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Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children

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Summary

Background The specificity of allergen skin prick testing to diagnose clinically relevant food allergy is controversial.
Objectives To determine the specificity of the allergen weal diameter to correctly identify children who react on formal open food challenges.
Methods Over a 9-year period children referred to a tertiary allergy clinic for the evaluation of suspected food allergy were prospectively studied. Allergen skin prick testing to cow milk, egg white and peanut extracts (Dome-Hollister-Stier, Spokane, WA, USA) was undertaken using a lancet technique. All children underwent open food challenges to the relevant food(s) in a hospital clinic. Challenges were classified as positive, if objective signs were seen; negative, if the child could tolerate normal quantities of the food, daily, for one week; or inconclusive if none of the former criteria were met.
Results Five hundred and fifty-five challenges were undertaken in 467 children: 339 challenges to cow milk, 121 to egg, and 95 to peanut. Fifty-five percentage of challenges were positive, 37% negative, and 8% inconclusive. For each food it was possible to identify a skin weal diameter at, and above, which negative reactions did not occur: cow milk, 8 mm; egg, 7 mm; peanut, 8 mm. In contrast, positive reactions could occur with a skin wheal diameter of 0 mm.
Conclusions In this high risk referral population it was possible to define skin weal diameters to egg, milk and peanut above which open oral food challenges were positive (100% specificity). By utilizing these measurements the need for formal food challenges can be reduced.

Keywords: food allergy, paediatric, allergen skin test, diagnosis, cow milk, peanut, egg, likelihood ratios, ROC curves

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Introduction

The specificity of allergen skin testing to diagnose clinically relevant food allergy is controversial [1–7]. A positive allergen skin test reaction indicates the presence of allergen-specific IgE (sensitization). Most investigators follow American and European recommendations which classify a positive reaction as consisting of a weal of ≥ 3 mm [8–10]. While this indicates sensitization it may not be associated with adverse reactions to that food [11]. A clinically more relevant end point would be to attempt to define a weal diameter invariably associated with adverse reactions. In a previous study we reported that all children with a strongly positive skin test reaction to a cow milk extract developed an adverse response on a formal cow milk challenge [12,13]. To confirm this observation a prospective study of an additional group of children was undertaken and other foods were also studied.

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Subjects and methods

Over a 9-year period (1989–98) the results of 555 food challenges and skin prick tests undertaken on 467 children

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(median age 3.0 years) in this tertiary referral centre were prospectively recorded. The children were referred for the investigation of food allergy and there was generally a high index of suspicion that they had, or were prone to, adverse reactions to food. In addition a small number of children were being re-tested after having had a positive food challenge at least 12 months previously.

Skin tests

Allergen skin prick tests (SPT) were performed using a lancet technique using extracts of the relevant foods: cow milk; egg white; and peanut (Dome-Hollister-Stier, Spokane, WA, USA) [14]. Histamine (1 mg/mL) and a negative control solution were also used. Skin tests were performed on the backs of infants and on the forearm of older children. The skin weal diameter (mm) to histamine was measured between 10 and 15 min and to allergen between 15 and 20 min. In addition the histamine equivalent score was calculated [14]. The majority of SPT were performed within a month of the food challenge. If SPT were performed more than 90 days before or after the food challenge the challenge result was not included in this analysis.

Food challenge procedures

Patients were on a diet free from the food in question and in symptomatic remission for at least 6 weeks prior to the food challenge. They attended the Royal Children's Hospital Allergy Centre for the first 2 days of the challenge procedure. Subsequent increases in the amount of food were undertaken at home. The parents were given a diary card to complete daily recordings of any adverse events. All patients were reviewed 1 week after commencing the challenge food, or sooner if an adverse reaction developed.

Cow milk Challenge procedures were undertaken once other causes of milk intolerance had been excluded, according to a previously published protocol [13,15]. The following volumes of fresh cows milk were administered. Day 1: 1 drop inside lip, 0.5, 2.5, 5, 10, 20, and 30 mLs at 30 minute intervals. Day 2: 30, 60 and 120 mLs at 30 minute intervals. Day 3: normal volumes of milk, i.e. > 450 mLs per day.

Egg The egg white of a lightly boiled egg (boiled for approximately 2 min) was used. The following amounts were administered. Day 1: a drop smeared on the buccal mucosa of the lower lip, then 1/8, 1/4, 1/2 and 1 teaspoon at 30 minute intervals. Day 2: 1 teaspoon, 2 teaspoons (approximately 1 egg white) at 30 minute intervals. Day 3: 1 egg white per day continued for a week. The procedure was repeated for egg yolk by challenge at home. 1/8 of a level (5 mL) teaspoon of egg white contains approximately 0.1 g of egg protein.

Peanut Peanut butter paste was used as the challenge food. The following amounts were administered. Day 1: a smear was wiped on the buccal mucosa of the low lip and if no reaction was followed by 1/8, 1/4, 1/2, and 1 teaspoon at 30 minute intervals. Day 2: 1 teaspoon, 2 teaspoons at 30 minute intervals. Day 3: 3 teaspoons, increasing by 1 teaspoon a day for 4 days. 1/8 of a level (5 mL) teaspoon of smooth peanut butter contains approximately 0.2 g of peanut protein.

If there was doubt as to whether a child had reacted to a certain dose, that dose was repeated. In a few instances where slow reactions were expected, the rate of food intake was increased more rapidly.

Criteria for classification of food challenge outcome

The children were regarded as having an adverse reaction to a food if (1) adverse reactions were observed on day 1 or day 2 in the Unit; (2) adverse reactions occurred after day 2 were confirmed by diary recording and clinical assessment. The children were considered as having a negative challenge if they tolerated normal amounts of the food, daily, for at least 1 week. Challenges were considered inconclusive if the above criteria could not be applied. An adverse reaction was considered to have occurred if any objective marker of change was seen–skin: urticaria, severe flaring of eczema; gastrointestinal, vomiting, diarrhoea; and respiratory, sneezing, rhinorrhoea, wheezing, cough; or anaphylaxis. Patients were deemed to be immediate reactors if they reacted within an hour of completing their challenge on day 1, slow reactors, if they reacted more than a hour after commencing challenge and before the end of the first week and delayed reactors, if they developed symptoms more than 1 week after commencing challenge procedures. Non-immediate reactions were confirmed by diary recording clinical assessment and were reproduced on two occasions before being regarded as positive [12,13].

Statistics

Results of the challenges were assigned into three groups; positive, negative, or inconclusive. The sensitivity and specificity of each weal diameter as a predictor of a positive challenge was calculated, with data from the inconclusive challenges excluded. This information is presented as likelihood ratios [16] for a positive challenge (i.e. the true positive rate or sensitivity the false-positive rate or 1-specificity) and graphically as Receiver Operating

Table 1. Clinical characteristics of children challenged

	Number of challenges	Number of children challenged (Boys)	Age in months Median (range)	Number (%) < 2 years
Cow milk	339	310 (220)	31.0 (1.0–192)	120 (26%)
Egg	121	111 (82)	36.2 (1.1–154)	39 (29%)
Peanut	95	92 (61)	57.8 (1.5–168)	18 (20%)

Table 2. Outcome of food challenges (% challenges)

	Number of challenges	Positive* challenges	Negative challenges	Inconclusive challenges
Cow Milk	339	42% (37%, 4%, 1%)	49%	9%
Egg	121	77% (72%, 5%, 0%)	17%	7%
Peanut	95	73% (73%, 0%,0%)	22%	4%

*Immediate (day 1), slow (day 1–6), delayed (>day 6) positive reactions.

Table 3. Major clinical features of positive food challenges

	Cow milk ($n = 143$)	Egg ($n = 93$)	Peanut ($n = 70$)
Urticaria	73%	84%	83%
Flare Eczema	7%	4%	11%
Vomiting	14%	10%	13%
Other G.I.*	21%	10%	3%
Respiratory†	14%	5%	7%
Rhinitis	15%	10%	10%

*Diarrhoea, colic, abdominal pain, †itchy throat plus cough and/or wheeze.

Characteristic (ROC) curves [16,17]. Likelihood ratios are a generalisable measure of the accuracy of a diagnostic test. They compare the frequency of the test correctly identifying those with the condition to the frequency of the test being positive in those without the condition. A value greater than 10 suggests a clinically useful test ROC curves present the data in a graphical form, the further the curve bows from the diagonal, the more useful the test is.

Results

The number of challenges for suspected allergy to cow milk, egg and peanut is shown in Table 1. Of these 120 (36% of all cow milk challenges), 39 (32% of all egg challenges) and 18 (19% of all peanut challenges) were undertaken in children aged less than 2 years. There were 306 (55%) positive, 206 (37%) negative, and 43 (8%) inconclusive challenges. The positive challenges usually developed within 2 h of commencing the procedure: cow milk (88% of positive challenges), egg (94%), and peanut (100%) (Table 2). The major clinical feature of these positive food challenges is shown in Table 3.

Positive food challenges were seen more frequently with increasing allergen skin weal size, whether expressed as a weal diameter (Fig. 1–3) or as a histamine equivalent score irrespective of whether the child was less than or more than 2 years of age. For each food, positive food challenges were always seen when the skin weal diameter was greater than a certain size: cow milk, 8 mm; egg, 7 mm; and peanut, 8 mm (Table 4). In children aged less than 2 years these diameters were correspondingly smaller: cow milk, 6 mm; egg, 5 mm; and peanut, 4 mm. This implies that weal diameters equal to or greater than these diameters were 100% specific in defining the outcome of food challenges (Fig. 4). In contrast for a 3-mm weal diameter [8–10] the specificity is reduced to 79% for cow milk, 70% for egg and 71% for peanut. For children less than 2 years of age the specificities were 91% (61/67), 75% (6/8) and 66% (2/3), respectively. Positive challenges were also seen in children with negative skin reactions.

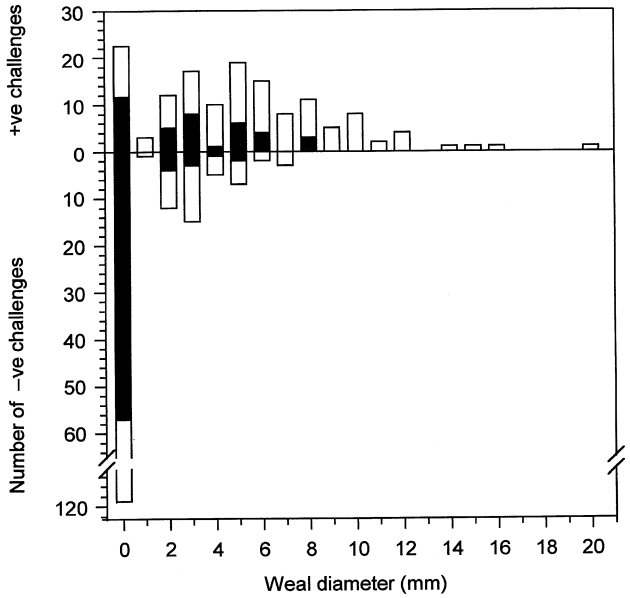

Fig. 1. The outcome of cow milk challenge and cow milk skin test weal diameter (mm). The solid bars represent the subgroup of children aged 0–2 years.

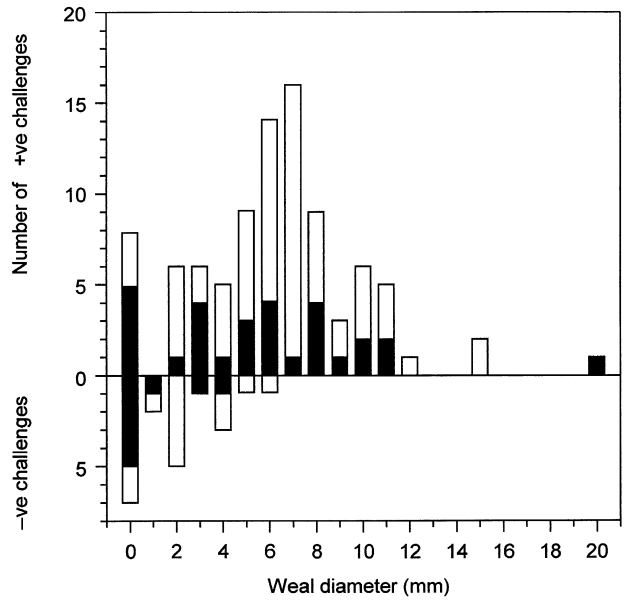

Fig. 2. The outcome of egg challenge and egg skin test weal diameter (mm). The solid bars represent the subgroup of children aged 0–2 years.

Discussion

In this study of children referred to a tertiary Institution for evaluation of food allergy we have found that a strongly positive skin reaction to cow milk (≥ 8 mm), egg (≥ 7 mm), and peanut (≥ 8 mm) was invariably associated with an adverse reaction on formal open food challenge. These results confirm and extend our previous observations [12]. In addition the results confirm observations by other groups that the presence of a positive allergen skin test, in itself, is a poor positive predictive marker of clinically relevant food allergy. It is only when the degree of sensitization is taken into account, is it of value. This increased certainty of correctly identifying a child who would react adversely to food (gain in specificity) is invariably accompanied by a reduction in the number of children correctly identified (loss of sensitivity). This is of clinical use however, in that it allows allergen skin prick tests to be used as a screening procedure, those with a strongly positive reaction do not require further evaluation. The findings are in agreement

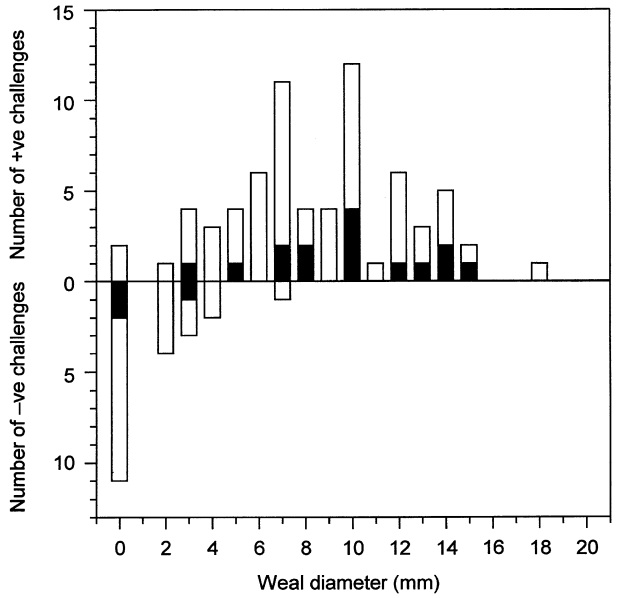

Fig. 3. The outcome of peanut challenge and peanut skin test weal diameter (mm). The solid bars represent the subgroup of children aged 0–2 years.

Table 4. Likelihood ratios of a positive challenge for a given weal diameter

Weal (mm)	Cow milk	Egg	Peanut
1	3.1	1.4	2.0
2	3.1	1.7	2.0
3	3.8	2.8	3.4
4	5.8	3.1	6.3
5	7.3	7.3	18.0
6	13.2	12.5	16.7
7	16.2	∞	∞
8	∞	∞	∞

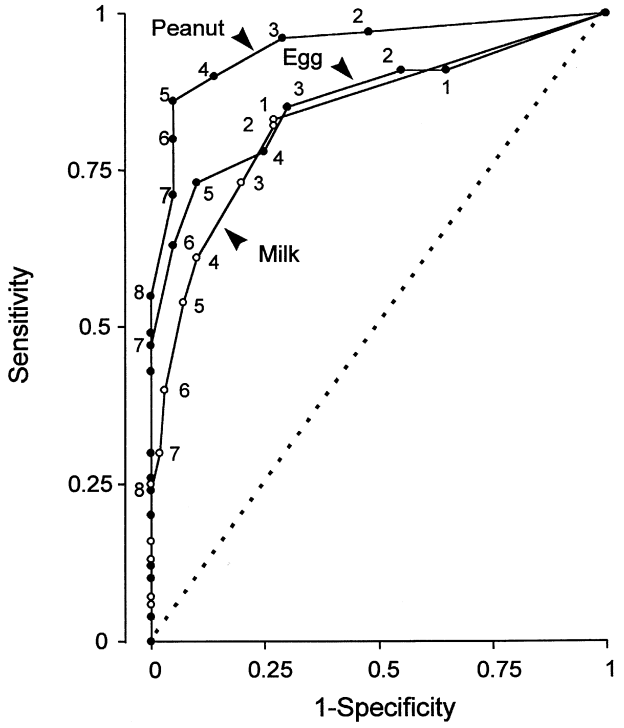

Fig. 4. Receiver operating characteristic (ROC) curves for skin weal diameter in predicting a positive food challenge for cow milk, egg, and peanut. The numbers on the curves represent the corresponding skin weal diameters (mm).

with recent work [15] which used the enhanced capabilities of the CAP system to measure food specific IgE antibodies, a test previously found to be a poor discriminator [1,18–20]. The investigators found it was possible to identify a subset of children with elevated food specific IgE who were highly likely to experience clinical reactions on double-blind placebo controlled food challenge [15].

A skin weal diameter of 3 mm has been recommended as a marker of hypersensitivity to foods in many North American and European and centres [8–10]. A 3-mm weal has high sensitivity, but the corresponding specificity is too low to be of value in identifying clinically relevant food allergy. Conversely a negative or < 3-mm weal is highly useful in excluding IgE-mediated food allergy. Sampson, in earlier studies, reported that a 3-mm weal had a 96% sensitivity in identifying children with cow milk, 98% egg, and 90% peanut allergy with a corresponding specificity of 51% for cow milk, 53% for egg and 29% for peanut [6]. The results from our study show that the corresponding sensitivity for a 3-mm weal would be 74% for cow milk, 84% for egg and 96% for peanut and with a specificity of 79% for cow milk, 70% for egg and 71% for peanut. The lower sensitivity for a 3-mm skin weal diameter in our children with cow milk allergy reflects the relatively high frequency of non-IgE reacting cow milk allergy in the first 2 years of life [13]. The median age of patients in our study was 3.0 years whereas Sampson's was 7.9 years [6].

A limitation of our study is that double-blind challenges (DBPC) were not routinely undertaken [10]. Although this has been regarded as the 'gold standard' for establishing the diagnosis of food allergy a number of exclusions are recommended by advocates of this procedure, e.g. patients with a history of anaphylaxis or severe anaphylaxis [2,6], and limitations of DBPC have been described [20–22]. Conversely open challenges while good at defining non-reactors are more likely to give false-positive results and inflate the apparent specificity of the skin tests. In our study the amounts of food given were small enough and the waiting periods between doses long enough to ensure generalized anaphylaxis did not occur. This has allowed the study of children with extreme sensitization who would previously have been omitted from study according to DBPC procedures. During the study period it was the unit's policy to attempt to define exactly all reported adverse reactions by formal challenge. This may explain why in previous reports from other Institutions such a clear correlation between sensitization and food challenge was not seen. The omission of highly sensitized children would bias the findings. While open challenges may have led to an observer bias, the end points of the challenges were clear and usually occurred within the first day of challenge; urticaria, usually circumoral; vomiting; and wheeze. Delayed reactions of diarrhoea and flaring of eczema were seen and documented by the use of a daily diary score card [23]. If the child became distressed but there was no objective evidence of an adverse reaction the challenge was considered inconclusive, and this ranged from 4 to 9% of all challenges.

Eigenmann and Sampson [24] have recently re-evaluated skin prick tests (SPT) in the diagnosis of food allergy in a group of older children with a history of atopic dermatitis. They used as a cut-off point weal sizes at the upper 95% confidence interval for food tolerant patients. These were 5 mm for cowmilk, 4 mm for egg, and 6 mm for peanut. The specificity of these SPT's were 68% for cow milk, 61% for egg and 71% for peanut. Applying Eigenmann and Sampson's cut-off points to our data gave specificities of 92% for cow milk, 75% for egg, and 95% for peanut. However, Eigenmann and Sampson's children were older, median age 4.6 years (personal communication), than our patients. Sampson [18] has highlighted the effect that the prevalence of food allergy in the general population has on reducing the predictive value of clinical tests calculated from highly selective populations seen in tertiary centres [25,26]. However in clinical practice this is not an important issue. The practising allergist needs to know, in their referred high-risk population, whether or not to undertake an expensive and potentially life threatening food challenge,

an SPT diameter above which all patients will react on challenge. This is achieved when the specificity of the test is 100%. In our study 100% specificity was reached for cow milk at an SPT diameter of 8 mm, for egg at 7 mm and peanut 8 mm for a group of children with a median age 3.0 years. For children 2 years of age or younger 100% specificity was reached at lower SPT weal diameters, 6 mm for cow milk, 5 mm for egg, and 4 mm peanut.

Our data highlights the need to consider how best to use SPT size in the evaluation of children with suspected food allergy. In the past single SPT weal cut-off sizes e.g. 3 mm [8–10], have been selected and the specificity and sensitivity calculated for that level. More appropriate use of such data is to link the cut-off diameter according to the clinical context in which it is to be used. Thus, an epidemiologist seeking evidence of sensitization to food proteins in a large population needs to have a SPT defined with a high sensitivity to identify most patients at risk for food allergy. By contrast, a clinician seeking to avoid unnecessary and potentially dangerous food challenges whilst avoiding unnecessary diet restriction will need to have a test with a high specificity. Our data has shown these SPT cut-off levels will be different according to the clinical context, the allergen extract used and the age of the child.

The findings from this prospective study have changed the food challenge policy of this unit. If a child has on initial evaluation a skin test weal to cow milk, egg, or peanut exceeding the above defined limits the child is considered allergic to that food and does not undergo further evaluation. Those with a lesser skin reactivity are challenged. Children are restudied annually and challenged according to the skin test results. All children with adverse reactions to other foods (such as soy and wheat) were the predictive value of skin testing has not been defined, and in our experience is not as clear, also require food challenges. This policy reduces the need for food challenges and permits simple monitoring of the commencement of tolerance [27,28]. However, caution needs to be applied in extrapolating our results with these allergen extracts to other populations, of different ages, where different skin test reagents of varying potency, and different methodologies may be used. Although no single test is likely to identify all children with food allergy, allergen skin prick tests to cow milk, egg, and peanut are a useful, inexpensive, and specific diagnostic tool readily applicable to clinical practice and epidemiological studies of food allergies [29].

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