



DR RU-XIN FOONG (Orcid ID : 0000-0001-7974-0068)

DR ALEXANDRA SANTOS (Orcid ID : 0000-0001-7974-0068)

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Authors: Foong RX^{1,2}, Santos AF^{1,2,3, 4}

¹Department of Women and Children's Health (Paediatric Allergy), School of Life Course Sciences, Faculty of Life Sciences and Medicine, King's College London, London, UK

²Children's Allergy Service, Guy's and St. Thomas' NHS Foundation Trust, London, UK

³ Peter Gorer Department of Immunobiology, School of Immunology and Microbial Sciences, King's College London, London, UK

⁴Asthma UK Centre for Allergic Mechanisms of Asthma, London, UK

Corresponding author:

Name: Dr Alexandra Santos

Postal address: Department of Pediatric Allergy, 2nd floor, South Wing, St Thomas' Hospital, 28 Westminster Bridge Road, SE1 7EH London, United Kingdom

Telephone: +44(0)20 71886424

Fax: +44(0) 20 74038640

Email address: alexandra.santos@kcl.ac.uk

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Abstract

Food allergy is increasing in prevalence, affecting up to 10% of children in developed countries. Food allergy can significantly affect the quality of life and well-being of patients and their families; therefore, an accurate diagnosis is of extreme importance. Some food allergies can spontaneously resolve in 50-60% of cow's milk and egg allergic, 20% of peanut allergic and 9% of tree nut allergic children by school age. For that reason, food allergic status should be monitored over time to determine when to reintroduce the food back into the child's diet. The gold-standard to confirm the diagnosis and the resolution of food allergy is an oral food challenge; however, this involves a risk of causing an acute allergic reaction and requires clinical experience and resources to treat allergic reactions of any degree of severity. In the clinical setting, biomarkers have been used and validated to enable an accurate diagnosis when combined with the clinical history, deferring the oral food challenge, whenever possible. In this review, we cover the tools available to support the diagnosis of food allergies and to predict food allergy resolution over time. We review the latest evidence on different testing modalities and how effective they are in guiding clinical decision-making in practice. We also evaluate predictive test cut-offs for the more common food allergens to try and provide guidance on when challenges might be most successful in determining oral tolerance in children.

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Key message: The diagnosis of food allergy is often made by a combination of clinical history and allergy tests, with oral food challenges, the gold-standard, used in the equivocal cases. The allergy tests commonly used to support food allergy diagnosis are skin prick test and specific IgE, with new tests, such as epitope mapping and the basophil and mast cell activation tests, currently emerging into clinical practice. These tests can also be used to monitor the spontaneous resolution of food allergy over time.

Background

Food allergies affect up to 10% of children in many parts of the world and have been on the increase over the last few decades (1). It is not clear whether this increase is due to an increase in new cases of food allergies or if allergies have remained more persistent or both. There is evidence that certain food allergies can be outgrown; for example 50-60% of children outgrow their milk and egg allergies anywhere from 2 to 6 years of age (2, 3) and approximately 22% of peanut allergy and 9-14% of tree nut allergies can be outgrown (4, 5). However, there is yet to be an effective cure for food allergies and with many food allergies persisting throughout life, it is likely that the burden of food allergic disease will increase with time.

Food allergic reactions can be classified into two main types: immunoglobulin-E (IgE) mediated reactions and non-IgE mediated reactions. IgE-mediated reactions are type 1 hypersensitivity reactions where the symptoms are usually of quick onset occurring within usually a few minutes to a couple of hours of exposure to the culprit allergen. The symptoms can manifest in many ways including cutaneous (hives, angioedema, rash, pruritus), respiratory (wheeze, stridor, difficulty breathing), cardiovascular (hypotension, dizziness, collapse), gastrointestinal (vomiting, diarrhoea, abdominal pain) symptoms or life-threatening anaphylaxis. Non-IgE mediated reactions tend to be more delayed with the onset of symptoms occurring more than 2 hours, often 24- 48 hours later and tend to affect the skin and gastrointestinal systems. The main focus of this paper is IgE-mediated food allergy.

Diagnosis of IgE-mediated food allergy

At the present time, the gold-standard for the diagnosis of food allergy remains oral food challenges (OFC). However, conducting OFC are labour-intensive and costly and do not come without the risk of life-threatening anaphylaxis. As a result, many food allergy diagnoses are made based on detailed clinical history and various allergy tests available including skin prick test (SPT), specific immunoglobulin-E (sIgE) levels to extract and component allergens and more novel diagnostic tests that are yet to be used regularly in the clinical setting, such as epitope mapping, the basophil activation test and the mast cell activation test. With a clear history of an immediate IgE-mediated allergic reaction to a food, most clinicians in the clinical setting would advise food avoidance. However, confirmation of the diagnosis of food allergy requires the documentation of

IgE to the culprit allergen, and if the history is not clear and levels of sIgE are not convincing, OFC may be required to clarify the diagnosis (see Figure 1).

Skin prick testing

Skin prick testing has been used to help guide allergy diagnosis for many years now. The sensitivity and specificity of SPT can be quite variable, ranging from 30-90% depending on the type and form of allergen (i.e. fresh food versus extract) and prick technique (6). Historically a SPT wheal of ≥ 3 mm has been considered as a positive SPT. Generally, SPTs have good sensitivity with larger wheal diameters being more likely to indicate true clinical food allergy. Numerous studies have looked at the diagnostic value of skin prick testing in comparison with OFC, and have defined positive cut-offs, i.e. cut-offs with high (95-100%) positive predictive value (PPV) and therefore useful to confirm the diagnosis of food allergy. Hill et al. reported the following SPT wheal diameters of ≥ 8 mm for milk, ≥ 7 mm for egg and ≥ 8 mm for peanut as having 100% PPV for the diagnosis of food allergy (7). SPTs are also useful and considered the standard for diagnosis of fruit and vegetable sensitisation with regards to pollen food allergy syndrome (PFAS). Fresh prick to prick tests have been shown to have higher sensitivity and specificity compared to commercial extracts, which is particularly important in patients with pollen sensitisation (8).

Specific IgE testing

Blood sIgE levels have also been used to support the diagnosis of food allergy. A mix of allergen extracts (e.g. Phadiatop, Thermofisher) can be used for screening of allergic sensitisation with the advantage of including various allergens in one test but at the expense of lower sensitivity compared with the single tests (9). However, similar to SPT, sIgE levels can indicate sensitisation. Following allergen exposure through ingestion, cutaneous, parenteral or inhalation, the allergen is taken up by antigen-presenting cells and through a complex interaction between T-cells, cytokine and chemokine secretion and B-cell lymphocytes, sIgE antibodies to that antigen are produced. These sIgE antibodies can be detected in the blood which indicate sensitisation to that allergen but the patient may not necessarily have clinical allergy or reaction to it and therefore, the interpretation of these tests should be used in conjunction with clinical history.

Historically, due to concerns about poor reactivity, SPT has been less preferred for determining atopic sensitisation in young infants. However, a study by Yang et al., has shown that infants <12 months with atopic dermatitis have SPT and sIgE that agree well with one another and that both can be used alongside each other to detect food sensitisation (10). Similar to SPT, the higher the sIgE level, the greater likelihood of IgE-mediated food allergy and comparing both these test levels for different allergens with the outcome of OFC can help determine their likelihood of clinical reaction (see Table 1) (11, 12). Hill et al. found that SPT and sIgE levels for cow's milk were similar in terms of diagnostic accuracy; however for peanut and egg, SPT was more sensitive (7).

From a clinical perspective, an $\text{IgE} \geq 0.35 \text{ kUA/L}$ is a widely accepted cut-offs to determine a test positive but the interpretation of their clinical relevance should be used in the context of an appropriate clinical history, consideration of the child's age, previous allergic reactions, atopic co-morbidities, such as eczema, and the food allergen in question as the sensitivity and specificity of the tests can vary. Komata et al., compared sIgE levels for egg and cow's milk in children <1 years old, 1 year old and 2 years old or greater and found that younger children react on low levels of sIgE compared to older children (13). Children with a history of reaction to a food were more likely to have a positive OFC result. Inoue et al., reported in a study on cashew allergy in children, all PPVs calculated were higher if the child had a previous clinical history of reaction to cashew compared to those that had no previous reaction (14). Atopic dermatitis is also common in children with food allergies and many of these children have elevated total IgE levels. There is little evidence to suggest that high total IgE level correlates with an increased risk of food allergies. In a study of infants with AD by Kerk et al., they reported approximately 80% of the children in their cohort had neither serological or clinical evidence of food allergy and although 11-50% of AD children had elevated IgE levels for the six common food allergens (peanut, egg, cow's milk, wheat, soy and fish) but fewer showed true allergy (15). In a retrospective study on OFCs performed in patients with varying degrees of AD who were avoiding foods due to positive SPT or sIgE, 89% of the children had no immediate reaction and of those who had a negative OFC none had a delayed eczematous flare post challenge (16). Higher total IgE levels can reduce the clinical relevance of a given specific IgE results and diagnostic cut-offs may vary in children with and without eczema (17). In a study assessing the utility of allergen to total IgE ratios in the

diagnosis of peanut and hazelnut allergies, a higher total IgE level shifted the probability curve of allergen-specific IgE to the right, meaning that the same allergen-specific IgE level tended to be less clinically relevant (18). The majority of studies where diagnostic cut-offs were defined were done in children with eczema as food allergy and eczema often co-exist. These are aspects to take into account when extrapolating such cut-offs from studies into clinical practice and when interpreting results from individual patients.

The shape of the probability curve for allergic reaction to a given food can be modulated by various factors, not only related to the patient but also to the challenge protocol. Yanagida et al., reported specifically on stepwise OFC outcomes with respect to sIgE levels for egg, milk and wheat where a 90% PPV for low dose OFC was determined at $>100\text{kUA/L}$. Their method of a 3-step challenge also gave information about whether patients could safely consume low or medium doses of allergen therefore avoiding complete elimination (19). This study also supports the fact that higher levels of IgE are associated with a lower threshold of reactivity, which has also been shown in other studies (20, 21).

IgE to components and to allergen peptides

The levels of sIgE can be determined against specific allergen or components, which, for some foods, can provide additional information to sIgE to allergen extracts, which is a mixture of different components. Examples of allergen components that can be very informative to distinguish clinical food allergy from clinically irrelevant sensitisation are Ara h 2 and Ara h 6 from peanut, Cor a 9 and Cor a 14 from hazelnut and Ana o 3 from cashew nut – see section below about examples of specific food allergies. Component testing is also particularly useful in diagnosing PFAS as it allows for discrimination between primary sensitization to food allergens from sensitization due to cross-reactive proteins (i.e. profilins, pathogenesis-related protein type 10 (PR-10) and non-specific lipid transfer proteins (LTP) (22).

One can go beyond individual allergens and investigate which part of the allergen molecule IgE recognises. Different methods can be used to identify IgE epitopes, including spot immunoassay, peptide microarray, peptide beads using the Luminex platform and methods coupling microarray with basophil assays have also been proposed (23). Informative epitope containing peptides have been identified in peanut allergens Ara h 1, Ara h 2 and Ara h 3 in different studies. Interestingly,

in a recent study, 10 different allergens were explored in a peptide peanut allergen microarray and key epitopes to distinguish allergic from sensitised tolerant subjects were found on the same three major allergens, which reinforces the importance of Ara h 1, Ara h 2 and Ara h 3 in peanut allergy (24). In the same study, the combination of 4 key peptides from Ara h 2 added diagnostic value to Ara h 2-sIgE when tested on the ImmunoCAP platform (24). In another study, machine learning allowed for the development of a method to determine whether a sensitised patient was allergic to peanut from data generated on a peptide microarray of Ara h 1-3 (25).

IgG₄/IgE ratios

Apart from levels of sIgE, other allergen-specific antibodies can modulate the reactivity of effector cells of food allergic reactions, like mast cells and basophils. *IgG₄* antibodies have been suggested to have a protective effect in food allergy. In a large peanut study, children who had IgE to peanut but were not peanut allergic had higher levels of peanut-s/*IgG₄* compared to allergic children and this was best appreciated when calculating *IgG₄/IgE* ratios, which were higher in sensitised but tolerant children. When removing *IgG₄* from the plasma samples, there was an increase in mast cell reactivity to peanut, supporting the protective role of peanut-*IgG₄* in inducing allergic reactions to peanut (26). In the LEAP intervention study, children who were exposed to peanut protein early were less likely to develop a peanut allergy and these children were found to have higher *IgG₄* levels and higher *IgG₄/IgE* ratios suggesting *IgG₄* had a protective role by blocking IgE binding to allergens (27). Also at the epitope level, *IgG₄/IgE* ratios were associated with the development of tolerance despite the presence of IgE in peanut allergy (24). Diagnostically, however, *IgG₄/IgE* ratios may not add a huge amount to sIgE. Datema et al., looked at specific IgG and *IgG₄* antibody levels in peanut allergic versus sensitised but tolerant patients and although IgG and *IgG₄/IgE* ratios were inversely associated with a positive oral peanut challenge and symptom severity, the ratios did not have better diagnostic value than sIgE to Ara h 2 (28). In fact, a study looking at predictive values of allergy tests in relation to peanut OFC outcomes in children from cohorts in Australia, UK and Ireland found that an Ara h 2 sIgE ≥ 0.35 kUA/L had the best positive predictive value (87%) and a peanut SPT < 3 mm had the best negative predictive value (94%) to diagnose peanut allergy (29).

Basophil activation test

Novel tests to meet the need for better diagnostic tests for peanut allergy have been developed, namely the BAT which has been mostly used in the research setting. The BAT is an in-vitro functional assay which detects the ability of IgE to activate live basophils that have been stimulated with an allergen. The basophils of allergic patients express the activation markers CD63 and CD203_c, in a dose-dependent way, when exposed to allergen whereas the basophils of those who are sensitized but tolerant either do not upregulate these markers or have lower expression levels (17).

In a study focusing on peanut allergy, BAT was found to have a PPV of 95% and a 98% NPV suggesting that a positive BAT to peanut could confirm peanut allergy without the need for a peanut OFC (30). Furthermore, BAT may be useful in estimating severity of allergic reactions. Santos et al., found that greater basophil activation was independently associated with severity of reactions during OFC to peanut (20). Further BAT testing on a large cohort of children participating in the LEAP and associated studies confirmed the diagnostic utility of BAT (with 98% specificity) and identified BAT as being the most specific and sensitive test, 97% and 100% respectively, in terms of identifying severe peanut reactions. In this study, participants with lower threshold of reactivity during OFC had higher basophil activation in vitro (21).

Mast cell activation test

The BAT has a high sensitivity and high specificity to diagnose food allergy. However, the test requires whole fresh blood within 4 hours of sampling with no prior storage and 10-15% of individuals have BAT results that are uninterpretable due to non-responding basophils to IgE-mediated stimulants (31). These limitations of BAT can be addressed with the use of the mast cell activation test (MAT). The principle of MAT is similar to BAT however it uses patients' plasma to sensitise LAD2 mast cells (a human mast cell line) before stimulation with allergen or controls and followed by flow cytometry.

Santos et al. have shown that MAT is comparable to BAT in terms of high specificity (98%) but has lower sensitivity (73%) to diagnose peanut allergy (31). MAT also identified patients at risk of severe allergic reactions during OFC, who have higher proportion of activated mast cells (31). Other groups have utilised primary mast cells to diagnose allergic patients using passive

sensitisation experiments and found the test to be highly sensitive but this in vitro system is less standardised as the cell reactivity varies between cell donors (32).

The use of BAT and MAT in the clinical context is yet to be established and requires appropriate standardisation, validation and quality assurance of the test. Future prospects with its diagnostic reliability suggest it may be particularly helpful in patients with equivocal assessment following clinical history, SPT and/or sIgE, before referral for OFC. This way, BAT and MAT can reduce the number of patients experiencing allergic reactions during OFC and deciding when an OFC is most useful in terms of determining allergic tolerance (30, 33, 34).

Diagnostic SPT, sIgE and component testing for specific food allergens

Peanut allergy

In terms of specific food allergies in children, one of the allergenic foods that has had greater focus in terms of diagnostic studies over the last decade has been peanut (35) but recent data has emerged on other foods, including hazelnut, cashew, and sesame. The focus on peanut may have been influenced by the increasing prevalence of peanut allergy in the last decade with approximately 0.4%-3% of children in developed countries being affected as well as it being one of the most common causes of anaphylactic reactions in children (35).

Various studies in the UK, Australia and the USA have looked at diagnostic cut-off points and have shown a positive predictive value of 95% with peanut SPTs being ≥ 8 mm (35). This has also been seen in a large study looking at biomarkers of allergic reactions to peanut in which a SPT > 8 mm was 100% sensitive and 92% specific to identify severe reactors (21). The Australian HealthNuts cohort have reported age-specificity 95% positive predictive cut-off values of peanut sIgE 34 kUA/L at 12 months but 2.1kUA/L at 4 years of age (4).

Specific component testing has also been shown to be helpful in predicting peanut allergy, especially Ara h 2-specific IgE. sIgE to Ara h 2 and Ara h 6 in particular have been associated with more severe reactions (36). In a study conducted by Beyer et al., children who had a peanut Ara h 2-sIgE of 14.4 kUA/l had a 90% probability for a positive peanut oral food challenge and a 95% positive predictive value if Ara h 2-sIgE was 42.2 kUA/l. Hemmings et al. reported that 84% of peanut allergic patients had an IgE to Ara h 2 and Ara h 6 but Ara h 2 induced greater inhibition of IgE binding and mast cell degranulation compared to Ara h 6 (37). Although these may provide

a useful guide in avoiding challenges, there is still a false positive rate of 1 in 10 or 1 in 20 children for the 90% and 95% probability respectively, and false-negative for patients who are sensitised to other allergens (38).

Table 2 compares various diagnostic cut-off criteria for the different modalities of testing used to diagnose peanut allergy.

Tree nut and sesame seed allergies

There has also been an increase in information available about the utility of SPT, specific IgE to extracts and component testing to diagnose tree nut and sesame seed allergies (39).

Ho et al. looked at SPT cut-offs in the diagnosis of tree nut allergy and found that a SPT $\geq$ 8mm had a specificity of 100% for cashew, hazelnut, walnut and 98% for sesame (40). In a study that conducted testing and OFC for hazelnut, component testing showed that Cor a 9 $\geq$ 1kUA/L or Cor a 14 $\geq$ 5kUA/L had a sensitivity of 83% and specificity of 93% in predicting hazelnut allergy (41). Kattan et al. reported similar results where a Cor a 9 $\geq$ 2kUA/L or Cor a 14 $\geq$ 1kUA/L had a sensitivity of 92% and specificity of 93% in terms of diagnosing clinical reactivity (42). Another study found that a diagnostic cut off of >0.72 kUA/L for Cor a 14 was able to diagnose hazelnut allergy 87% correctly (43). More recently, Datema et al. reported that patients sensitised to Cor a 9 $\geq$ 0.35kUA/L had sIgE levels that were significantly higher in the patients who had severe symptoms during hazelnut OFC (44). Recent systematic reviews of the literature summarise the evidence about component testing in peanut and hazelnut allergy diagnosis (45, 46).

A study of Greek children with cashew and pistachio allergy found that a specific cashew component Ana o 3 cut off of 0.16kUA/L had a 97.1% accuracy in diagnosing cashew and/or pistachio nut allergy (47). Another study also reported a 95% positive predictive value for a positive cashew OFC outcome using an Ana o 3 cut off of 2.0kUA/L and had it been used, 60% of the children in the study would have been identified correctly as having a cashew allergy without an OFC (48). Component testing has also been used in sesame allergy which has become an allergen of growing interest with increasing prevalence globally (49). Maruyama found that specific component testing to Ses i 1 had the best predictive value with a clinical sensitivity of 86.1% and sensitivity of 85.7% if a 3.96kUA/L cut-off was used (50).

The resolution of food allergy

Diagnostic tools such as specific component testing for more common continue to be at the forefront of allergy research to help guide clinicians in the management of their patients' allergies. In a study looking at *in vitro* testing for egg allergy, the authors described a sequential approach of calculating the net sensitivity and net specificity of various egg component test combinations (i.e. native egg white, native ovomucoid, denatured egg white). This combination of tests could increase the sensitivity of in determining egg tolerance or allergy compared to native egg white testing alone (51). The use of predictive models based on several diagnostic tests is becoming more common. Datema et al., (44) described a model that combined component-resolved diagnosis (CRD) with clinical background and extract-based serology and found it to be superior compared to CRD alone in identifying severe reactions to hazelnut. Santos et al., (21) generated multivariate models combining various tests to predict the severity and threshold of allergic reactions during peanut OFC and found them more informative than individual tests.

Understanding the resolution of allergies and the timing of when this may occur is an important part of food allergy management. Some food allergies, such as milk and egg allergies, are more commonly outgrown in childhood compared to others, like peanut and tree nut allergies, which tend to be more persistent. The immune mechanisms of food allergy resolution are still not fully understood. There is evidence to suggest that lower levels of allergen sIgE at diagnosis and decreasing sIgE levels or SPT wheal size over time are predictive of food allergy resolution (52) – Table 3. The age of the patient at the time of diagnostic testing is another important factor to consider as well with studies reporting a change in 95% PPVs as in the Australian Healthnuts cohort where peanut IgE cut-off level was 34kUA/L in infants less than 2 years old but 2.1kUA/L at 4 years of age (4, 53).

Immunological changes with natural resolution, induction of tolerance and desensitisation

Prevention studies have looked at whether oral tolerance can be influenced by early food consumption. The LEAP study demonstrated an 81% relative risk reduction of developing peanut allergy by 60 months of age if peanut was introduced and consumed regularly up to 5 years of age (27). Allergen-specific immunotherapy studies have shed some light into how immune tolerance might develop (54). On repeated regular exposure to the allergen, there is an initial

decrease in mast cell and basophil degranulation that occurs within hours or days accompanying clinical desensitisation. Following this, there are allergen specific T-regulatory cell and B regulatory cell processes that occur leading to the relative suppression of effector T-cells. Over the following weeks to months, a dose-dependent increase in allergen specific *IgG₄* is seen which acts by blocking antibodies in the IgE-mediated pathways of effector cell activation (54, 55).

In two separate studies looking at cow's milk allergy, tolerance to cow's milk was associated with high levels of cow's milk specific *IgG₄* and high *IgG₄* to low sIgE ratios (56, 57). In a study conducted by Esmaeilzadeh et al., the consumption of regular baked milk in milk allergic children's diets aided in the development of milk tolerance when challenged 12 months later; however, there was no significant association between sIgE levels to milk and milk tolerance (58). Leonard et al. found that egg allergic children who initiated and regularly consumed baked egg in their diet were more likely to outgrow their egg allergy and develop tolerance compared to those who strictly avoided egg. They also found that ingestion of baked egg was associated with decreased egg white SPT wheal diameter and egg sIgE levels as well as an increase in egg-specific *IgG₄* levels 3 months after consuming baked egg (59).

Although *IgG₄* increases with allergen exposure, there is no strong evidence to suggest it is predictive of natural resolution. *IgG₄* is dependent on allergen exposure; however, it has not been found to be a useful tool in monitoring or predicting clinical response during immunotherapy. Similarly, although allergen sIgE increases within the first few weeks of immunotherapy, it also does not correlate with clinical improvement and therefore, is not a useful predictor for monitoring immunotherapy outcomes (55). Further studies looking at biomarkers affected during peanut OIT have shown that only IgE to peanut Ara h 2 and Ara h 6 and *IgG₄* levels to peanut Ara h 1, Ara h 2 and Ara h 6 change with OIT (60). Authors have noticed that during OIT, there is a significant reduction in basophil reactivity and sensitivity in relation to the allergen. The reduction in basophil activity seems to correspond with desensitisation as seen in studies looking at OIT in peanut (61) and egg (62).

Other cellular markers have been investigated in the context of natural acquisition of tolerance. Turcanu et al. looked at peanut specific T-cell responses in children who were previously peanut allergic but became tolerant (63). In this study they found that peanut allergic children exhibit a Th (T helper)2-biased response when exposed to peanut antigen whereas exposure of food

antigens in non-allergic children induces a Th1-skewed response. In the children who were previously allergic to peanut but then became tolerant, their peanut specific cytokine profile was very similar to the children with no history of peanut allergy (i.e. a shift to a Th1 cytokine profile). The role of T-regulatory cells has also been investigated in terms of allergy resolution. In allergic individuals, exposure to an antigen causes emergence of an allergen-specific CD4⁺ Th2 cell response, generation of allergen sIgE and recruitment of effector cells to the gastrointestinal tract.

Studies of children who have outgrown their milk allergy have shown a link between oral tolerance development and higher numbers of milk-specific CD4⁺ CD25⁺ T-regulatory cells (64, 65). Another study investigated T-regulatory cell responses in children who developed natural tolerance to egg or peanut and found that the children who developed natural tolerance had significantly increased IL-10 protein levels which are expressed by CD4⁺ T-regulatory cells, CD25⁺CD127^{lo} cells and Foxp3⁺ cells (66). IL-10 is an immunosuppressive cytokine which has been shown to suppress type 2 immune responses and allergic inflammation. It can help induce tolerance by suppressing activation of IgE production and increase allergen-specific IgG₄ antibody responses (67). Fishbein et al. also found that there was an increase in IL-10 in children who developed natural tolerance to egg compared to those who had persistent egg allergy and this upregulation was antigen-specific (68). This was in contrast to oral immunotherapy studies which do not show an increase or transient increase in IL-10. IL-10 was also increased in control patients with no history of egg allergy, which further supports the finding that IL-10 differentiates between allergy and tolerance.

Monitoring SPT size and sIgE levels over time

A reduction in SPT wheal diameter size and/or sIgE levels to food allergens can be monitored over time and often the reduction of these allergy tests coincides with a clinical picture of allergic resolution and acquisition of tolerance. Shek et al. compared trends in sIgE blood levels of children who underwent at least two OFC, although majority had yearly OFC up to 10 years of age, to determine tolerance or continued allergy to egg or milk. Through the application of logistic models for both egg and milk, they found a significant relationship between decreasing sIgE levels and the probability of becoming clinically tolerant for both egg and milk. They also

calculated the degree of decrease in sIgE levels over time and found that if the decrease in sIgE occurred over a shorter period of time, this was greater indicative of the child outgrowing their food allergy (69).

By following the trends of sIgE levels across time, it may help guide clinicians in determining which children are not only ready to have an OFC but also which children are more likely to pass it. Vazquez-Ortiz et al. conducted a study on egg allergic children to determine cut-offs sIgE to egg components to help predict the outcome of OFC to help determine the timing of when would be best to conduct them. They determined that an ovalbumin sIgE $<1.45\text{kUA/L}$ was the most accurate cut-off point in identifying children with a high probability of uncooked egg tolerance but ovalbumin sIgE $<2.49\text{kUA/L}$ was the best cut-off point in identifying children with a high probability of cooked egg tolerance (70).

Having studies with cut-offs that help determine when a child may be successful in passing a challenge is useful although there are many factors that can influence this. De Boer et al. (71) looked at optimum cut-off points for determining 50% PPV for challenges and reported a sIgE cow's milk of 3.06kUA/L having a PPV of 92% for baked milk OFC and a sIgE egg white of 2.81kUA/L having a PPV of 84% for baked egg OFC.

At the epitope level, changes in IgE binding accompany different clinical phenotypes of milk allergy, with the IgE of patients allergic to all forms of milk binding more peptides with higher affinity compared to the IgE of patients who tolerate baked milk and that of patients who have resolved their milk allergy(72). These changes are likely to be similar in children undergoing the process of resolving egg allergy. There are also children who, despite an improvement in test results, still continue to have clinical reactions; thus, it is important to assess the changes in SPT and sIgE over time in light of the clinical context of the patient and consider other factors, such as age, time to last reaction and importance of the food in the child's diet, to decide when to re-challenge.

It is also important to be cautious when reviewing SPT and sIgE levels as research has shown discrepancies in diagnostic agreement using the different modalities. Schoos et al., reported a substantial disagreement between sIgE and SPT when diagnosing food sensitisation in children across time (age 6 months, 18 months, 4 years and 6 years). More specifically, they found that

the prevalence of food sensitisation increased in childhood if diagnosis was based on sIgE but decreased in prevalence if diagnosis was based on SPT (73).

Predicting prognosis of food allergy

A large SPT or a high specific IgE level can have prognostic value about whether food allergy is likely to be persistent (see Figure 2). In a Korean study, milk and egg sIgE levels at the first reaction was found to be a significant prognostic factor for future oral tolerance of cow's milk and egg (74). This was similar in a large cohort study looking at resolution of cow's milk allergy where two of the baseline characteristics most predictive of milk allergy resolution were milk sIgE and SPT to milk. There was a significant difference in the rate of resolution when comparing baseline SPT wheal sizes of <5mm, 5-10mm and 10mm greater as well as when comparing baseline sIgE milk levels of <2kUA/L, 2-10kUA/L and >10kUA/L with a smaller SPT wheal and/or sIgE milk level detecting a higher rate of resolution by 6 years of age. They also reported that milk specific *IgG₄* levels at baseline were not predictive of resolution (75). Sicherer et al. found baseline egg IgE <2kUA/L to be most predictive of egg resolution by 72 months of age and persistent elevated egg sIgE were strongly associated with persistent egg allergy, in a cohort of egg allergic children (76). In the Australian HealthNuts cohort of children, a 95% positive predictive value for persistent peanut allergy at 4 years of age was found for SPT≥13mm and peanut sIgE ≥5.0kUA/L when children were 1 years old (4). In a study looking at the natural history of treenut allergy, it was found that by using a sIgE cutoff of <5kUA/L, 58% of the children passed their OFC and 63% passed an OFC if their treenut sIgE was <2kUA/L (77).

Conclusion

Our understanding of food allergy has come a long way over the last few decades in terms of the diagnosis and management of allergic children. The diagnostic tests we have are relatively good at diagnosing patients with definite allergic reactions, there is still a clinical need for better tests especially for those children with a more ambiguous allergy history. Further research in understanding how allergic resolution occurs and if any biomarkers can be used to help guide when OFC may be appropriate from a safety and cost perspective would be extremely valuable for clinical practice.

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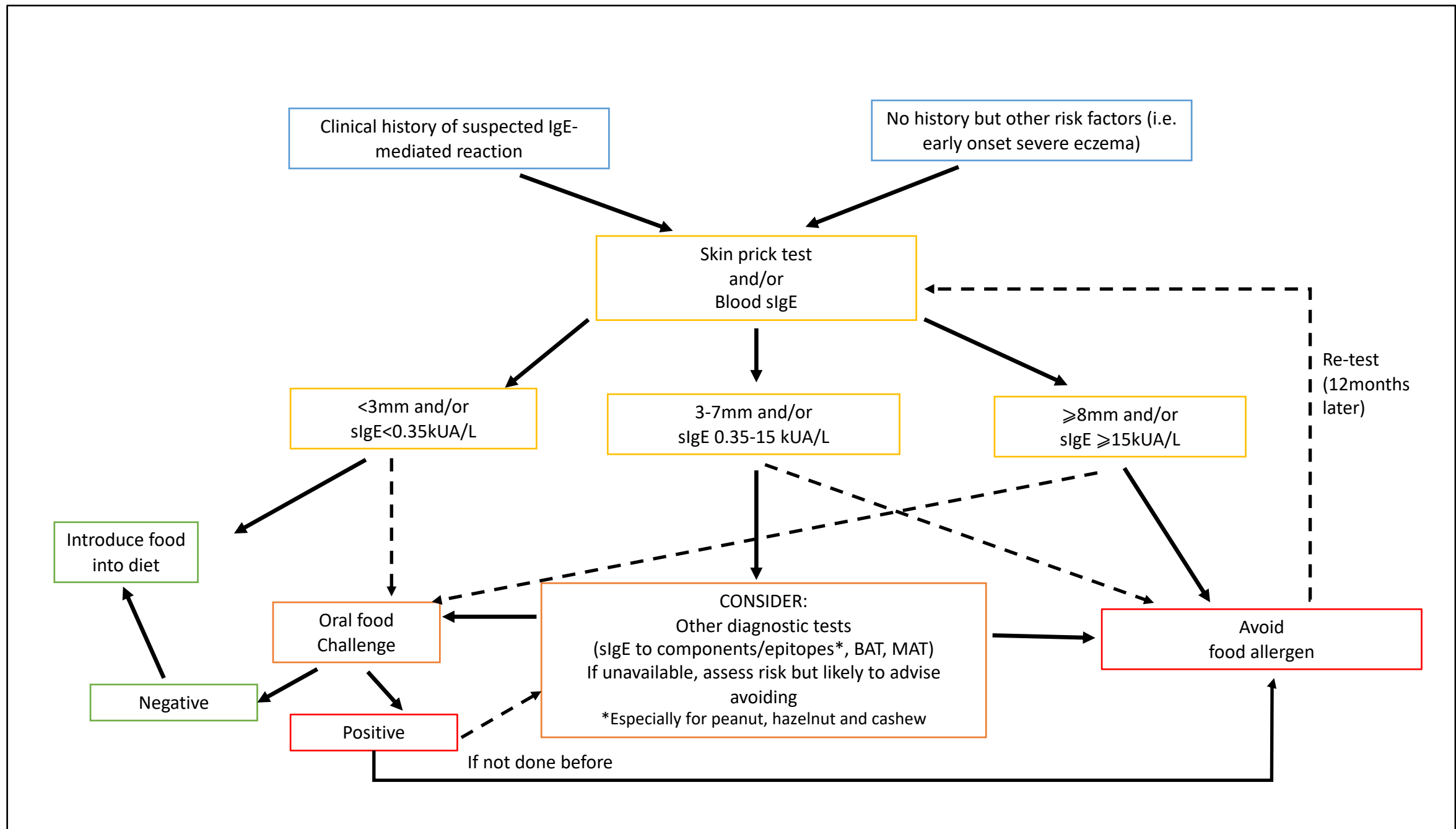
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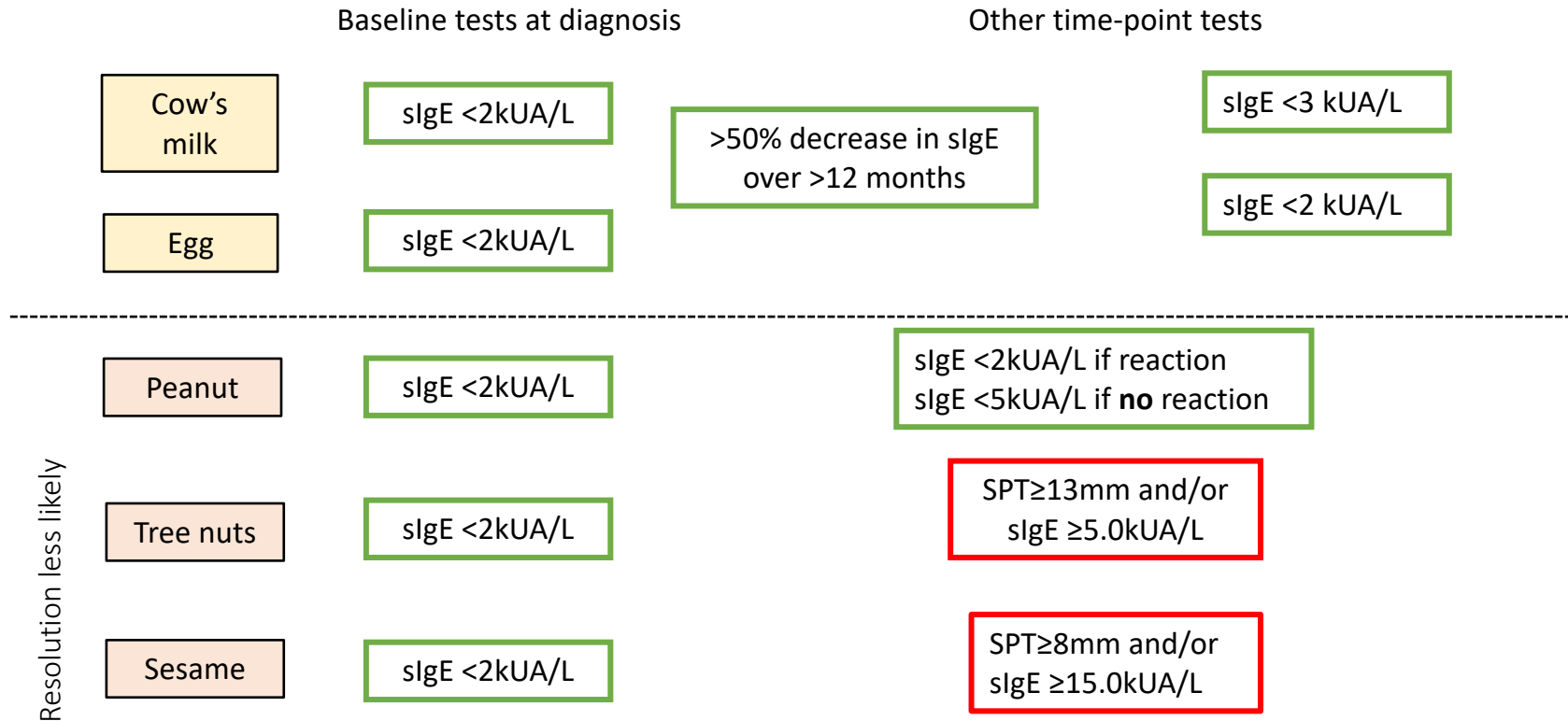
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*The age timeline arrow does not directly correlate with the boxes but is designed to show progression over time and suggest possible times when tests could be conducted.

Title: Biomarkers of diagnosis and resolution of food allergy

Authors: Foong RX^{1,2}, Santos AF^{1,2,3, 4}

¹Department of Women and Children's Health (Paediatric Allergy), School of Life Course Sciences, Faculty of Life Sciences and Medicine, King's College London, London, UK

²Children's Allergy Service, Guy's and St. Thomas' NHS Foundation Trust, London, UK

³ Peter Gorer Department of Immunobiology, School of Immunology and Microbial Sciences, King's College London, London, UK

⁴Asthma UK Centre for Allergic Mechanisms of Asthma, London, UK

Corresponding author:

Name: Dr Alexandra Santos

Postal address: Department of Pediatric Allergy, 2nd floor, South Wing, St Thomas' Hospital, 28 Westminster Bridge Road, SE1 7EH London, United Kingdom

Telephone: +44(0)20 71886424

Fax: +44(0) 20 74038640

Email address: alexandra.santos@kcl.ac.uk

Tables

Table 1. Sensitivity and specificity of skin prick test (SPT) and specific-IgE to common allergenic foods with 95% confidence intervals

Foods	SPT $\geq 3\text{mm}$		Specific IgE $\geq 0.35\text{kUA/L}$	
	Sensitivity	Specificity	Sensitivity	Specificity
Cow's milk ⁽¹⁾	88% (95% CI: 76-94%)	68% (95% CI: 56-77%)	87% (95% CI: 75-94%)	48% (95% CI: 36-59%)
Egg ⁽¹⁾	92% (95% CI: 80-97%)	58% (95% CI: 49-67%)	93% (95% CI: 82-98%)	49% (95% CI: 40-58%)
Wheat ⁽¹⁾	73% (95% CI: 56-85%)	73% (95% CI: 48-89%)	83% (95% CI: 69-92%)	43% (95% CI: 20-69%)
Soya ⁽¹⁾	55% (95% CI: 33-75%)	68% (95% CI: 52-80%)	83% (95% CI: 64-93%)	38% (95% CI: 24-54%)
Peanut ⁽¹⁾	95% (95% CI: 88-98%)	61% (95% CI: 47-74%)	96% (95% CI: 92-98%)	59% (95% CI: 45-72%)
Hazelnut ^(2, 3)	100%*	36%*	98%*	21%*
Cashew nut ^(2, 4)	96%*	52%*	95%*	58%*

Footnote: *95% confidence intervals were not determined.

Table 2. Positive diagnostic cut-offs for food allergens, i.e. cut-offs with high positive predictive value and therefore useful to confirm the diagnosis of food allergy

Food	Test modality	Cut-off	Sensitivity	Specificity
Any food (general)	Skin prick test	8mm ⁽⁵⁾	77%	81%
	Specific IgE	15kUA/L ⁽⁵⁾	49%	69%
Cow's milk	Skin prick test	8mm ⁽⁶⁾	30%	100%
	Specific IgE	32 kUA/L ⁽⁷⁾	34%	100%
	Component specific-IgE	Casein-sIgE ≥20 kUA/L ⁽⁸⁾	30%	95%
	Basophil activation test	SI CD203 _c Casein ≥1.3 ⁽⁹⁾	67%	71%
Egg	Skin prick test	7mm ⁽⁶⁾	52%	100%
	Specific IgE	6kUA/L ⁽⁷⁾	64%	90%
	Component specific-IgE	Ovomucoid-sIgE ≥3.7kUA/L ⁽¹⁰⁾	67.4%	95%
	Basophil activation test	SI CD203 _c (raw egg diagnosis) ⁽⁹⁾ - Egg white ≥1.7kUA/L - Ovomucoid ≥1.6kUA/L	77% 83%	63% 83%
Peanut ^(1, 11, 12)	Skin prick test	8mm ⁽⁶⁾	51%	100%
	Specific IgE	15 kUA/L ⁽⁷⁾	57%	100%
	Component specific IgE	Ara h 2-sIgE >0.28kUA/L ⁽¹¹⁾	82%	94%
		Ara h 6-sIgE >0.32kUA/L ⁽¹¹⁾	82%	90%
	Basophil activation test	4.78% CD63+ basophils ⁽¹²⁾	75%	99%
	Mast cell activation test	17.2% LAD2 cells ⁽¹³⁾	73%	98%
Hazelnut	Skin prick test	8mm ⁽²⁾	8%	100%
	Specific IgE	3.15 kUA/L ⁽¹⁴⁾	70.8%	90.6%
	Component specific IgE	Cor a 9 ≥2kUA/L and Cor a 14 ≥1kUA/L ⁽¹⁵⁾	92%	93.1%
	Basophil activation test	CD-sens >1.7 ⁽¹⁶⁾	100%	85%
Cashew nut	Skin prick test	8mm ⁽²⁾	39%	100%
	Specific IgE	27.0 kUA/L	PPV = 95%*	
	Component	rAna o3 ≥0.16kUA/L ⁽⁴⁾	96.8%	94.4%

	specific-IgE			
Sesame	Skin prick test	10mm (extract) ⁽¹⁷⁾	79%	75%
	Specific IgE	≥7kUA/L ⁽¹⁸⁾	14.3%	96.2%
	Basophil activation test	10.9% CD63 cells ⁽¹⁷⁾	86%	85%

*sensitivity and specificity not reported.

39 **Table 3.** Cut-offs of sIgE and SPT for the resolution of food allergy
40

Foods	Cut-off predictive of resolution or persistence at diagnosis	Cut-off for 50% NPV at follow-up
Cow's milk ⁽¹⁹⁾	Resolution: <2kUA/L	<2kUA/L
Egg ⁽²⁰⁾	Resolution:<2kUA/L	<2kUA/L
Peanut ⁽²¹⁾	Persistence: - SPT≥13mm - Peanut-sIgE ≥5.0kUA/L	Peanut-sIgE<2kUA/L (if history of reaction) Peanut-sIgE <5kUA/L (no history of reaction)
Tree nuts ⁽²²⁾ Sesame	Resolution:<2kUA/L	<2kUA/L

Figure Legends

Figure 1. Proposed diagnostic work-up for peanut allergy. The cut-offs indicated can be replaced with the 95% NPV and 95% PPV cut-offs for other foods to apply the algorithm for the diagnosis of other food allergies. Thick lines indicate more likely pathway and dashed lines indicate alternative pathways depending on the clinical information available for the individual patient being assessed.

Figure 2. Suggested algorithm to approach resolution of food allergy. Boxes in green are associated with likely resolution and boxes in red with likely persistence. Foods in yellow and orange boxes are associated with more likely transient and persistent food allergies, respectively.

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