM). The Kruskale Wallis test was used for comparisons among the three groups. A p-value of <0.05 was considered statistically significant.

reported that IgE to egg white and IgE to ovomucoid were associated with sustained unresponsiveness.[9]

We conclude that the use of egg white components is clinically helpful for distinguishing between sensitization and clinical allergy. Furthermore, it is helpful for distinguishing between allergy to cooked and raw egg, or exclusively to raw egg.

Milk (Table 1)

Cow's milk proteins are among the most common causes of IgE-mediated food allergic reactions in children.[10] Proteins in cow's milk have a high sequence homology (>80%) with milk proteins from goat and sheep and are highly clinically cross-reactive (>90%) with these species. In contrast, the laboratory and clinical cross-reactivity is low with milk from donkey, mare, buffalo or camel.[11] The majority of patients allergic to milk are sensitized to several cow's milk proteins. However, the profile of the IgE response to these components varies greatly. The most important allergens in milk are caseins (Bos d 8), β-lactoglobulin (Bos d 5), and α-lactoglobulin (Bos d 4), although allergies to other minor proteins such as bovine serum albumin (Bos d 6) have also been reported.[12] Whey is a mixture of Bos d 5, Bos d 4, Bos d 6, and immunoglobulins, which are soluble in their native forms, more sensitive to heating and lose their IgE binding following 15–20 min of boiling at >90 °C.[13] Bos d 6 is independent of other cow's milk proteins and may reflect cross-reactivity with beef. Homologs of all of allergens present in cow's milk are also present in human breast milk, with the exception of Bos d 5.

CRD can be useful for monitoring natural tolerance development in cow's milk allergy. Boyano-Martínez and co-workers have been following children with cow's milk allergy over time.[14] They have observed that casein is the protein that best discriminates between persistent and transient allergy. Lower serum levels of IgE to casein were associated with higher chances of resolution of CMA. Ito *et al.* have found that high levels of casein-specific IgE antibodies are strongly associated with milk allergy in children and might be associated with prolonged allergy.[15] Ahrens *et al.* have shown that children with lower levels of IgE to cow's milk, Bos d 4, Bos d 5, kappa-casein, and alpha s1 casein had better odds of outgrowing their cow's milk allergy over an 80 month period.[16]

Nowak-Wegrzyn *et al.* reported that the majority (75%) of the cow's milk allergic children tolerate cow's milk as an ingredient in the baked products[17,18] and Caubert *et al.* has found that high levels of IgE antibodies to casein were predictive of clinical reactivity to baked milk.[19]

A treatment option for persistent cow's milk allergy is oral-, sublingual-, and epicutaneous-immunotherapy. Not all patients develop tolerance during therapy, and markers to identify those who will benefit from it are needed. Kuitinen *et al.* have found that high baseline IgE levels to α-lactalbumin, β-lactoglobulin and casein are associated with lower maintenance dose reached.[20] An increase in the IgG4 concentration to milk components during treatment indicated effective desensitization. Yanagida *et al.* performed a low-dose-induction OIT for milk in a high risk population known to have had severe reactions to very low amounts of milk.[21] Despite the low levels of intake, casein-specific IgE levels were significantly reduced. They also found that the casein-specific IgG4 levels were significantly elevated as an indicator of tolerance induction.

CRD can be helpful for the clinicians when evaluating their cow's milk allergy patients for monitoring and predicting the resolution of the disease. It might also be useful as markers to identify those who will best benefit from oral immunotherapy.

Wheat (Table 1)

Wheat allergy is worldwide common and varies depending on age and region from 0.4% to 4%.[22,23] Sensitization to wheat among

children was <1% in a systematic review taking into account a number of studies in different geographical regions.[24] However, the sensitization rates seem to vary a lot. Venter *et al.* described a sensitization rate for wheat of 0.4% in six-year-old children in Britain.[25] Östblom *et al.* found a corresponding rate of 6% among 8-year old children in Sweden.[23] Patelis *et al.* found a prevalence of 18% among young asthmatics in Sweden.[26] This variation is due to geographical location, selection of study population, and cross-reactions to pollen. A recent study of 108 atopic Finnish children demonstrated that a wheat IgE of 10.5 kU_A/L had a 72% PPV of wheat allergy.[27] Additionally, younger children may react at lower wheat IgE-level compared to older children.[28] One factor that contributes to the decreased specificity of IgE wheat testing is cross-reactivity between grass pollen and wheat; thus often grass pollen allergic but wheat tolerant individuals may have elevated wheat IgE levels.[29] Venter *et al.* has recently demonstrated very low prevalence of IgE-mediated wheat allergy and high levels of cross-sensitization between grass and wheat in the Isle of Wight cohort using CRD.[30]

This is well noticed in the clinic since a positive test result to wheat-flour extract may not always correlate with clinical symptoms of wheat allergy. This is likely to cause problem with over-diagnosis of wheat allergy. Nilsson *et al.* recently described that only half of the children with a doctor's diagnosis of wheat allergy, confirmed sensitization to wheat and on a wheat elimination diet, reacted in oral wheat challenges.[31] These patients are unnecessarily on a wheat-free diet most likely due to tolerance development or misinterpretation of wheat allergy tests.

This indicates that *in vitro* diagnosis of allergy to wheat may be improved by using wheat allergen components.

A child with wheat allergy is likely to become tolerant with a similar pattern to that of milk or egg allergy. Studies of the natural history of wheat allergy indicate that high levels of wheat-specific IgE predict slower resolution. Keet *et al.* also were able to show that children with IgE negative wheat allergy were clinically tolerant by age three.[32] CRD can also be helpful in predicting tolerance development because high IgE levels to gliadins have been show to correlate with persistent wheat hypersensitivity and the development of asthma in children.[33]

The best-characterized single component of wheat is omega-5 gliadin. It is also the most well-documented of the wheat components in WDEIA and IgE-mediated form of food allergy to wheat.[29,34–42] Two studies have also documented that there is an association between the levels of IgE to omega-5 gliadin and severity of reaction during wheat challenge.[31,37]

Many wheat-allergic individuals have been shown also to be sensitized to α-, β-, and/or γ-gliadins as well as high and low molecular weight glutenins (HMW-glutenin and LMW-glutenin, respectively).[27,31,34,43]

Alpha-amylase inhibitors and lipid transfer proteins have been associated with Baker's asthma and wheat allergy[27,44,45] and challenge proven wheat allergy.[46] A new form of wheat-related allergic disease which seems IgE-mediated is contact urticaria caused by hydrolyzed wheat protein.[47] Nilsson *et al.* found that majority (96%) of children with IgE-Ab to wheat had also IgE-Ab to hydrolyzed wheat protein.[48]

It seems that a combination of gluten-derived components tests improves the prediction of clinical reactions both for the diagnosis of WDEIA and immediate reactions to wheat. There are so far no commercially available IgE tests for α-, β- or γ-gliadins, HMW or LMW-glutenin.

Soy (Table 2)

Soybean is an important allergen source due to the common use in processed foods, sometimes representing a hidden allergen. Fermented soy products such as soy sauce and miso are much less allergenic compared with soy milk and tofu. Allergenic reactions have been described after exposure to both highly processed soy as well as unprocessed soy beans.[49]

Soybean allergy in children is known to be mediated primarily by contact *via* the gastrointestinal tract, often in the form of soya-

Table 2
Clinical characteristics of food allergen components (soybean and peanut).

Antigen	Component to food allergens	Author	Published year	Results	Ref#
Soybean	Gly m 4 (Bet v 1 homolog)	Fukutomi *et al.*	2012	High level of Gly m 4-sIgE was associated with adult soybean allergy	58
		Berneder *et al.*	2013	Gly m 4 was a risk factor for severe oral allergy syndrome or systemic reactions to soybean in patients allergic to birch	60
	Gly m 5 (7S Globulin) Gly m 6 (11S Globulin)	Holzhauser *et al.*	2009	Gly m 5 or Gly m 6 were diagnostic markers for severe allergic reactions to soy	50
		Ito *et al.*	2011		51
	Gly m 8 (2S albumin)	Ebisawa *et al.*	2013	Gly m 8 was the best diagnostic marker for soybean allergy	52
		Klemans *et al.*	2013		53
		Kattan *et al.*	2015		54
Peanuts	Ara h 1 (7S globulin) Ara h 2 (2S albumin) Ara h 3 (11S globulin)	Lieberman *et al.*	2013	Ara h 1, 3 and 2 were associated with severe reactions to peanut	65
	Ara h 2 (2S albumin)	Ebisawa *et al.*	2012	Ara h 2 was the best diagnostic marker for peanut allergy	64
		Dang *et al.*	2012		66
		Lieberman *et al.*	2013		65
		Klemans *et al.*	2013		68
		Eller *et al.*	2013		72
		Beyer *et al.*	2015	A 95% probability for a positive peanut challenge was estimated for an Ara h 2 sIgE of 42.5 kU/L	78
	Ara h 2 (2S albumin) Ara h 6 (2S albumin)	Klemans *et al.*	2013	The diagnostic value of sIgE to Ara h 6 on population level was as good as sIgE to Ara h 2	68
		Kukkonen *et al.*	2015	Co-sensitization to Ara h 2 and Ara h 6 was associated with severe reactions to peanut	73
	Ara h 8 (Bet v 1 homolog)	Sicherer *et al.*	2013	Ara h 8 was associated with no or very mild local symptoms	70
	Ara h 8 (Bet v 1 homolog) Ara h 9 (LTP)	Ballmer-Weber *et al.*	2015	Ara h 8 and Ara h 9 were major allergens for central/western and southern Europeans	71
	Ara h 9 (LTP)	Sicherer *et al.*	2013	Ara h 9 was associated with mild to severe symptoms in Mediterranean patients	70

based milk substitute products, particularly in infants allergic to cow's milk. The primary sensitizers seem to be the most important soya proteins Gly m 5 (7S Globulin), Gly m 6 (11S Globulin) and Gly m 8 (2S albumin).[50–54] The latter protein has recently been identified as a major relevant allergenic molecule and officially accepted by the IUIS Allergen Nomenclature Sub-Committee. Ebisawa *et al.* studied soy sensitization in symptomatic and non-symptomatic children on ingestion of soy and found that Gly m 8 is a major allergen in soy allergic children.[52] Klemans *et al.* studied adult patients with suspected soy allergy and found that Gly m 8 sIgE had the greatest accuracy in the diagnosis of soy allergy.[53] Kattan *et al.* was also able to verify that clinical reactivity to soy is best identified by component testing to Gly m 8.[54]

Since the turn of the century, allergic reactions to moderately processed soy powder have increasingly been recognized among birch pollen allergic individuals. This form of soy allergy may be acquired following primary sensitization to birch pollen, due to IgE cross-reactivity between the most important birch pollen allergen Bet v 1 and its homologous protein in soybean, Gly m 4.[55] Gly m 4 is underrepresented in diagnostic soybean extracts leading to vast differences between extract and component IgE tests. Recent data from Germany show that soy sensitization can be as prevalent as six percent in a normal population based on large cohort of approx. 13,000 individuals aged 3–17 years of age.[56] This is mostly like due to high prevalence of birch sensitization. Haftenberger *et al.* have recently shown that prevalence of Gly m 4 sensitization was as high as 10.3% and to soybean extract 3.7% in a similar adult cohort with approx. 7000 individuals.[57]

Unlike the other Bet v 1 homolog Gly m 4 has been shown to be a risk factor for severe oral allergy syndrome or systemic reactions to soy in patients allergic to birch.[58–60] Fukutomi *et al.* have studied the clinical impact of respiratory sensitization to pollen-derived pathogenesis-related class 10 protein on the development of adult soybean allergy.[58] They found that soy milk was the allergen that most prevalently induced symptoms in those who had been exposed, whereas none of the soybean-allergic patients reacted to fermented soybean products. The explanation is that Gly m 4 is heat-labile to a degree but also apparently susceptible to degradation by fermentation. This condition is also present in children and Kosma *et al.* reported similar clinical picture in children allergic to birch pollen.[59] The children also experienced severe allergic reactions following ingestion of soy milk during the pollen season. Berneder *et al.* reported recently Gly m 4 as a marker for severe food allergic reactions to soy.[60] Klemans *et al.* reported that subjects who only reacted to soy milk, but tolerated other forms of soy, had significantly higher sIgE levels to Gly m 4.[53]

Severe reaction to soy allergens can be caused by a primary soy allergy as well as to birch pollen – related soy allergy. In the first case, it is important that the patient avoid allergenic soy products and carry an emergency kit. In the second case, the patient needs only to avoid soy products that have not been extensively processed. Component testing for soy can help differentiate between these two conditions.[49]

Peanut (Table 2)

The best documented CRD area regarding clinical utility of measuring IgE is peanut components. The reason for this might be that peanut allergy is major public health problem and is known to be over diagnosed due to its cross-reactivity. For example, approximately 8% of the US population test positive for peanut but clearly the vast majority of these individuals tolerate this food[61] CRD studies have been carried out at all continents with similar results.[62–66]

Klemans *et al.* recently systematically searched the literature to assess the diagnostic value of sIgE to peanut components in diagnosing peanut allergy.[67] Data on sensitivity, specificity, and positive and negative likelihood ratios were extracted or calculated for a descriptive analysis and they found twenty-two studies eligible for inclusion.

Specific IgE to Ara h 2 (2S albumin) showed the best diagnostic accuracy of all tests for peanut allergy. Compared to the currently used SPT and sIgE to peanut extract, sIgE to Ara h 2 was superior in diagnosing peanut allergy and the authors suggest that the peanut extract-based tests should be replaced in daily clinical practice, especially in children. Sensitization to Ara h 1 (7S globulin), 3 (11S globulin) and 2 (2S albumin) have also been associated with severe reactions.[65,66,68,69] Sensitization to Ara h 9 (LTP) has been associated with mild to severe symptoms in Mediterranean patients and sensitiztion to Ara h 8 (Bet v 1 homolog) with no or very mild local symptoms.[70]

Areas that are recently in focus for peanut CRD studies are whether the concentration of IgE antibodies to Ara h 2 is associated with severity of symptoms upon peanut ingestion.

Ballmer-Weber *et al.* found that IgE to Ara h 2 $\geq$ 1.0 kU_A/l is significantly associated with the development of systemic reactions to peanut in the Europrevall study.[71] Klemans *et al.* found that higher levels of sIgE to Ara h 2 and peanut extract were associated with a larger proportion of patient groups reacting to a dose increase with objective symptoms in both adults and children.[68] Eller and Bindslev-Jensen documented that symptom severity elicited during challenge correlated significantly with the levels of Ara h 2 ($\rho(s) = 0.60$, $P < 0.0001$), but large individual variation was found.[72]

Kukkonen *et al.* were able to show that co-sensitization to Ara h 2 and Ara h 6 was associated with severe reactions distinguishing severe allergy from mild symptoms.[73] This finding is in line with a number of studies suggesting that severity correlates with binding to multiple proteins, Ara h 1–3.[74–77] Also Klemans *et al.* were able to verify the clinical utility of measuring IgE to Ara h 6 in adults.[68] The diagnostic value of sIgE to Ara h 6 on population level was as good as sIgE to Ara h 2. On individual level, however, 5% of the subjects showed contradicting results between both tests, leading to a risk of misdiagnosis if only one of both tests is used. It therefore seems that also Ara h 6 should be used in the clinic in order to optimize the diagnostic work-up.

Despite the consistent finding of Ara h 2 as the best available predictor of peanut allergy, no universal sIgE cut-off level has been established as predictive of clinical reactivity. Recently Beyer *et al.* published a 95% probability for a positive peanut challenge was estimated for an Ara h 2 sIgE as high as 42.5 kU_A/L.[78] The range of cut-offs proposed is most likely due to variations in geographic location and patient selection criteria.

CRD has also started to be used in oral immunotherapy in peanut allergic patients. Vickery *et al.* found that lower baseline levels of IgE to Ara h 2, Ara h 3 and peanut were associated with successful desensitization.[79] Also studies from Japan have shown that Ara h 2 specific antibodies seem to dominate the temporary IgE and sustained IgG4 response during OIT treatment.[80]

Recent findings in processing of peanuts have brought light upon the issue of allergenicity which is of importance for the peanut allergic individual. Roasting at high temperatures likely promotes the formation of compact globular protein aggregates that can increase allergenicity of Ara h 1 and 2, whereas cooking might reduce their allergenicity. Peanut oil is commonly used and in its unrefined form, may contain sufficient amounts of allergens to trigger reactions. Petersen *et al.* could demonstrate that roasting modifies the Ara h 8 molecule and increases the IgE reactivity as well as thermal and proteolytic stability.[81]

Tree nuts (Table 3)

In epidemiology of tree nut allergy, clear geographical patterns are found. In most studies, estimates of the prevalence of tree nut allergies are based on sensitization and/or a convincing history. The prevalence rates are rarely based on a standardized food challenge which explains the large heterogeneity of published prevalence data.

A recent meta-analysis has shown that in continental Europe, the most prevalent tree nut allergy is to hazelnut.[82] Walnut and cashew allergies are the most common tree nut allergies in the USA, while Brazil nut and walnut allergy were among the most frequent nut allergies in the UK. Imamura *et al.* have shown in Japan that walnut was the most common tree nut causing anaphylaxis.[83] Johnson *et al.* reviewed emergency visits during the first decade of 2000 and found that peanuts and tree nuts accounted for 50% of the food allergy emergency incidents.[84] They were able to show that the number of cashew nut reactions increased significantly over the study period whereas the number of reactions to peanut and other tree nuts remained constant.

Hazelnut has been shown to be the most prevalent food sensitization in the adult EuroPrevall cohort at 9.3%, with a large variation between the European countries (lowest Iceland 1.3% and highest Switzerland 17.8%).[85] Sensitization to hazelnut is more common in northern Europe and can be explained by extensive cross-reactivity to birch pollen but also to other tree nuts. Hazelnut allergy is the tree nut allergy that has been most extensively studied using CRD. There are several relevant allergens in hazelnuts. Cor a 1 is a homolog of the major birch pollen Bet v 1. Cor a 8 is lipid transfer protein. Cor a 9, an 11S globulin- and Cor a 14 is a 2S albumin seed storage protein.

Recent studies in the USA and Europe have demonstrated an important role for Cor a 9 and Cor a 14 in predicting clinical reactivity to hazelnut.[78,86–91] A Dutch study by Masthoff *et al.* were the first to describe the clinical utility of combining IgE tests for Cor a 9 with Cor a 14.[86] Sensitization to Cor a 9, Cor a 14, or both was strongly associated with having objective symptoms on ingestion of hazelnut. In children, sIgE levels to Cor a 9 of $\geq$1 kU_A/L or Cor a 14 of $\geq$5 kU_A/L, and, in adults, sIgE levels to Cor a 9 of $\geq$1 kU_A/L or Cor a 14 of $\geq$1 kU_A/L had a specificity of >90% and accounted for 83% of children and 44% of adults with hazelnut allergy with objective symptoms. Brandström *et al.* showed in their DBPCFC study that children positive in challenge also had higher levels of IgE-ab to Cor a 9 and Cor a 14 ($P < 0.01$ and $P < 0.001$, respectively) compared with those with a negative challenge.[87] In a US cohort, Kattan *et al.* reported that a sIgE result $\geq$2.0 kU_A/L for Cor a 9 or $\geq$1.0 kU_A/L for Cor a 14 had a sensitivity of 92% and specificity of 93% for clinical reactivity.[89]

In a recent German multicenter study, Beyer *et al.* evaluated 143 children with suspected hazelnut allergy.[78] All children underwent an OFC to hazelnut, and sIgE to whole hazelnut and Cor a 1, Cor a 8, Cor a 9, and Cor a 14 were analyzed. With an AUC of 0.89, sIgE to Cor a 14 was found to distinguish between allergic and tolerant children better than sIgE to whole hazelnut (AUC 0.71, $p < 0.001$). The AUC for Cor a 9 was 0.80. A Cor a 14 level of 0.35 kU_A/L was found to reach a sensitivity of 85% and a specificity of 81%, with a 90% probability of reaction found at 47.8 kU_A/L.

Hazelnut component testing to Cor a 9 and Cor a 14 has demonstrated improved utility in differentiating patients with birch pollen sensitization who are not clinically reactive or have mild oral symptoms from those more likely to have severe allergic reactions. These tests show greater specificity than traditional SPT and sIgE to hazelnut extract, though it is difficult to determine diagnostic cut-off values as predictive sIgE levels vary among the different studies.

Cashew is now used in industrial food as replacement for more expensive pine nuts and for its properties of improving texture. Reactions to cashew nuts are as common as reactions to milk and egg in early life in Sweden[92] and is associated with an equivalent or even greater risk for anaphylaxis than peanuts in children.[93,94]

Savvatianos *et al.* have studied sensitization to cashew nut 2S albumin, Ana o 3, and found it to be highly predictive of cashew and pistachio allergy in Greek children.[95] IgE sensitization to rAna o 3 ($\geq$0.35 kU_A/L) was detected in 93 of the allergic children (93%). In contrast, only 2 (6%) of the tolerant patients were found to be positive to rAna o 3.

The molecular components identified and recognized as allergens in English walnut, Juglans regia, are Jug r 1, Jug r 2, and Jug r 3, Jug r 4.[96,97] Jug r 1 is a 2S albumin seed storage protein and is inherently allergenic. Jug r 2 is a vicilin storage protein and is thought to have low clinical significance. Jug r 3, a commonly recognized lipid transfer protein (LTP), is associated with local symptoms and systemic reactions. Jug r 1, Jug r 2 and Jug r 3 are commercially available walnut components. Ciprandi *et al.* have shown that high levels of IgE to raw walnut and positivity to Jug r 1, 2, and 3, mainly if multiple, may be considered marker of severe walnut allergy.[98] Rayes *et al.* have studied Brazil nut allergy and found sIgE to recombinant allergen component Ber e 1 may provide higher sensitivity than whole Brazil nut extract.[99] They acknowledge that the use of a combination of SPT and sIgE to Ber e 1 might further enhance the diagnostic accuracy and reduce the need for oral challenge for the diagnosis of Brazil nut allergy. Goikoetxea *et al.* have studied if microarray analysis is useful and sufficient to diagnose nut allergy in the Mediterranean area.[100] They found that the diagnostic performance of ISAC was adequate for hazelnut and walnut allergy.

Table 3
Clinical characteristics of food allergen components (tree nuts, sesame, and buckwheat).

Antigen	Component to food allergens	Author	Published year	Results	Ref#
Hazelnut	Cor a 9 (11S globulin)	Masthoff *et al.*	2013	Cor a 9 and Cor a 14 were predicting clinical reactivity to hazelnut	86
	Cor a 14 (2S albumin)	Kattan *et al.*	2014		90
		Beyer *et al.*	2015		78
		Brandström *et al.*	2015		87
		Buyuktiryaki *et al.*	2016		89
	Cor a 14 (2S albumin)	Carraro *et al.*	2016	Cor a 14 was predicting clinical reactivity to hazelnut	91
		Eller *et al.*	2016		88
Cashew nut	Ana o 3 (2S albumin)	Savvatianos *et al.*	2015	Ana o 3 was highly predictive of cashew and pistachio allergy	95
Walnut	Jug r 1 (2S albumin)	Sordet *et al.*	2009	Jug r 1 was inherently allergenic	97
Brazil nut	Ber e 1 (2S albumin)	Rayes *et al.*	2015	Ber e 1 predicted clinical reactivity to Brazil nut	99
Sesame	Ses i 1 (2S albumin)	Maruyama *et al.*	2016	Ses i 1 was the best diagnostic marker for sesame allergy	104
Buckwheat	Fag e 3 (7S globulin)	Maruyama *et al.*	2016	Fag e 3 -sIgE improved the diagnostic accuracy for buckwheat allergy	108

Sesame and buckwheat allergy (Table 3)

Sesame is the most prevalent cause of allergic reactions to seeds. Sesame allergy is an increasingly recognized health burden, especially in developed countries including European countries, United States, and Japan.[101] Most cases occur in infancy and early childhood and the clinical presentation includes significant numbers of patients with severe reactions.[83] Sesame is a potent allergen and anaphylaxis has been reported in up to 30% of sesame-allergic children. Sesame allergy is also present in adults and can be preceded by *de novo* sensitization. The gold standard of objective evidence, that is assessment of sensitization and oral food challenge (OFC) have been used in few sesame studies.[101] The prevalence of sesame allergy seems to differ between communities due to different food habits. Sesame allergy was reported to be the third most common allergy in Israeli children, exceeded only by milk and egg allergy.[102] The specific IgE tests and skin prick tests presently available for diagnosis of sesame allergy are all based on natural sesame extract. Seven sesame allergen components have been registered by the WHO/IUIS Allergen Nomenclature Subcommittee, including two 2S albumins (Ses i 1 and Ses i 2), one vicilin-like 7S globulin (Ses i 3), two oleosins (Ses i 4 and 5), and finally two 11S globulins (Ses i 6 and Ses i 7). The 2S albumins and 11S globulins are dominating seed storage proteins in sesame, whereas 7S globulin is a minor component (Fig. 3). All together, they represent 80–90% of the total sesame seed proteins.[103]

Maruyama *et al.* studied almost one hundred Japanese children sensitized to sesame and diagnosed their sesame allergy through OFC or a convincing history.[104] Recombinant Ses i 1 was shown to have the best diagnostic performance of the allergen components based on area under curve (AUC) values from receiver operating characteristic (ROC) analysis. The experimental rSes i 1 ImmunoCAP test had larger AUC (0.891; 95% CI, 0.826–0.955) compared to the commercially available sesame test (0.697; 95% CI, 0.589–0.805). The clinical sensitivity and specificity for the rSes i 1 ImmunoCAP test at optimal cut-off (3.96 kU_A/L) were 86.1% and 85.7%, respectively.

Buckwheat is one of the leading causes of food anaphylaxis especially in Japan and Korea, even though buckwheat sensitization and allergy has a low prevalence.[105–107] Buckwheat allergy normally develops during school age or later, unlike allergies caused by eggs and dairy products that occur during early childhood. This might be because families introduce buckwheat late into the diet compared to egg and milk. The prevalence of buckwheat allergy in Japanese and Korean schoolchildren is estimated to be 0.1%–0.2%.[106,107] If buckwheat allergy is suspected, food avoidance is often initiated without performing OFCs, owing to the high risk of anaphylaxis. Therefore, there is a need among clinicians to identify the allergen components associated with symptomatic buckwheat allergy. Reported buckwheat allergen components of importance include Fag e 1 (13S globulin), Fag e 2 (2S albumin), Fag e 3 (7S globulin) and Fag e 10 kD (2S albumin).[105]

Maruyama *et al.* recently examined sixty-five children with suspected buckwheat allergy.[108] They were divided into two groups according to their clinical reactivity to buckwheat based on challenge outcome and/or clinical history. The symptomatic group comprised subjects with a positive challenge result (n = 21) and subjects with a convincing history of buckwheat allergy (n = 7), among which 4 subjects previously experienced anaphylactic symptoms. The authors found that measurement of sIgE antibodies to Fag e 3 improved the diagnostic accuracy owing to a significantly higher clinical specificity than that achieved with measuring sIgE to buckwheat.

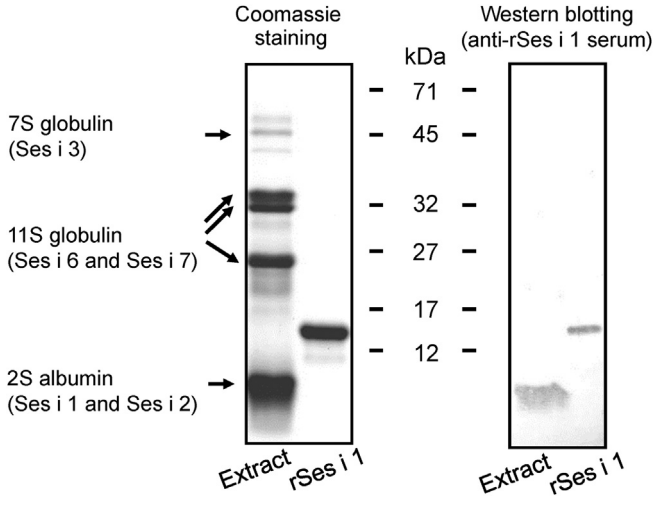


Fig. 3. Sesame seed extract and recombinant Ses i 1. The molecular size of sesame 2S albumin is smaller than that of recombinant Ses i 1 (rSes i 1) under the reducing condition on SDS-PAGE, because 2S albumin is processed into the subunits.

Clinical conditions when CRD is relevant for the food allergic patients

The first line approach to the diagnosis of food allergy is a thorough clinical history and physical examination of the patient. Sensitization to a food allergen can be confirmed with an allergen extract-based *in vitro* test or an *in vivo* skin prick test. However, an increasing proportion of patients have inconclusive clinical history, symptoms profiles and allergen extract test results. In many cases, such ambiguities may be resolved by including CRD to the diagnostic workup.[1]

CRD can contribute to efficient identification of primary sensitization to symptom-eliciting allergens and reveal co-sensitization and/or cross-sensitization. Using IgE testing with a microarray assay in a retrospectively study of 74 patient cases, Cardona and colleagues observed that underlying sensitizations to plant-derived foods, mainly vegetables and cereals, accounted for the reactions in 92% of patients sensitized to LTPs (Pru p 3).[109]

Further, molecular testing can be used to characterize the severity and assess the future risk of a reaction as have been discussed in the allergen sections above. CRD can also be helpful when there is co-sensitization to inhalant and food allergens and the symptomology is unclear. Pascal *et al.* studied Spanish patients with a complex clinical history and multiple sensitizations to plant-foods and pollens and could demonstrate that a broad spectrum of clinical reactions can be explained by LTP sensitizations using a microarray test.[110] Romano *et al.* found that LTP was the most frequent sensitizer in Italian subjects with food-dependent exercise-induced anaphylaxis.[111]

CRD can be used to optimize the decision process of food challenge tests, avoiding costly, time consuming, and potentially life-threatening reactions and improve allergen avoidance recommendations. Our personal reflection is that CRD allows the clinician to perform safe challenges. This seems to increase the number of OFCs, especially for peanut and hazelnut due to the fact that these allergies are heavily over diagnosed.

When suspecting idiopathic anaphylaxis allergen microarrays allow the simultaneous detection of patients' antibody profiles towards a large variety of each of the molecular allergens. Heaps *et al.* used the microarray and identified hitherto unknown sensitizations in up to 20% of patients with idiopathic anaphylaxis, that were highly likely to cause anaphylaxis. In the majority of cases,

sensitization to wheat omega-5 gliadin and shrimp components were demonstrated.[112]

Conclusion

The use of allergen components is rapidly evolving and increases our possibility to treat food allergic patients with a more individual approach. Using molecular allergology, we can already now better diagnose, prognose, and grade the food allergy. We can also get help in deciding which patient should receive OFC or not. Daily routine molecular allergy diagnostics offers a number of benefits that give us a higher diagnostic precision and allow for better management of the patient.

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Conflict of interest

MPB is medical director at Thermo Fisher Scientific. The rest of the authors have no conflict of interest.

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