

In Vivo Diagnosis: Skin Testing and Challenge Procedures

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KEY CONCEPTS

- The medical history is the cornerstone for establishing an accurate diagnosis of food allergy.
- Prick skin tests determine sensitization (presence of food-specific IgE) and provide significant diagnostic value when considered in the context of the medical history.
- Increasingly larger skin test wheals are associated with increasing risks for clinical reactions.
- Response to elimination diets may provide presumptive evidence of food-related disease.
- The oral food challenge, in particular when double-blind and placebo-controlled, is the most definitive modality available to diagnose a food-related illness.

Introduction

This chapter focuses on food allergen skin tests, obtaining an informative medical history, and decision making in regard to undertaking physician-supervised oral food challenges (OFCs). These *in vivo* modalities provide immediate diagnostic information that is crucial in the evaluation of an individual with suspected food allergy. The OFC is the tool at the physician's disposal that can most definitively diagnose an adverse reaction to food. The results of an OFC are informative and do not depend on the specific etiology or immunopathology of an adverse reaction to a food, whether the problem is due to intolerance, a pharmacologic response, an allergic (immunologic) reaction, or a psychological one. While potentially definitive, OFCs carry risks because severe reactions may be induced. The clinician, therefore, must also rely upon patient histories and a number of additional tests to help determine the likelihood of a true allergy or adverse reaction to food prior to, and sometimes in place of, undertaking an OFC. For allergic reactions that are mediated by IgE antibody, the tests most familiar to the allergist are the prick skin test (PST), a focus of this chapter, and the determination of serum food-specific IgE antibodies (Chapters 19 and 21), and possibly patch tests (Chapter 22). Numerous additional tests

may be needed in various clinical scenarios (e.g. stool culture, endoscopy with biopsy, pH probe, breath hydrogen) to assist in determining if an adverse reaction to food is the cause of a clinical problem. In addition, refinements on currently available tests, clinical evaluations of proposed tests (e.g. patch tests with food), and additional novel tests are under investigation to improve and expand the diagnostic armamentarium. Despite the potential for inaccurate histories and various limitations of *in vitro* and *in vivo* tests, the OFC can provide a final diagnostic answer. Oral challenges designed to be double-blind and placebo-controlled so as to reduce subject and observer bias are considered the "gold standard" for the diagnosis of food hypersensitivity.

Historical background

The typical diet includes several meals and snacks distributed throughout the day. Since the frequency of food intake is high, any sudden adverse physiologic event or chronic illness could incorrectly be ascribed to food. Once a patient makes an erroneous association between a food and a symptom, it may be difficult to dissuade the patient from their notion of cause and effect. In a paper published in 1950, Graham and colleagues [1] performed experiments that would be difficult to undertake today for ethical reasons. Subjects with strong beliefs regarding their reactions to foods were given water by nasogastric tube and told they were receiving the test food and were given the test food and advised that the water was being instilled. Reactions to the tests

correlated with suggestion. To address the evident subject bias, masked ingestions were introduced by Loveless in several studies in the 1950s [2,3]. In an accompanying editorial, Lowell [4] emphasized the need for blinded challenges to demonstrate cause–effect relationships in the evaluation of adverse reactions to foods. Charles May is credited with bringing double-blind, placebo-controlled oral food challenges (DBPCFCs) into routine clinical practice and research use [5].

By the late 1980s, a number of seminal points concerning the epidemiology of food hypersensitivity were confirmed and refined through the use of masked and placebo-controlled OFCs. Challenges confirmed the role of food allergy in chronic disease such as childhood atopic dermatitis [6] and in immediate reactions [7], and determined that 6–8% of young children experience genuine adverse reactions to foods, but that most of the sensitivities resolved in early childhood [8]. The types of symptoms elicited by foods were confirmed to be most commonly associated with the skin (hives, atopic dermatitis), gut, and respiratory tract, and not commonly with behavioral problems [6–11].

The foods involved were generally confined to a rather limited number with children affected by allergy to cow’s milk, egg, soy, wheat, peanut, and tree nuts, and older individuals to peanut, nuts, and seafood. The inaccuracy of the patient’s history in regard to the relationship of food allergy to chronic disease was also underscored by several studies with an accuracy generally under 40% when compared to blinded food challenges [7,12–14]. In addition, food additives/preservatives were not a frequent cause of problems [11].

DBPCFCs are now a fundamental tool for scientifically establishing a number of important features of food hypersensitivity reactions. Studies have broadened our understanding of the spectrum of food hypersensitivity disorders. A growing number of studies point out the role of food hypersensitivity in isolated gastrointestinal disorders [15]; however, food allergy is not a frequent cause of isolated chronic respiratory disease [16]. The number of foods proven to cause reactions is ever expanding and includes seeds, fruits, vegetables, meats, and virtually every type of spice [17,18]. Despite advances in *in vitro* and *in vivo* diagnostic tests, the OFC has remained the final endpoint (“gold standard”) to determine clinical tolerance or reactivity to food. Table 20.1 summarizes the early and recent advances in our understanding of food hypersensitivity obtained through OFCs.

Prick skin tests

Tests to detect food-specific IgE antibody are central to identify or exclude foods responsible for immediate type, and some chronic disease-inducing food-allergic reactions (e.g. atopic dermatitis and eosinophilic gastroenteropathies). The most familiar, convenient, and commonly used method is prick/puncture skin testing (PST). The intradermal form

Table 20.1 Features about adverse reactions to foods determined through studies using OFCs

Epidemiology	6–8% of children 1–2% of adults Most common foods: egg, milk, peanut, tree nut, seafood, soy, wheat Increasingly wide variety of foods
Associated disorders	Anaphylaxis (acute skin, gut, respiratory and cardiovascular reactions) Atopic dermatitis (~35% with moderate skin disease) Numerous gastrointestinal disorders
Infrequently associated disorders (2–5%)	Isolated chronic respiratory disease Chronic urticaria
Clinical symptoms only rarely, or possibly not associated	Behavioral disorders Neurologic disorders

of allergen skin testing was introduced by Blackley [19] over 100 years ago, and the prick test was described by Lewis and Grant in 1924 [20]. The technique of PST is simple, but specific variations exist. While the patient is off antihistamines for an appropriate length of time, a device such as a needle, bifurcated needle, probe, or lancet is used to puncture the epidermis through an extract of a food. Appropriate positive (histamine) and negative (saline–glycerine) controls are also placed. The test site is examined 10–20 minutes later. A local wheal and flare response indicate the presence of food-specific IgE antibody. A mean wheal diameter 3 mm or greater compared to a saline control is generally considered positive [21,22], but interpretation will be discussed in more detail below. Of course, the test would not be expected to be positive for food reactions that are not mediated by IgE antibodies, such as several of the infantile gastrointestinal disorders including food-protein-induced enterocolitis syndrome and proctocolitis. Clearly, the PST is an invaluable screening tool for the allergist. However, the clinician using PSTs for the diagnosis of food hypersensitivity must be aware of the utility and limitations of the test in order to use it to the best advantage for clinical and research purposes.

Technical considerations

Skin test results are influenced by variables such as test reagents, type of skin test device, location of test placement, patient factors, and methods of measuring results. The selection of skin test reagent is of primary importance. Unfortunately, standardized food extracts are not currently available despite a clear, long-standing recognition for the need [21,22]. Commercial extracts are usually prepared as glycerinated extracts of 1:10 or 1:20 dilution. With the lack of standardized extracts, it is well recognized that variations exist in allergen distribution and concentration between lots

and manufacturers [23,24]. The problem of protein stability must also be considered. An example demonstrating the liability of certain food extracts is the evaluation of food allergy in pollen-food syndrome (oral allergy syndrome to fresh fruits and vegetables). Patients may react to the uncooked, but not the cooked form of the food and this may similarly be reflected in skin test results as commercial extracts may lack the ability to display the labile proteins involved [25]. For the evaluation of allergy to fresh fruits and vegetables, and possibly other foods, many authorities have suggested the use of fresh foods (e.g. fresh milk, egg white, fruits, and vegetables) [26]. The PST can be performed using liquid foods, by creating an in-house extract, or using a prick-prick technique (pricking the fruit and then the patient, thereby transferring the juice) [27]. Presumably, such in-house reagents are more concentrated and this may increase sensitivity, a possible deficit in some circumstances, and may increase the risk for side effects from the test itself. The impact of allergen concentration on wheal size is somewhat tempered by the fact that wheal size increases by a factor of ~ 1.5 for each logarithmic increase in concentration [28].

The device used for pricking the skin, and the technique used with any given device, may also influence the results. A variety of devices are on the market for introducing the allergen into the epidermis. As may be imagined, the more penetration, the more likely there will be a response and so the area and depth to which the allergen is introduced is pertinent. Therefore, the configuration of the device, the pressure applied by the operator, and the time over which pressure is applied must be considered [29]. Test results also vary according to the location on the body on which they are placed. For example, the back is $\sim 20\%$ more reactive than the arm [30]. Studies that evaluate histamine reactivity indicate that wheals become detectable in early infancy and increase in size with age until adulthood [31,32]. These physical and patient variables become relevant when comparing study results, and for clinical decision making. In practice, consistency of materials and procedures, and review of precision (coefficient of variation should be $<20\%$ for wheal diameter) should be undertaken by comparing repeated tests by personnel administering them. Various single- and multi-headed devices are available, with significant differences in all areas of device performance among all devices examined. In one study, multi-headed devices were judged more painful than single devices and had larger reactions on the back, whereas single devices had larger reactions on the arms [33].

Additional variations concerning PSTs are the timing at which they are read and the manner in which they are measured and reported. The histamine test peaks at 10 minutes while allergen wheal size generally peaks at 15–20 minutes [34]. One suggested method of measurement is to determine the greatest wheal (or flare) diameter, its perpendicular maximum diameter, and to determine the mean of these

Table 20.2 Aspects that impact sensitivity of PSTs

Feature	Correlation with sensitivity
Extract concentration	Direct
Device used	Variable
Pressure applied during application	Direct
Location	Back > volar aspect arm
Reporting progressively larger reaction sizes (e.g. wheal 4 mm instead of 3 mm) as categorically positive	Inverse

two measurements [34]. However, many researchers report the longest diameter, which is less time consuming to measure but presents, on average, a higher value than the mean diameter. Presenting the result in millimeters is preferred, with additional presentation of the size of the histamine and saline controls for comparison. The measurement of the saline control is typically subtracted from the allergen and histamine results to account for dermographism. Thus, a positive test (reflecting IgE) is generally regarded as one with a mean wheal diameter at least 3 mm greater than the saline control. In practice, reporting of results often varies by investigator and may be reported as mean diameter, mean diameter compared to histamine control categorically (e.g. 1+, 2+, etc.), or as a calculated area. Studies must be evaluated carefully because individual investigators may be reporting data based on a variety of methods that may not be directly comparable (e.g. mean wheal diameter versus largest diameter). Despite the numerous potential confounding variables involved in the PST procedure, the clinical utility is excellent. Technical issues that can impact PST sensitivity are summarized in Table 20.2.

Diagnostic value

The ability of a test to indicate the presence or absence of disease depends on intrinsic characteristics of the test itself and also features of the population on which it is being applied. The PST is excellent for detecting food-specific IgE antibody and when it is negative, it is highly likely that there is none, and that no IgE antibody-mediated allergic reaction would occur to the tested food (excellent negative predictive accuracy, NPA). However, this conclusion, when considering the individual patient, depends strongly on the prior probability of true allergy, a concept discussed further below. Obviously, a negative result does not exclude the possibility of cell-mediated allergic reactions or intolerance. To complicate matters for the allergist and patient, the presence of IgE to a food often does not equate with clinical reactions; that is, there is often ($\sim 50\%$) clinically inconsequential sensitization. Again, this statement depends on the prior probability of risk of true allergy in the study population. For example, skin testing to peanut in the general

population (no selection for allergy) performed in the United States showed 8% had a positive PST [35], yet population-based studies of true allergy to peanut show that only 0.6% are allergic [36]. Therefore, in unselected patients, one could conclude that nearly 93% of positive tests are “false positive.”

The sensitivity and specificity of a test provide information about its ability to identify a known condition. Sensitivity refers to the proportion of patients with an illness who test positive, and for IgE-mediated food allergy, the sensitivity of the PST is usually high (>80%). Specificity refers to the proportion of individuals without the disorder who test negative, and for IgE antibody-mediated food allergy, specificity of the PST is usually lower than the sensitivity but usually better than 50% [26,37,38]. Sensitivity and specificity are impacted by intrinsic properties of the test (Table 20.2), but the clinical question of import to the physician concerns the probability that a patient has food allergy if the test is positive (positive predictive accuracy, PPA) or does not have food allergy if the test is negative (negative predictive accuracy, NPA). The predictive accuracy is impacted by the prevalence of the disorder in the population being tested (or as applied to the individual, the prior probability that the person being tested has the disorder). In studies using referred patients with an increased probability of disease, and a definition of positive PST as one with a mean wheal diameter of ≥ 3 mm, PSTs have an excellent NPA (~90%) but the PPA is on the order of only 50% [26,37,38].

The definition used to indicate a positive test (or degree of positive) will additionally impact the PPA and NPA. For example, increasing skin test size correlates directly with increasing IgE antibody and the risk of clinical reactions. Therefore, if one were to analyze skin test sizes (rather than just labeling them categorically as positive or negative at a mean wheal size of 3 mm), there would be variation in sensitivity and specificity with each incremental change in size. In general, as the definition of a positive test requires a larger wheal, specificity increases and sensitivity decreases. Receiver operator curves are used to display the association of test size defined as positive with sensitivity and specificity that must be determined experimentally (Fig. 20.1). The uppermost left quadrant on the curve would be the point where combined maximum sensitivity and specificity could be achieved. Similarly, as “cut-off” for positive increases, so does PPA while the NPA simultaneously decreases. Since these indices of predictive value are population dependant, the predictive value drops (illness is overestimated) when results obtained in a referral center (high prevalence) are applied to unselected individuals.

An additional way of considering the meaning of a test is to consider the chance that a person with food allergy would have a positive test compared to the chance that one without food allergy would have a negative test. This ratio

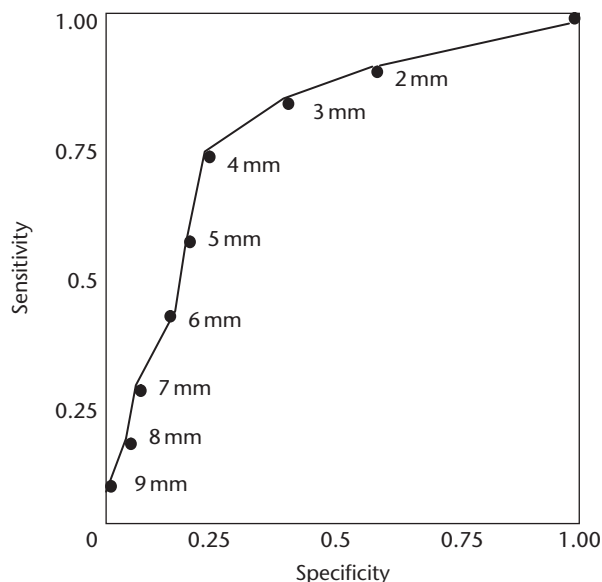


Figure 20.1 A receiver operator curve showing a hypothetical experiment in which PST sensitivity and specificity were determined for various wheal sizes. When different skin test sizes are considered as a positive “cut-off,” there is a trade-off between sensitivity and specificity. The single point at which sensitivity and specificity is maximized is the one closest to the upper left corner (4 mm in this example). When the skin test size meets and exceeds 9 mm in this example, there is 100% specificity and all patients would be expected to react to this food.

is termed a likelihood ratio. This ratio is independent of population prevalence, but in order to use it for predicting food allergy, one must have a sense for pre-test probability in the individual tested (i.e. the impact is similar to population prevalence of disease on PPA and NPA). If one knows the likelihood ratio of a skin test and the pre-test probability of food allergy, it is possible to calculate a post-test probability by multiplying the likelihood ratio by the pre-test probability [39]. While the specific data is not worked out for most foods, the concept is clinically vital to appreciate and underscores the importance of a careful history. Consider, for example, three individuals: one had three severe allergic reactions to egg requiring epinephrine, another has atopic dermatitis and no history of a reaction to egg, and a third sometimes has headaches when he eats egg. Each patient is tested by PST to egg white and has a 4-mm wheal. The meaning of a 4-mm wheal to egg when there has been recurrent anaphylaxis to egg is that it confirms reactivity because the pre-test probability is high. In a chronic condition like atopic dermatitis, a modest size skin test may reflect clinical reactivity in only about half of patients (depending also on age) and may be a relevant positive in this scenario needing confirmation by other means. The test result in the situation of isolated headaches is most likely of no clinical concern as the pre-test probability is essentially zero. Considering again the patient with multiple episodes

of egg-related anaphylaxis, if there were no wheal to egg the clinician would not be likely to trust the result because the pre-test probability is so high and the correct course of action would be to repeat the test and consider a supervised OFC if the test were negative. These features underscore the importance of considering the medical history when evaluating test results. Likelihood ratios can be calculated for increasing skin test wheal sizes which in turn can assist in broadening the ability to predict reactions in various clinical scenarios, but more studies are needed to provide reliable data for a large number of foods [40]. Such data would be particularly helpful for the interpretation of skin tests performed to foods with homologous proteins (see Chapters 4 and 25) in persons who have a bona fide allergy to one of a group of related foods.

It has been observed by some investigators that particularly large PSTs may have 100% positive predictive value. This concept was demonstrated in a study [40] showing that for young infants, reactions to egg, milk, and peanut were certain to occur if the skin test wheal was ≥ 8 mm for cow's milk and peanut and ≥ 7 mm for egg. The scenario reflects increasing likelihood ratio with increasing sizes of skin tests (likelihood ratios over 12.5 for all three allergens with wheals ≥ 6 mm in the referral population). This result requires replication in further studies. These investigators [41] also evaluated children of 4 months to 19 years of age referred for suspected nut allergy with a single lancet technique and commercial extracts (except whole food for sesame and pistachio) and compared skin test sizes to food challenge outcomes. Positive challenges were associated ($>95\%$ accuracy) with wheal sizes ≥ 8 mm for cashew, hazel, walnut, and sesame. Correlation was poor for almond, pistachio, pecan, and brazil nut though fewer subjects were tested.

When considering the clinical use of such study results, it is also important to consider the variables mentioned previously concerning method of interpretation, skin test device, reagents, study population, etc. Table 20.3 summarizes predictive values of skin tests from representative international studies [40,42–44]. As indicated in the table, the populations differed but included various groups with an elevated prior probability of allergy. It is important to recognize that skin test sizes reflecting “100% specificity,” or diagnostic value, varied by the study. The clinical utility of PSTs is maximized when two decision point wheal sizes are considered in the interpretation: one with high NPA and another with high PPA. When considered together, this may reduce the need for further evaluations (e.g. OFCs).

Risks of prick skin tests

PSTs are typically considered of low risk because allergen exposure is minute and a generalized systemic allergic reaction is rare. In a review of a database of 34,905 skin tests to foods in 1138 patients, the systemic reaction rate was 0.008% and there were no severe reactions [45]. Devenney

et al. [46] identified six infants with generalized reactions representing a rate of 521 per 100,000 tested children or 6522 per 100,000 tested infants. All of the reactions identified were in infants under 6 months of age and they were tested with fresh foods rather than extracts. There is one fatality associated with PST; an adult with food allergy and asthma with a recent exacerbation was tested to 90 foods at one time and experienced a fatal respiratory arrest [47]. In general, these studies support the notion that PSTs are low risk, but caution is needed for infants, use of undiluted extracts, and application of excessive numbers of tests. The physician performing allergy tests should appreciate the low but possible risk of anaphylaxis and be prepared to identify and treat reactions. Intradermal allergy skin tests with food extracts give an unacceptably high false-positive rate, have been associated with systemic reactions including fatal anaphylactic reactions, and should not be used [48].

Pitfalls of skin tests and future diagnostic possibilities

It is not clear why PSTs are occasionally negative despite apparent acute allergic reactions during a food challenge. As has been indicated for venom testing [49], it may be prudent to perform both serum IgE and PST to a food to improve sensitivity when suspicion of reactions are high based on history. In addition, it has been suggested, when suspicion of allergy is high and before proceeding to OFC, to follow a negative PST performed with a commercial extract with a fresh extract of the same food. Presumably this procedure is more sensitive because labile proteins are displayed, compared to commercial extracts, and proteins that may not have been represented during aqueous extraction in creating a commercial extract may be presented as well [50]. For example, Hauswirth and Burks [51] described a patient with systemic anaphylaxis to banana whose commercial, but not fresh extract, skin testing was positive. The *in vitro* tests for detection of serum food-specific IgE antibodies are also very sensitive and specific, but may not display the very same allergen profile as the skin tests. The tests can therefore be used in a complementary fashion when needed. For example, Knight *et al.* [43] challenged children to egg when their serum IgE concentrations to egg white was favorable, approximately 50%, to pass an OFC, for example around 2 kU_A/l. The size of the wheal to a commercial egg extract PST correlated with the outcome of the food challenges: 20% of those with a negative skin test reacted to OFC while 90% with a wheal size of 9 mm reacted.

Improvement in the diagnostic accuracy of PSTs for the future will require additional studies to better characterize the test utility over a broad spectrum of disease, patient age, and types of foods. Standardization of commercial extracts is needed, but development of extracts using bio-engineered, well-characterized proteins may also prove beneficial. Test results currently do not correlate well with

Table 20.3 Predictive values of skin tests from various studies

Allergen	Age	Skin test wheal	Probability of reaction/ reaction rates	Comments	Reference
Milk	Median 3 years	8 mm	~100%	Australia, referred for suspected allergy, lancet technique, commercial extracts, wheal diameter, open OFC	[40]
	<2 years	6 mm	~100%	Same as above	
	Median 3 years	0 mm	~15%	Same as above	
	Median 22 months	12.5 mm	95%	Germany, all OFC, suspected allergy, 87% atopic dermatitis, fresh foods, mean wheal diameter, lancet technique	[42]
Egg	Mean 5 years	0 mm	20%	United States, children without recent egg reaction and serum IgE typically below 2.5 kU _A /l, bifurcated needle, commercial extract, mean wheal diameter	[43]
		3 mm	50%	As above	
		9 mm	90%	As above	
	Median 3 years	7 mm	~100%	Australia, referred for suspected allergy, lancet technique, commercial extracts, wheal diameter, open OFC	[40]
	<2 years	5 mm	~100%	Same as above	
	Median 22 months	13 mm	95%	Germany, all OFC, suspected allergy, fresh foods, mean wheal diameter, lancet technique	[42]
Peanut	Median 3 years	8 mm	~100%	Australia, referred for suspected allergy, lancet technique, commercial extracts, wheal diameter open OFC	[40]
	<2 years	4 mm	~100%	Same as above	
	Mean 7 years	8 mm	~95%	United Kingdom, mixed suspected allergy, ages 1–16 years, lancet technique, extract, longest diameter	[44]
		3 mm	~50%	As above	
		0 mm	~13%	As above	
			~28%	As above	
			92%	As above	

severity of a reaction or level of patient sensitivity, but diagnosis using specific proteins to which IgE binding is associated with severe reactions may allow future diagnosis that is more sensitive and specific with additional predictive value in regard to severity [52–54].

Additional diagnostic steps prior to oral food challenges

An OFC can determine whether a specific food triggers disease, but it is time consuming and carries risk. Therefore, additional diagnostic steps are taken to determine if an OFC would be of utility and, if so, additional consideration about risks/benefits are considered prior to proceeding. To determine a diagnosis and to determine whether an OFC is needed for a diagnosis requires consideration of patient history, test results, the immunopathology of the disorder under consideration, the physical examination, and the results of elimination

diets. These diagnostic steps are considered in more detail in Chapter 24. Specific information about diagnostic tests such as determination and interpretation of serum IgE (Chapters 19 and 21), and the atopy patch test (Chapter 20) are described elsewhere. Here, the specific components of the history and physical examination that are of import for diagnosing food allergy will be described. The value and limitations of diet diaries and elimination diets will be explained. Lastly, decisions about undertaking an OFC and the type of OFC will be reviewed. Specific details about undertaking an elimination diet and performing an OFC will be described in Chapter 23.

The history and physical examination

The history and physical examination are undertaken prior to the selection of any diagnostic tests. The clinician must consider from the history whether the complaints are likely

to be associated with food allergy, intolerance or toxic effects, or not related to foods whatsoever. Furthermore, the physician is interested in constructing *a priori* assessments of the chance that foods are playing a role, which foods may be involved and whether the pathophysiology, if it is related to a hypersensitivity reaction, is IgE antibody mediated, cell mediated, or a combination. The physical examination may confirm atopic dermatitis, growth problems, urticaria, and other symptoms of atopic disease, or may exclude them. A careful history should focus upon: the symptoms attributed to food ingestion (type, acute versus chronic), the food(s) involved, consistency of reactions, the quantity of food required to elicit symptoms, the timing between ingestion and onset of symptoms, the most recent reaction/patterns of reactivity, the manner in which the food was prepared (raw, cooked), potential contamination with known allergens, and any ancillary associated activity that may play a role (i.e. exercise, alcohol ingestion). The importance of these queries, many of which are self-evident, derive from various observations about food-allergic reactions. For example, consistent reactions raise prior probability that a suspect food is causal. If a food is an infrequent part of the diet, it is more likely a culprit than a food eaten often. Proteins may be altered through cooking resulting in variations in reaction. Sometimes a rather large amount of a food needs to be ingested for a reaction to occur, or ancillary activities such as exercise or ingestion of medications is required [55]. A person with a prior known allergy may have reacted to contamination of their food with a known allergen, rather than have developed a new allergy, so careful discussion is required. Details about the meal may disclose nuances of note. Consider, for example, an allergic reaction to ingestion of fish in a person where fish-allergic reactions have not been consistent. Canned tuna and salmon are typically tolerated by those who react to fish that is not canned, though allergy to canned tuna is also described [56]. Various fish preparation methods (e.g. boiling versus frying versus eating raw) may have different outcomes on protein allergenicity for different types of fish [57]. Use of antacids (concomitant medications) may reduce digestion and may result in reactions despite prior tolerance [58–60]. The part of the fish ingested can have different levels of the major allergen, such that dark or red muscle may lack the allergen compared to white muscle [61,62]. Lastly, allergy to parasites in fish, specifically to *anisakis simplex*, represents another potential confounding diagnostic issue [63–66]. A thorough history is needed to appropriately address these possibilities.

It is convenient, and possibly quite illuminating, to have patients keep a symptom diary and chart the foods they consume with and without symptoms and also to collect ingredient labels from the foods they eat. For example, they may chart 3–7 days of meals and snacks, showing the time of ingestions, the amount eaten, brands, preparation methods, and any symptoms. The accuracy and diagnostic utility of

such records have not been evaluated. To improve the quality of the information, patients/families should be reminded to record all foods and medications ingested, as they may be prone to neglect beverages, snacks, medications, and condiments. The information gathered from the general history, physical examination, and diet records are used to determine the best mode of diagnosis or may lead to dismissal of the problem from the history alone.

In the case of acute reactions following the isolated ingestion of a particular food with classical food-allergic symptoms, such as acute urticaria or anaphylaxis, the history may clearly implicate a particular food and a positive test for specific IgE antibody (PST/RAST) would be confirmatory and exclude the need for OFC. In the context of an acute reaction to a food to which IgE has been detected, elimination is not considered diagnostic, but rather for purposes of treatment. If the ingestion was of mixed foods and the causal food was uncertain (i.e. a meal with five ingredients), the history may help to eliminate some of the foods. For example, foods frequently ingested without symptoms are generally excluded as potential triggers when evaluating symptoms associated with acute reactions. Tests for food-specific IgE antibodies may help to further narrow the possibilities.

Diagnostic elimination diets

In chronic disorders such as atopic dermatitis, eosinophilic gastroenteropathies, or asthma, it is more difficult to pinpoint causal food(s) [67]. The history is helpful, but since these disorders have a waxing and waning course, and considering limitations in diagnostic laboratory tests, false associations to food ingestions are common. The evaluation of these disorders may require a period of dietary elimination to observe for symptom resolution. This period of trial diet requires selection of foods to be eliminated (based usually on history, test results, epidemiology of the disorder, etc.). The diet trial could be confounded if additional therapies are simultaneously undertaken (e.g. steroids for eosinophilic gastrointestinal disorders, an improved skin care regiment for atopic dermatitis, etc.). Therefore, it is usually prudent to alter one variable at a time. Chronic symptoms should resolve during a period of elimination and if they do, OFCs may be needed to determine which food(s) were causing the chronic symptoms. If symptoms do not resolve, then the eliminated foods are not causal. Elimination of foods to which IgE antibodies are demonstrable, but to which acute reactions are not observed, may carry a risk of loss of a desensitized state, where reintroduction later can trigger more evident acute reactions (e.g. urticaria, anaphylaxis) [68,69]. The frequency of this occurrence is unknown and the length of time for elimination to warrant this concern is currently unclear, but the risk should be considered in decisions to begin dietary trials. Additional details about elimination diets are provided in Chapter 23.

Oral food challenges

OFCs are typically undertaken to identify a causal food when allergy is otherwise unclear (e.g. tests are equivocal or irrelevant), or to monitor for resolution of an allergy. The specific routine for deciding upon OFC and undertaking them is described in Chapter 23. OFC is usually the only means to evaluate disorders or complaints where ancillary tests are irrelevant. For example, the evaluation of reactions to food dyes and preservatives usually requires OFCs. Similarly, patients may attribute a host of medical complaints to food ingestion in disorders that are not proven to be patho-physiologically linked to food allergy (e.g. arthritis, fatigue, behavioral problems, etc.). In all of the circumstances where chronic complaints are involved, the OFC is capable of revealing or excluding relationships to foods. Such determinations are crucial because patients may undertake unnecessary dietary alterations that can have nutritional and social consequences [70,71]. Overall, the approach to diagnosis in chronic disorders, where most readily available diagnostic tests are of limited value, requires elimination diets and OFC to confirm suspected associations.

In regard to undertaking an OFC when there is supporting evidence of allergy, the decision requires consideration of risk, nutritional need, social need, and other factors. The issues to consider when deciding whether to undertake an oral challenge and what challenge setting (e.g. open, single, or double masked) are summarized in Table 20.4. Diagnostic tests considered in this chapter and elsewhere, results of elimination diets, and historical points are central to decision making. There are settings in which oral challenges may be optional or contraindicated. Severe anaphylaxis to an isolated ingestion, with a positive test for specific IgE antibody to the causal food, is one example of a relative contraindication for oral challenge. On the other hand,

in some circumstances even a patient with this convincing history may require a challenge; for example, if enough time has passed and laboratory indices are favorable for the possibility that tolerance has developed. If the food being eliminated is not nutritionally or socially important (e.g. star fruit), then challenge may be unwarranted. These same rules may apply if several members of a food family are being eliminated, but the food family is not a major part of the diet (e.g. elimination of all tree nuts when an allergy to one is certain). An evaluation of the history and test results may allow an assessment of a probability that a challenge would be tolerated. Depending on patient preferences and physician judgment, the decision to proceed may vary. For example, an estimation of an 80% risk of a reaction to peanut for a 2 year old is not as likely to result in a decision to challenge as it might be for a 16 year old. Overall, a variety of safety and social issues should be considered.

There are three general modalities to perform OFC and their selection depends on various considerations [12,72–74]. Challenges can be done “openly” with the patient ingesting the food in its natural form, “single-blind” with the food masked and the patient unaware if the test substance contains the target food or double-blind and placebo-controlled (DBPCFC) where neither patient nor physician knows which challenges contain the food being tested. In the latter two formats, the food must be hidden in some way, such as in another food or opaque capsules. When challenges are undertaken for research purposes, the DBPCFC is the preferred format because there is the least chance for bias from either the patient or physician who must monitor for symptoms. The false-positive and false-negative rate for the DBPCFC, based primarily on studies in children with atopic dermatitis, is 0.7% and 3.2%, respectively [75,76]. Because the food is masked, it is sometimes difficult to provide meal sized portions in a foods’ natural state. To help exclude false

Table 20.4 Issues to consider for undertaking an OFC

Category	Variables	Factors
Indication to challenge	Probability to pass (risks)	History, physical examination, test results, nature of allergen, natural history of disease
	Needs (benefits)	Social Nutritional
Challenge type	Open	Numerous foods to screen, disorder with objective symptoms, allowance for bias
	Single-blind	Less prone to bias than open
	DBPCFC	Least prone to bias, most definitive approach for subjective symptoms
Challenge location	Home	Adding foods in chronic or behavioral disorders with no risk of acute/severe reactions
	Office	Challenges at low risk for severe reaction
	Hospital/ICU	Challenges that are more likely to elicit reactions requiring medical intervention

negatives, it has long been suggested to include an open feeding under supervision of a meal size portion of the tested food prepared in its usual manner as a follow-up to any negative DBPCFC [76]. Increasing the number of challenges (additional placebo and true foods) helps to diminish the possibility of a random association, but this can be a very labor-intensive approach [77,78].

There are several factors that weigh in deciding which type of challenge to use, and DBPCFC, a labor-intensive format, may not be the initial choice. Although the open challenge is most prone to bias, it is easy to perform since no special preparation is needed to mask the food. Indeed, if the patient tolerates the ingestion of the food, there is little concern about bias. It is only when symptoms, especially subjective ones, arise that the issue of bias come into play. Therefore, open challenges are a good option for screening when several foods are under consideration and if a food is tolerated, nothing further is needed. If there is a reaction to an open challenge used in the clinical setting, and there is concern that the reaction may have been psychological, the format could be altered to include blinding and controls. Single-blind challenges help to alleviate patient bias and may be an option to increase efficiency (since a second placebo arm is not always needed). Additional information about undertaking OFC is presented in Chapter 23.

Summary

In vivo tests are primary tools among the armamentarium available to the clinician for the diagnosis of adverse reactions to foods. The medical history, perhaps supplemented with diet records, is the cornerstone of diagnosis. Skin testing is safe, cost effective, and when properly performed and interpreted, highly informative for the diagnosis of IgE antibody-mediated disorders. Elimination diets may provide presumptive evidence of a food-responsive disease. OFCs are the most definitive test available for the final confirmation of these disorders. While oral challenges are time consuming and may elicit severe reactions, they can be safely and efficiently performed with the proper preparation and remain the mainstay of diagnosis for clinical and research settings.

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