

REVIEW ARTICLE

Peanut allergy diagnosis: Moving from basic to more elegant testing

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Abstract

Peanut allergy (PNA) is an IgE-mediated immune disorder, which merits particular attention due to its impact on the health and quality of life of millions of patients worldwide. PNA tends to develop in early life and resolves in only 20% of peanut-allergic children. It accounts for the majority of severe food-related allergic reactions. An accurate diagnosis of PNA is vital. In this review, we present the approach to the diagnosis of peanut allergy, starting from the history and proceeding to measures of overall sensitization and then to component-resolved diagnostics and oral food challenges as indicated. Additional testing in development includes basophil activation testing and determination of epitopes for peanut-allergic responses. Based on the literature, stepwise approaches and predictive models for diagnosing PNA are also presented.

KEYWORDS

basophil activation test, component-resolved diagnosis, epitope, food allergy, oral food challenges, peanut allergy, skin prick testing, specific IgE

1 | INTRODUCTION

Peanut allergy (PNA) is an IgE-mediated immune disorder, which merits particular attention due to its impact on the health and quality of life of millions of patients worldwide.

Peanut allergy is particularly widespread in the younger population and affects almost ¼ of children with food allergy (FA).¹ Although PNA tends to develop in early life, it resolves in only 20% of children.² The overall prevalence of PNA is estimated as 0.4% in Europe and 0.6% in the United States.^{3,4} The frequency of PNA by self-report is higher in the United States (1.2% to 8% of children)^{5,6} than in Europe (from 0.5% to 2.5%).^{3,7} Studies based on oral food challenge (OFC) have estimated the prevalence of PNA in Europe as 0.2%–1.6%,^{8,9} in Australia to be 3% at 12 months of age and 1.9% at 4 years.¹⁰ On the basis of stringent criteria (peanut IgE > 14 kUA/L and prescribed epinephrine autoinjector), its prevalence among US school children was found to be 2%.¹¹

Overall, the prevalence of PNA appears to be increasing, especially in developed countries. A large telephone-based survey found

rates of PNA in US children rising from 0.6% in 1997 to 1.2% in 2002 and 2.1% in 2007.^{2,5,6} Similar trends have been reported from the UK⁸; however, studies from Canada¹² and a newer one from the UK suggest the prevalence has stabilized.¹³

Peanut allergy also represents the most common cause of anaphylactic reactions in children, with its incidence increasing between five and sevenfold over the past 10 years.^{14,15} It accounts for the majority of severe food-related allergic reactions and has the highest lifetime risk for both anaphylaxis and anaphylaxis mortalities.¹⁶ Exposure to even trace amounts of peanut can induce a serious reaction, including anaphylactic shock.

These facts result in significant effects on quality of life of patients and their families. In addition, the widespread distribution of peanuts (PN) in food products not only complicates their avoidance but also increases the risk of allergic reactions through accidental consumption.^{2,16} Hence, an accurate diagnosis of PNA is vital. However, while the diagnosis is often obvious just based on the clinical history, in many cases it is far less clear given the imperfect nature of food allergy

testing. For example, it has been shown that fewer than 30% of pediatricians and family practice physicians felt comfortable interpreting laboratory tests to diagnose FA.¹⁷ Therefore, there is great interest in developing techniques to improve the diagnosis of PNA and identifying tools that provide better prognostic information.

2 | HISTORY

The cornerstone of the diagnosis of any food allergy is the history. If characteristic clinical signs of an allergic reaction correlate clearly with PN consumption, the history itself may be enough to diagnose PNA, especially if there is confirmation of sensitization by skin or serologic testing. However, for patients without a clear history, further diagnostic procedures may be necessary.¹⁸

It has been found that most children with PNA react to the first oral allergen exposure.¹⁹ As in other typical IgE-mediated reactions, symptoms typically develop within seconds to two hours after consumption. A biphasic or late-phase reaction may also be observed two and eight hours later, and this has been found in about 1/3 of patients with fatal or near-fatal anaphylactic reactions.²⁰

The inducing dose can vary, even from reaction to reaction in the same patient. Reactions may occur after the ingestion of even a few milligrams of peanut protein (one peanut has about 300 mg of protein).²¹ A previous severe reaction is a risk factor for future severe reactions,¹⁸ but overall the ability to predict reaction severity is very limited.

Although the history is a very important element of diagnosis, the information provided by the patient does not always correlate with the results of provocation tests, which are the diagnostic gold standard. It is important to emphasize that when an individual can ingest peanut without symptoms, no further diagnostic testing should be performed given the risk of false-positive test results.

3 | SKIN PRICK TEST

Skin prick tests (SPT) are one means of defining peanut sensitization. While larger SPT reactions are more like to be indicative of true PNA, they do not allow for an assessment of its severity. In general, SPTs have excellent sensitivity and negative predictive value (NPV). However, they also demonstrate poor specificity and positive predictive value (PPV). It is important to emphasize that a negative test result does not always exclude the possibility of PNA, but far more importantly that a positive test without supporting clinical information can by no means be trusted to diagnose PNA.

Many attempts have been made to identify the "cutoff points" of the SPT, that is, SPT values corresponding with a 95% probability of a positive result of an OFC. A PN-SPT wheal ≥ 8 mm has been found to have a 95% PPV in studies in Australia, the UK, and the United States, while a size ≥ 16 mm has been demonstrated in France (Table 1).²²⁻²⁸ Even smaller SPT reactions may be predictive in very young children.²⁵

Key Message

Peanut allergy (PNA) merits particular attention due to its impact on the health and quality of life of millions of patients worldwide. PNA tends to develop in early life and resolves in only 20% of peanut-allergic children. It accounts for the majority of severe food-related allergic reactions. An accurate diagnosis of PNA is vital. In this review, we present the current approach to the diagnosis of peanut allergy, starting from the history, SPT, sIgE, OFC and proceeding to component-resolved diagnostics, basophil activation testing, determination of epitopes for peanut-allergic responses, and suggesting stepwise approaches and predictive models for diagnosing PNA.

4 | SPECIFIC IGE

Similar to the SPT, the presence of peanut-specific IgE (PN-sIgE) in the blood indicates sensitivity to PN, but not necessarily the presence of an allergy. In fact, it has been shown that up to 95% of sensitized individuals from unselected population are not clinically allergic²⁹ and that this is especially common in children with atopy.³⁰

Multiple studies have attempted to define predictive decision points using PN-sIgE.^{5,26,31} In one Dutch study of 103 atopic children with suspected PNA who underwent DBPCFC, the presence of 21.8 kU/L PN-sIgE had a 90% predictive value, while 43.8 kU/L had a 95% predictive value.²⁷ In an Australian birth cohort study of 5276 infants, a PN-sIgE of 34 kU/L had a 95% predictive value.²²

Despite attempts to establish such "cutoff points" for SPTs and sIgE concentrations, it has not been possible to calculate reliable reference values for children which are independent of age, geographical area, or the population under study, that is, referral center vs unselected populations.²²⁻²⁶ These 95% PPVs have proved useful in aiding diagnosis and reducing the number of OFCs that need to be performed. However, a significant proportion of children undergoing investigation for PNA do not yield results that are equal to or above the 95% PPV, falling into an "immunologic gray zone." As always, it is essential that the clinical history be integrated with SPT and sIgE results, still acknowledging that the test results are not always diagnostic and that some patients will need an OFC to confirm or rule out a diagnosis of PNA.³²

5 | COMPONENT-RESOLVED DIAGNOSIS

Another method to characterize peanut sensitization is component-resolved diagnosis (CRD).³³ With this technology, instead of using crude allergen extracts to detect sensitization, CRD measures IgE to purified or recombinant allergenic peanut proteins. The specific pattern of sensitization is important with regard to diagnostic

TABLE 1 PN-SPT and PN-sIgE cutoff points (measured by ImmunoCAP) to assess the likelihood of obtaining a positive or negative OFC and LR in children with PNA²²⁻²⁸

Population	SPT wheal mm			sIgE kU/L (ImmunoCAP)		References
	~95% PPV	~50% NPV	LR	~95% PPV	LR	
UK, Mean 7 y	≥8	≤3	16.9	≥15	8.87	Roberts et al ²⁸
US, Median 4 y				≥14		Sampson et al ²⁶
Australia						
>2 y	≥8		∞	-		Sporik et al ²⁵
<2 y	≥4		∞			
Australia, Mean 4.5 y	≥15		∞	≥10 KU/L	∞	Wainstein et al ²⁴
France, Median 4 y	>16	≤3	∞	>57	∞	Rance et al ²³
Dutch, Median 6.7 y	-			>24.1	24	van Nieuwaal et al ²⁷
Australia, Mean 1 y	≥8		22.2	>34	14	Peters et al ²²

Abbreviations: LR, likelihood ratios (LR ≥ 10 is strongly suggestive of peanut allergy, and a LR ≤ 0.1 strongly allow to exclude allergy); ; NPV, negative predictive value; PPV, positive predictive value; sIgE, specific immunoglobulin E; SPT, skin prick test; y, year.

accuracy and potentially with regard to prognostic implications.³⁴ When used effectively, CRD may reduce the need to perform OFCs.

Of the 17 peanut proteins that have been identified, nine have been recognized as having clinical significance. The characteristics of these peanut components are shown in Table 2.³⁵⁻⁴⁰

The most significant components of peanuts were found to be Ara h 1, Ara h 2, and Ara h 3, also known as specific components associated with primary sensitization to peanuts. Ara h 5, Ara h 8, and Ara h 9 are cross-reacting components, that is, markers for secondary sensitization via primary sensitization to birch and alder tree pollen.

More than 90% of patients with PNA have sIgE to Ara h 1 and 2, whereas between 45% and 95% have sIgE to Ara h 3.⁴¹ Other studies have shown that 80% of patients with PNA are sensitive to Ara h 2,⁴² although cases of allergy to Ara h 2 alone are uncommon. The EuroPrevall study showed that sensitization to Ara h 1, 2, and 3 was usually acquired in childhood and that peanut-tolerant subjects were frequently sensitized to Ara h 8 or 9 but not to storage proteins.⁴³

Few studies compare SPT with CRD. A Dutch study found that CRD is not superior to sIgE to peanut extract or to SPT.⁴⁴ However, other studies suggest that PN-SPT and PN-sIgE should be replaced by Ara h 2 as the routine screening test for PNA.^{42,44-46}

Sensitivity to Ara h 2 typically gives the most accurate prediction for clinical allergy.^{36,37,44} Unfortunately, however unequivocal cutoff values for Ara h 2-sIgE are not clear, with 90% to 95% PPV ranging from 0.35 to 42.2 kU/L.³⁶ The diagnostic value of Ara h 2-sIgE for different cutoff points is presented in Table 3.⁴⁷

Other peanut components, including Ara h 1, 3, and 6, may have diagnostic value,³⁸ and allergic patients without sIgE to Ara h 2, but with Ara h 1 or Ara h 3 sensitization, have also been reported.⁴³ Ara h 6 sIgE appears to have similar diagnostic significance as Ara h 2.⁴⁸ Ara h 8 is homologous to the labile birch protein PR-10 Bet v 1, and

Ara h 8 sensitization is typically associated with tolerance or mild local symptoms.⁴⁹ In addition, individuals who have isolated Ara h 8 sensitization typically have lower PN-sIgE and are sensitized to birch pollen, with only 17% exhibiting clinical symptoms when eating peanuts or demonstrating pollen-food syndrome (PFS).^{39,49}

5.1 | Severity prediction

The significance of CRD in predicting the severity of allergic reactions remains unclear. While studies show that higher concentrations of Ara h 2-sIgE are clearly associated with an increased risk of true PNA, the relationship to reaction severity is inconclusive based on the current data.^{36,50,51} Van Erp et al⁴⁰ concluded that the level of sIgE to Ara h 2 or other components should not be used to classify individual patients at higher risk for severe allergic symptoms during challenge or in daily life.

5.2 | Geographical differences

When interpreting the results, it is important to note that there may be geographical differences of both types and levels of sIgE for individual PN components.^{39,52} Geographical differences were observed for Ara h 8 and Ara h 9, which were major allergens for central/western and southern Europeans, respectively.⁴¹ In the study performed in Southern France, IgE to Ara h 6 were found in 64% PNA children, followed by Ara h 2 (63%), Ara h 1 (60%), and Ara h 9 (52%). Ara h 6 and Ara h 2 were the best predictors of PNA at diagnosis in Mediterranean pediatric patients.⁴⁸ In the United States, patients with PNA were most commonly sensitive to Ara h 1, 2, and 3, while

TABLE 2 Characteristics of peanut allergens³⁵⁻⁴⁰

Peanut allergens	Protein family Type	Heat stability	Clinical implication	Cross-reactivity	Related proteins	% of sensitization in PNA patients
Ara h 1	Cupin 7S globulin Seed storage proteins	+++	Correlated with reactivity	+	Cor a 11 (hazelnut) Gly m 8Gly m 5 (soybean) Ana o 1 (cashew) Pis v 3 (pistachio) Jug r 2 (walnut) Ses i 3 (sesame) Len c1 (lentil) Pis s 1 (pea) Lup an 1 (lupin)	26-92
Ara h 2	Conglutin 2S albumin Seed storage proteins	+++	Best correlation with reactivity/severity	+	Cor a 14 (hazelnut) Gly m 8 Gly m 1 (soybean) Ana o 3 (cashew) Pis v 1 (pistachio) Jug r 1 (walnut) Ber e1 (pecan) Ses i 1, Ses i 2 (sesame) Sin a 1 (mustard)	60-100 (Western Europe, US) 60 (Mediterranean region)
Ara h 3/4	Cupin 11S globulin Seed storage proteins	+++	Correlated with reactivity	+	Cor a 9 (hazelnut) Gly m 9 Ana o 2 (cashew) Pis v2 (pistachio) Pru du 6 (almond) Jug r4 (walnut) Ber e 2 (pecan) Ses i 7, Ses i 6 (sesame) Sin a 2 (mustard)	20-80
Ara h 5	Profilin Pollen-associated	-	Not correlated with reactivity	+++	Bet v 2 Gly m 3 (soybean) Cor a 2 (hazelnut) Pru du 4 (almond) Hel a 2 (sunflower) Sin a 4 (mustard)	
Ara h 6	Conglutin 2S albumin Seed storage proteins	+++	Correlated with reactivity	+	Cor a 14 (hazelnut) Gly m 8 Gly m 1 (soybean) Ana o 3 (cashew) Pis v 1 (pistachio) Jug r 1 (walnut) Ber e 1 (pecan) Ses i 1, Ses i 2 (sesame) Sin a 1 (mustard)	60-100
Ara h 7	Conglutin 2S albumin Seed storage proteins	No data	No data	No data	Cor a 14 (hazelnut) Gly m 8 Gly m 1 (soybean) Ana o 3 (cashew) Pis v 1 (pistachio) Jug r 1 (walnut) Ber e 1 (pecan) Ses i 1, Ses i 2 (sesame) Sin a 1 (mustard)	
Ara h 8	Bet v1 family (PR-10, Bet v 1 homolog)	-	Not correlated with significant reactivity, Pollen-associated	+++	Bet v 1 (birch) Mal d 1 Cor a 1 (hazelnut) Cas s 1 (chestnut) Gly m 4 (soybean)	

(Continues)

TABLE 2 (Continued)

Peanut allergens	Protein family Type	Heat stability	Clinical implication	Cross-reactivity	Related proteins	% of sensitization in PNA patients
Ara h 9	Lipid transfer protein Plant pan allergen	++	Correlated with reactivity mainly in the Mediterranean area	+++	Pru p 3 (peach) Art v 3 (mugwort) Cor a 8 (hazelnut) Pru du 3 (almond) Jug r 3 (walnut) Cas s 8 (chestnut) Hel a 3 (sunflower) Sin a 3 (mustard) Len c 3 (lentil) Pha v 3 (green bean)	
Ara h 10 Ara h 11 Ara h 14 Ara h 15	Oleosin		Marker allergens for severe reactions		Cor a 12 Cor a 13 (hazelnut) Ses i 4 (sesame) Ses i 5 (sesame)	

those in Spain were sensitive to Ara h 9, those in Sweden to Ara h 8, and those in Austria to Ara h 2 and Ara h 6.^{37,53} Geographical differences also apply to cutoff values, for example: A 90% PPV for Ara h 2 was found for 14.4 kU/L in a German study,³⁶ whereas the same cutoff was closer to 2 kU/L in the United States.³⁹

5.3 | Stepwise approach

It has been suggested that a stepwise approach can be employed for the use of component testing. If patients have PN-sIgE levels >25 kUA/L or <0.35 kUA/L, or if they are not sensitized to birch tree pollen, component testing may not add additional information.³⁹

Although CRD does not eliminate the need for OFC in many cases, it increases the chance of establishing a correct diagnosis of PNA. It is important to recognize that CRD must be used and interpreted in the context of each individual patient, with age, place of residence, other allergic sensitivities, and many other factors under consideration.³⁹ Sicherer and Wood emphasize that CRD should be used judiciously and indicate when component testing is more or less useful (Table 4).³⁹

6 | ORAL FOOD CHALLENGE

Peanut food challenges (PN-FC) are performed in the same way as other oral food challenge (OFCs). A number of papers discuss the methodology of FC, such as the European EAACI position paper,⁵⁴ the AAAAI Work Group,⁵⁵ and the joint EAACI/AAAAI PRACTALL.⁵⁶ However, although the PRACTALL protocol is used for research purposes, there is no international consensus on OFC methodology in clinical practice. A retrospective analysis of data from 200 patients from pediatric allergy units in Switzerland and the UK highlights how differences in performing PN-FCs might influence outcomes, demonstrating the need for standardization of OFC protocols.⁵⁷

Although the search continues for newer and better methods of diagnosing FA, the double-blind placebo-controlled oral food challenge (DBPCFC) remains the gold standard. Usually, DBPCFCs are conducted in research settings, while open challenges are better suited to clinical practice.⁵⁸ Although OFCs have great diagnostic value, they carry significant risk. Therefore, the aim of the preliminary diagnostic stage is to determine whether PNA is highly likely with little reason to do an OFC, or whether the OFC should be performed with the aim of ruling out PNA.³⁹ For patients without any clear clinical history or laboratory evidence that points toward PNA, a physician-supervised OFC is necessary to confirm or exclude PNA. The other important indication for peanut OFC is the introduction of peanuts in patients who are sensitized but have not eaten peanuts, or in children with PNA after a period of avoidance, to evaluate the possibility of oral tolerance acquisition. OFCs are also used in research, even if a positive result is expected, with the aim of broadening knowledge on the pathophysiology of PNA, to determine a threshold for reactivity, or evaluate the effects of novel immunotherapies for PNA. Another indication for peanut FC applies to infants following the new recommendations based on the results of the LEAP study.⁵⁹

6.1 | Interpretation of results

There is no consensus definition for a positive OFC. While an OFC is considered positive when objective allergic symptoms are observed, subjective symptoms like behavior changes, itchy skin, throat tightness, nausea, or abdominal pain, especially if repetitive or persistent, may also indicate a positive OFC. The severity of OFC reactions may also be graded based on the constellation of symptoms.⁶⁰

When interpreting the results of PN-FC, it is also important to consider the possibility of obtaining false-negative or false-positive results, as well as indeterminate results. Negative DBPCFCs usually need to be subsequently confirmed by an open challenge to rule out

false-negative results.⁴ However, reintroduction fails in a subset of children.⁶¹ Furthermore, sometimes the OFC outcome is doubtful, especially in very young children who start to vomit or cry, or in children who display changes in behavior.⁵⁷

6.2 | Risk factors for severe OFC reactions

Several studies suggest that the level of sIgE can sometimes correlate with the severity of a PNA reaction,^{62,63} although others show opposite results.^{23,28} Although usually SPT or sIgE does not predict clinical severity, if a patient is found to be sensitized only to PR-10 homologues and has a clinical history of only mild reactions, it may be reasonable to assume that the risk of a future severe reaction is low and PFS is possible.

Gupta et al indicate that the sIgE/total IgE ratio is more accurate than sIgE alone in predicting outcomes of challenges performed to confirm PN tolerance.⁶⁴ Another ratio which may be used to predict severity of reaction during peanut OFC is the BAT CD63 ratio (ratio of peanut-stimulated basophils to anti-IgE-stimulated basophils), together with the history of exercise-induced asthma, and FEV1/FVC ratio at the time of DBPCFC.⁶⁵

6.3 | Doses, intervals, new protocols

The initial and total dose of the challenge should be adjusted according to the clinical history and age. Bird et al suggest that the majority of open OFCs conducted in clinical practice in carefully preselected, low-risk patients may be performed more safely and in a more cost- and time-effective manner, by administering fewer doses, that is, three rather than six or seven doses (PRACTALL),⁵⁴ and administered at intervals less than 30 minutes apart.⁶⁶

The findings concerning the relationship between threshold dose and reaction severity are varied. According to Ludman et al,⁵⁷ the higher doses of PN do not increase the severity of reactions. Three patterns of reactivity in patients undergoing OFC were reported as follows: those with non-anaphylactic symptoms despite consuming the highest dose, those with initially mild symptoms with anaphylaxis upon increasing doses, and those with anaphylaxis as an initial symptom, often without prior subjective symptoms.⁶⁷

Oral food challenge studies have shown that low individual reaction thresholds do not necessarily correlate with severe reactions. Recently, novel FC protocols were introduced which allow better evaluation of thresholds. In the PAT (Peanut Allergen Threshold) study, a novel single-dose protocol was used to assess the ED05, that is, the dose that elicits a reaction in 5% of allergic subjects. It was estimated that a dose of 1.5 mg of peanut protein (or 6 mg of whole peanut or approximately 1/100th of a peanut kernel) constituted the ED05. They have shown that the single-dose protocol does not replace current clinical OFC, but would provide extra clinical information about patient's level of risk, related to dose. Single-dose challenges could be performed before starting a progressive clinical OFC to identify the most sensitive patients and reduce any risks associated with the use of higher doses used in clinical OFC. The protocol was very acceptable to families and was clinically very safe. In addition, this single-dose protocol is easier to perform than routine diagnostic OFCs and it improved food allergy-related quality of life.⁶²

Therefore, OFC allows not only for PNA to be confirmed or ruled out, but also for the threshold of reactivity to be established. The approach has important clinical implications, including identifying the possibility of tolerance of peanut traces in foods, evaluating the feasibility of peanut immunotherapy, and improving quality of life. The proposed diagnostic algorithm for PNA in clinical settings is presented on Figure 1.

6.4 | Other methods of provocation

The peanut conjunctival allergen provocation test (CAPT) was recently shown to be feasible and of diagnostic value in PNA and could potentially be a safe in vivo alternative to OFC. The sensitivity and specificity of the CPT were 0.96 and 0.83.⁶⁸ Although not sufficient to replace other diagnostic methods, both CRD and CAPT may improve diagnostic accuracy.^{18,68,69}

7 | THE BASOPHIL ACTIVATION TEST

The basophil activation test (BAT) is a flow cytometry-based assay which measures the presence of activation markers, such as CD63 and CD203c on the basophil cell surface. CD63 detection is highly

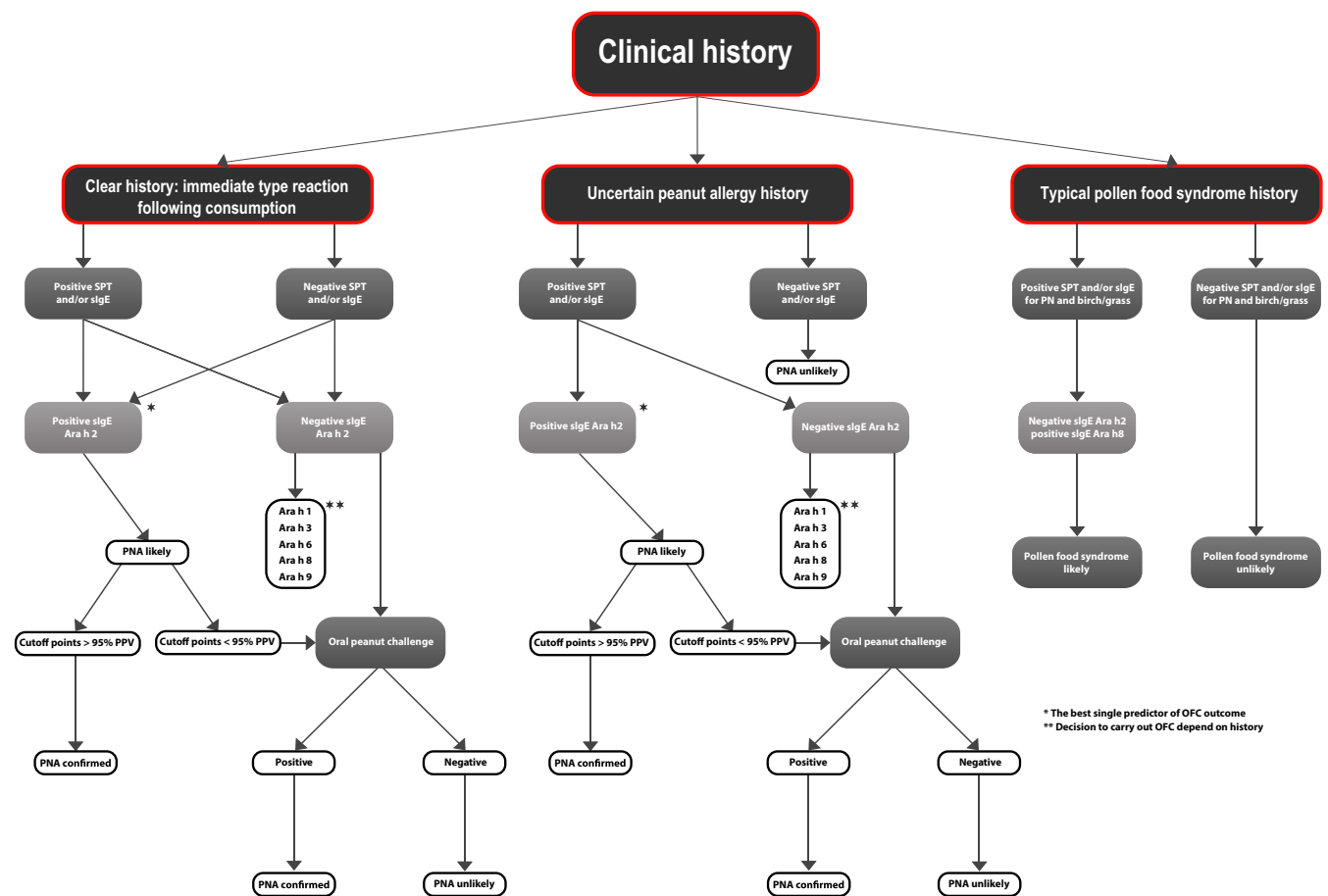
Cutoff point (kU/L)	PPV (%)	NPV (%)	Sensitivity (%)	Specificity (%)
≥0.2	71	92	94	66
≥0.35	74	90	91	72
≥0.5	75	83	83	75
≥1	81	79	74	85
≥2	87	77	70	91
≥5	96	71	55	98
≥10	100	66	43	100

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

TABLE 3 Diagnostic value of the predictor sIgE to Ara h 2 for different cutoff points⁴⁷

TABLE 4 Considerations in obtaining peanut component tests³⁹

Factors that make component testing LESS likely to be informative	Factors that make component testing MORE likely to be informative
A recent convincing clinical reaction	Mild reactions or no reaction history
A remote significant clinical reaction in a patient with PN-IgE > 15	Remote clinical reaction with development of birch sensitization over time
PN-IgE > 25 or < 0.35 kUA/L	PN-IgE 0.35-15 kUA/L
Lack of birch sensitization	Birch sensitization
Younger children	Older persons

**FIGURE 1** Diagnostic algorithm for PNA in clinical setting. Ara h 1, 3, 6, 8, and 9, peanut components; OFC, oral food challenge; PNA, peanut allergy; PN, peanut; PPV, positive predictive value; slgE, specific immunoglobulin E; SPT, skin prick test

specific for basophils anaphylactic degranulation. When stimulated with specific food allergens that cross-link FcεRI and prompt granule exocytosis, CD63 becomes highly expressed on the surface of activated basophils. A 4% reduction (from 5% to 1%) in false-negative results can be achieved with the addition of CD203c (the basophil-specific marker) to the gating strategy. This altered gating strategy has been shown to enhance the sensitivity of BAT from 88% to 98% and its specificity from 94% to 96%. This translates to a 6% improvement in PNA diagnosis by BAT (from 91% to 97%).⁷⁰ As a result, Santos et al suggest that BAT may be regarded as OFC in a test tube, becoming an alternative for standard OFC, by exposing basophils involved in acute allergic reactions to a food extract in a test tube, instead of eating the food by a child.⁷⁰

The largest study to date assessing the use of BAT in the diagnosis of PNA was published in 2014 by Santos et al. The study involved 105 children, who received BAT and SPT, slgE. The use of BAT made it possible to reduce the amount of OFC by 2/3. The sensitivity of the test was found to be 97%, and the PPV and specificity were almost 100%. The authors of the study conclude that BAT will significantly improve PNA diagnosis with standard skin and IgE testing.⁷¹

The high specificity of BAT is an important addition to existing allergy tests, such as SPT and slgE. A positive BAT would confirm the diagnosis of PNA with a high degree of certainty, resulting in a reduction in positive OFCs, which would be most desirable in clinical practice.⁷²

TABLE 5 The advantages and disadvantages of diagnostic methods for PNA

Method	Advantages	Disadvantages	Comments
History	• If it is unambiguous, it may be enough to confirm • Provides information about epidemiologic factors, hence can be used to improve diagnostic utility • Allows for determination of pretest probability • Is the most important test for all patients who have previously consumed peanut	• In some patients, there is no history of peanut ingestion/ prior reactions • The history may not be clear even with prior ingestion	Must address the essential questions: • Is there a relationship between PN consumption and the occurrence of a reaction, • After what time the symptoms occurred, • After what dose of allergen, • What symptoms were observed, • When did the last similar reaction occur are there comorbid conditions
SPT and/or sIgE	• Positive tests confirm IgE sensitization • High sensitivity and NPV: Negative tests usually rule out clinical PNA • The cutoff points allow determination of 90 or 95% PPV, making OFCs unnecessary in some children • Increasing SPT/ sIgE levels are associated with increased likelihood of true PNA	• Low specificity and PPV • Set cutoff points differ depending on the studied population (unselected, ie, birth cohort vs. selected, ie, referral center) • The cutoff points with PNA risk are not always diagnostic • Negative tests with convincing clinical history warrant caution and further evaluation • Full spectrum of severity can occur even at low PN-sIgE levels • IgE levels do not accurately predict reaction severity	• They are necessary to confirm the diagnosis of PNA • They require standardization • Highly predictive cutoff values are dependent on country and population tested
OFC	• A positive result confirms PNA • A negative result excludes PNA	• Time-consuming • Expensive • Risk of anaphylaxis • Sometimes difficult to interpret results • False-negative and false-positive results can occur • Require specialized personnel • OFCs need to be performed in a supervised environment with the facilities and expertise to treat allergic reactions and anaphylaxis	• Still it is the "golden standard," although studies are still under way to reduce the need to perform OFCs
CRD	• Ara h 2 may provide better predictive accuracy than SPTs and sIgE • Improves decision-making on whether or not to carry out an OFC • May predict severity of reaction • Able to identify positive tests due to cross-reactivity • Might be able to predict resolution of peanut allergy	• Does not replace OFCs • Cutoff points of sIgE to Ara h 2 that predict tolerance or allergy vary with age, geographical region and study populations, and validation studies performed in different settings would be necessary to implement cutoffs in daily practice • Levels of sIgE to Ara h 2 or other components are correlated to severity but cannot be used to classify individual patients at higher risk for severe allergic symptoms	• Geographical differentiation should be taken into account when interpreting the results (peanut-allergic patients have different sensitization patterns in different countries) • It should be noted that some currently available cutoff points were estimated in a selected groups of referral patients and, therefore, cannot be generalized to other centers without validation studies. • Data in young children (<4 years) are currently lacking

(Continues)

TABLE 5 (Continued)

Method	Advantages	Disadvantages	Comments
BAT	• Determines actual basophil reactivity in the presence of all the antibodies in serum • May replace OFCs in many patients • Basophil activation can be determined up to high doses of allergen regardless of disease severity • Possible to perform in children with extensive eczema • Safe • Requires a small volume of blood (about 1 mL)	• Promising, but not yet feasible for clinical practice (available only in research settings) • BAT requires expertise to perform a flow cytometry-based assay • The test requires fresh blood (<24 h) since blood collection	• Needs to be performed on fresh blood relatively soon after collection • Basophils may be already be activated in some patients • Requires further standardization and validation • Likely much cheaper and certainly safer than an OFC, but more expensive than SPT or sIgE
Epitope testing	• May be a better marker of clinical reactivity • May add more information about prognosis, including reaction severity and persistence of allergy	• Only for research studies at the present time	• Improved technologies should allow this to move to the clinical arena if value is confirmed with larger studies

BAT can also provide information about the severity and the threshold of allergic reactions. These data, in addition to the presence of other clinical characteristics known to be risk factors (eg, persistent asthma), might enable the clinician to identify high-risk allergic patients who require closer follow-up and more intensified education.

Similar to other laboratory methods, van Erp et al⁴⁶ propose that better prediction of peanut-allergic status can be achieved using a stepwise approach based on Ara h 2-sIgE followed by BAT. Unfortunately, due to the need to use fresh blood in the BAT assay, it has been difficult to adapt this test into clinical practice, remaining primarily a research procedure.

7.1 | IgE-BINDING EPITOPES

Thanks to advances in molecular biology and analytical chemistry, a number of allergen molecules have been identified, and their three-dimensional structures have been determined. Epitope mapping of T- and B-cell epitopes is an important tool for understanding antigen components involved in food allergic reactions that could possibly lead to novel diagnostic tools.^{73,74}

As a result of the studies based on the analysis of peptide microarrays, after the exclusion of a few immunodominant epitopes, a significant differentiation in the epitope binding patterns between individual allergic patients has been demonstrated. Upon close evaluation of the epitopes, it was found that certain amino acids were key drivers of reactivity in some patients, while others were critical for antibody binding in all patients. The authors conclude that the use of a new generation of peptide microarrays enabled the description of linear epitope patterns at the single amino acid level. The method is scalable to large patient cohorts and can be used as a standardized tool to follow the immune response in great detail.⁷⁵

According to several studies, sequential IgE-binding epitopes may play a role of biomarkers for characterizing numerous phenotypes of FA. In a retrospective study of peanut-allergic patients, higher IgE sequential epitope diversity to Ara h 1, Ara h 2, and Ara h 3 was found

in patients with a history of more severe allergic reactions to peanut.⁷⁴ In addition, sensitization of effector cells with sera containing more diverse IgE antibodies conferred greater effector response to specific allergens according to BAT. A positive correlation between IgE epitope diversity, as represented by the number of epitopes recognized, and clinical sensitivity was confirmed by Flinterman et al,⁷³ who also found that a higher ratio of sequential epitopes than conformational epitopes predicted a more persistent and severe course of FA.

The application of IgE epitope mapping in the diagnosis and prognosis of FA is currently limited. Although positive IgE binding demonstrates sensitization to a peptide or protein, it is not the same as the allergens cross-link IgE on mast cells or basophils *in vivo*. As such, IgE binding to single epitopes of allergenic components may not be sufficient to provoke clinical symptoms. Therefore, several studies of IgE-binding epitopes have tried to assess *in vitro* IgE binding with a more biologic system, such as the BAT.⁷⁴ A basophil-mediated microarray immunoassay was recently developed, in which the printed spots of protein or peptide-containing allergenic epitopes were not detected with patient's serum IgE but with human basophils passively sensitized with patient's serum.⁷⁶

While still a test in development, the measurement of serum IgE antibodies specific to epitope peptides may eventually be useful for the diagnosis (better diagnostic efficiency than the use of whole allergen) and prognosis (correlation has been found between linear IgE epitope diversity and severity and persistence of PNA) of FA in clinical practice. The identification of epitopes is also of potential value in the development food protein modifications that can reduce or eliminate IgE binding of the allergen while still retaining immunoreactivity for the development of peptide immunotherapies for the treatment of FA.

The advantages and disadvantages for each of the diagnostic methods for PNA are presented in Table 5.

8 | PREDICTIVE MODELS

Considering the ongoing difficulties faced in the diagnosis of PNA, as in other types of FA, attempts have been made to create

mathematical/computer models incorporating a range of factors (including sex, age, history, SPT, sIgE, and total IgE minus PN-sIgE), with the aim of increasing the accuracy of diagnosing and assessing the risk of occurrence of severe PNA. It was found that the proposed algorithm had a better specificity and sensitivity for predicting allergy than testing sIgE, or using SPT or a combination of the two.⁷⁷ Klemens et al⁴⁷ evaluated whether additional candidate predictors such as atopic dermatitis, allergic rhinitis, and sIgE to the peanut-specific components Ara h 1, 2, 3, and 8 could increase the diagnostic accuracy.

In addition, the analysis of sIgE for peanut extract and peanut-specific components constituted the basis for developing another prognostic model. The comparison of the diagnostic value of this model to DunnGalvin's validated model⁷⁷ shows that both models can reduce the number of OFC by at least 50%.⁴⁷ Although the predictive models may be a promising tool in the diagnosis of FA, it is currently not recommended to use it in practice due to the large diversity of results, various factors included in the models, lack of uniformity, precision, different populations, and they are time consuming. Moreover using mathematical models for estimating the risk of disease, we must be aware of limitations, both methodological and of data quality, that make it difficult to estimate individual risk.

It is important to bear in mind that although allergy tests are useful in diagnosing FA, a number of factors influence pretest probability, such as age, concomitant atopic disease, population type, and geographical location.

9 | CONCLUSIONS

Even with the wide range of diagnostic tests for the diagnosis of PNA, the history and OFC remain most important. With new tests in development, the diagnosis of PNA is likely to improve over the coming years. Although not available today for clinical practice, the combination of CRD, BAT, IgE epitope mapping, and high-throughput microarray platforms may be the assays of the future, offering improved diagnostic and prognostic accuracy.

CONFLICT OF INTERESTS

We certify that there is no actual or potential conflict of interest in relation to this article.

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