

Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community?

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Summary

Background The severity of allergic reactions to food appears to be affected by many interacting factors. It is uncertain whether challenge-based reactions reflect the severity of past reactions or can predict future risk.

Objective To explore the relationship of a subject's clinical history of past reactions to the severity of reaction elicited by a low-dose, double-blind, placebo-controlled food challenge (DBPCFC) with peanut.

Method Cross-sectional questionnaire assessment of community-based allergic reactions and low-dose DBPCFC in self-selected peanut-allergic subjects. Reaction severity was assessed using a novel scoring system, taking account of the dose of allergen ingested.

Results Forty subjects (15 males, 23 children, 23 asthmatics by history) were studied. Only the most recent community reaction predicted the severity of reaction in the DBPCFC, but even this association was weak ($r = 0.37$, $P = 0.03$). Peanut-specific IgE (PsIgE) and skin prick test (SPT) weal size were not associated with community score but PsIgE level correlated well with the challenge score ($r = 0.6$, $P = 0.001$). Asthma did not affect the eliciting dose or challenge score directly but the association of PsIgE and challenge score was stronger in those without asthma ($r = 0.72$, $P = 0.001$) than in those with asthma ($r = 0.48$, $P = 0.02$).

Conclusions The scoring system developed appears to improve the sensitivity of assessment of reactions induced by DBPCFC. This is the first prospective study showing an association between PsIgE levels and clinical reactivity in DBPCFC, an effect that is more pronounced in non-asthmatics. This finding has important implications for the clinical care of subjects with food allergy. There is a poor correlation between the severity of reported reactions in the community and the severity of reaction elicited during low-dose DBPCFC with peanut.

Keywords anaphylaxis, double-blind, food allergy, peanut, placebo-controlled food challenge, threshold

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Introduction

Food allergy affects up to 8% of pre-schoolchildren and up to 2% of the general population. Diagnosis can be made on the basis of a convincing clinical history and supportive *in vivo* and *in vitro* assessment of specific IgE but a double-blind, placebo-controlled food challenge (DBPCFC) is considered the gold standard test [1, 2].

Allergists can now use 'decision points', relating to levels of skin prick test (SPT) reactivity and specific IgE at which clinical reactivity in DBPCFCs can be predicted, to determine whether an individual challenge is clinically indicated [3–6]. It appears that challenges are being used less often to confirm

a clinical diagnosis and more often to test for clinical resolution of allergy, which is the norm in milk and egg allergy but less common in allergy to peanut, tree nuts, fish and shellfish [1].

Opinions vary on the issue of using levels of IgE (however measured) to predict the severity of clinical reactivity and persistence or resolution of food allergy [7–13]. In a low-dose DBPCFC study with peanut, Wensing et al. [14] showed that severe reactors reacted to lower doses (with 50% of the group reacting to doses ≤ 3 mg of peanut protein) but no report was made of any correlation between specific IgE levels and threshold dose. Osterballe and Bindslev-Jensen [15] showed no association between IgE levels and challenge threshold in egg-allergic subjects. Multi-centred international efforts are underway to apply a single approach to the investigation of eliciting doses/thresholds of several key foods: egg, milk and peanut [16, 17].

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There also remains clinical uncertainty about the relevance of a result of an allergen exposure during a food challenge to the reactions that subjects experience at home or elsewhere in the community. It is well known that most significant allergic reactions to foods occur outside the home environment [18, 19]. It is therefore likely that there are other factors such as asthma, exercise, consumption of alcohol and anxiety that may exacerbate reactions in the community and render them more severe than reactions undertaken during a supervised, titrated food challenge.

A history of asthma is associated with a fatal or severe, non-fatal outcome [19–22]. It is plausible that having an unstable, or potentially unstable, airway imparts higher risk. In a retrospective case–control study, food allergy was found more commonly (odds ratio of > 5) in severe asthmatics who required ventilatory and intensive care support than in subjects with less severe asthma [23].

Although the scoring of a single *reaction* has been defined, no gold standard exists for scoring *disease* severity, either to help the patient make decisions on their lifestyle or to aid researchers investigating mechanisms underlying severe reactions or resolution. Current scoring systems [24, 25] do not account for dose, which, in clinical practice, appears to be a major determinant of outcome. We have devised a new scoring system that combines dosage and symptom grades to give an overall score for each community reaction and the reaction elicited by low-dose DBPCFC (Table 1).

The primary aim of this study were firstly to compare a questionnaire evaluation of the clinical reactivity to peanut because of accidental exposure in the community with the gold standard of DBPCFC, and secondly to determine whether PsIgE is a predictor of clinical severity in community-based and low-dose DBPCFC-induced reactions. The secondary aim was to investigate the effect of asthma on this relationship of PsIgE and clinical reactivity.

Methods

This study was approved by the Southampton and South-West Hampshire local research ethics committee (LREC number 337/00). Written, informed personal and parental consent were obtained in every case.

Design

To compare the questionnaire-based assessment of community reactions with DBPCFC, we used a cross-sectional design using volunteers from local adult and paediatric allergy clinics and national advertisements that were targeted,

via the membership of the Anaphylaxis Campaign, at peanut-allergic people. Of those 151 who agreed to complete questionnaires, 40 agreed to further testing.

Study criteria

The inclusion criteria were: older than 6 years of age; a convincing clinical history of an allergic reaction to peanut in the last 3 years before challenge and a SPT (1:20w/v) for peanut of ≥ 6 mm, in the presence of a negative control (saline); and at least a 3 mm weal to histamine (1:10w/v. All skin test reagents were from ALK-Abello, Hungerford, UK). SPTs were performed with single-tine lancets, pricking the skin at an angle of 90°. The weal diameter recorded at 15 min was the mean of the two perpendicular diameters. Children less than 6 years, women who might be pregnant and adults over 65 years were excluded. Subjects who were unable to discontinue allergy medications, such as antihistamines, were not recruited.

Questionnaire to evaluate clinical reactivity

We designed a questionnaire to enquire about each individual's two most recent reactions and their most severe reaction, together with the estimated dose that elicited the reactions and the clinical symptoms and signs observed or reported. The questionnaire was adapted from one used in a previous study that had been validated with a sensitivity of 100% and a specificity of 87% for detecting peanut allergy compared with the gold standard of DBPCFC [26]. Community- and challenge-based reactions were scored using a 5 × 5 grid of five grades of increasing clinical severity and five increasing doses (Table 1). Scores of 1–5 were considered mild reactions, 6–13 were scored as moderate and 14 or more were considered severe reactions, as reported previously [7]. The amount of peanut ingested to cause each of these reactions was calculated using data obtained from food manufacturers and standard recipes for peanut containing foods, e.g. satay sauce. For example, a volunteer who experienced generalized urticaria after mucosal contact with peanut but without ingestion was given a score of 4, and a reaction of laryngeal oedema after a dose of peanut between 3 and 99 mg was scored as 15.

Volunteers came to the Wellcome Trust Clinical Research Facility (WTCRF) in Southampton for clinical evaluation, skin prick testing, measurement of specific IgE in serum, a bronchial hyper-responsiveness (BHR) test with histamine and a DBPCFC. The results of PsIgE measurements were not available at the time of challenge, and the person performing the specific IgE measurement was blinded to the outcome of the challenge.

Table 1. Scoring system for reactions to peanut in the community or during low-dose DBPCFC

	>3/4 nut eaten/ <100 mg peanut protein	1/4 – 3/4 nut eaten/ 3 – 99 mg peanut protein	Fragment swallowed/ <3 mg peanut protein	Mucosal touch, none eaten	Inhalation/skin contact only
Localized urticaria/pruritus	1	2	3	4	6
Generalized urticaria	5	7	8	10	12
Abdominal pain/vomiting/angioedema	9	11	13	16	18
Laryngeal oedema/mild wheezing	14	15	17	19	20
Collapse/severe respiratory symptoms	21	22	23	24	25

Score 1–5, mild; 6–13, moderate; 14–25, severe.

DBPCFC, double-blind, placebo-controlled food challenge.

Bronchial hyper-responsiveness test with histamine

Volunteers underwent a histamine challenge using incentive-based spirometry (Koko, Ferraris Respiratory Europe, Hertford, UK), if their forced expiratory volume in 1 s (FEV₁) was more than 75% of the value predicted for their age and height [27]. Volunteers had to discontinue anti-histamine medication for at least 1 week before assessment, and avoided caffeine-containing products (coffee, tea, chocolate) and inhaled short- and long-acting β_2 -agonist inhalers for 6–8 h before challenge. They were allowed to remain on any inhaled corticosteroid medication. After a baseline challenge with saline, volunteers had a titrated histamine challenge if the post-saline FEV₁ was not more than 10% lower than the baseline study. FEV₁ readings were taken in duplicate at 1 and 3 min post-histamine dose, and the lowest values at each time-point were compared. The lowest of these was used to calculate the change of airway reactivity (FEV₁) after histamine administration.

Double-blind placebo-controlled food challenge

We used a protocol developed in Southampton and published recently [28]. Eleven active doses of increasing magnitude from 1 mg to 4 g peanut protein were randomly interspersed with four placebo doses. Chocolate was used to blind the taste of peanut. Adequacy of blinding had been determined by tasting sessions that mimicked the challenge procedure using volunteers who were not food allergic. The challenge was scored using the same 5 × 5 scoring system as was used for the community-based reactions assessed by questionnaire (Table 1).

Measurement of peanut-specific immunoglobulin E

Serum concentrations of PsIgE were determined by CAP-FEIA (Pharmacia, Uppsala, Sweden). Absolute values are reported in kU/L. Where initial values were >100 kU/L on CAP-FEIA, samples were diluted appropriately with suitable dilution buffers as suggested by Pharmacia and then re-tested.

Statistical analysis

Data were manually entered (double entry) into an SPSS for Windows database (version 11, Chicago, IL, USA). Scores for DBPCFC (range 2–25) were considered as ordinal data and compared using Spearman's rank correlation to ordinal data such as mean weal diameter in SPT and continuous data such as PsIgE. Spearman's rank correlation was used to determine the association between the DBPCFC scores and the questionnaire of community reaction scores. A *P*-value of ≤ 0.05 was considered statistically significant. Mann–Whitney *U*-tests were used to compare the clinical scores between adults/children, asthmatics/non-asthmatics and gender.

Results

Characteristics of challenged volunteers

Demographic details of the 40 challenged volunteers and their reported allergies to other foods are shown in Table 2. They

Table 2. Demographic details

	Child	Adult	Total
Total	23	17	40
Gender (M:F)	8:15	7:10	15:25
Asthma	13	10	23
Eczema	12	9	21
Rhinitis	11	10	21
Positive SPTs			
Tree nut	12	11	23
Milk	1	3	4
Egg	1	3	4
Soya*	1	7*	8
Fish	0	2	2

**P* = 0.006. SPT, skin prick test.

appeared to be broadly representative of British peanut-allergic subjects, as previously reported [7, 8, 25]. The median number of previous reactions reported was 3 (range 1–20), with adults predictably reporting more reactions than children (adult median 5, range 1–20, children's median number of reactions 2, range 1–6, *P* = 0.002). Nearly all had appropriate rescue plans in place already: 38 subjects carried adrenaline autoinjectors, and eight (21%) had used their autoinjectors in the past in response to a reaction experienced in the community. Eleven subjects (27%) had attended hospital after their most severe reaction to peanut.

The median SPT weal to peanut was 9 mm, range 4–18 mm. Children had a smaller peanut SPT than adults (child median 8 mm, range 4–15 vs. adult 12 mm, range 5–18, *P* = 0.008). The range of serum PsIgE concentrations measured for the 40 challenged volunteers was 0–649 kU/L (mean 71.85 kU/L, median 5.65 kU/L). Values for children and adults did not differ significantly. Two of the challenged volunteers had no measurable PsIgE despite a history of symptoms, a positive SPT and a positive DBPCFC. Only four subjects demonstrated a fall in FEV₁ of more than 20% from baseline during the BHR, implying well-controlled asthma.

Correlation of peanut skin prick test and peanut-specific immunoglobulin E

There was no apparent correlation between the mean diameter of peanut SPT weal and PsIgE concentration. Age shows a weak correlation with the mean weal diameter (Spearman's rank correlation *r* = 0.40, *P* = 0.01, *n* = 40). Within the paediatric population, there was a weak association between PsIgE and SPT size (Spearman's rank correlation *r* = 0.40, *P* = 0.04, *n* = 23).

Cumulative eliciting dose of peanut during double-blind, placebo-controlled food challenge

The median cumulative eliciting dose of peanut was 36 mg (range 1–3936 mg, Fig. 1a). Three subjects reacted to the first dose of peanut, 1 mg, establishing this as the LOAEL in this study group, but we were therefore unable to identify a level of no adverse effect NOAEL [16, 17]. There was no difference between subjects with and without asthma in terms of

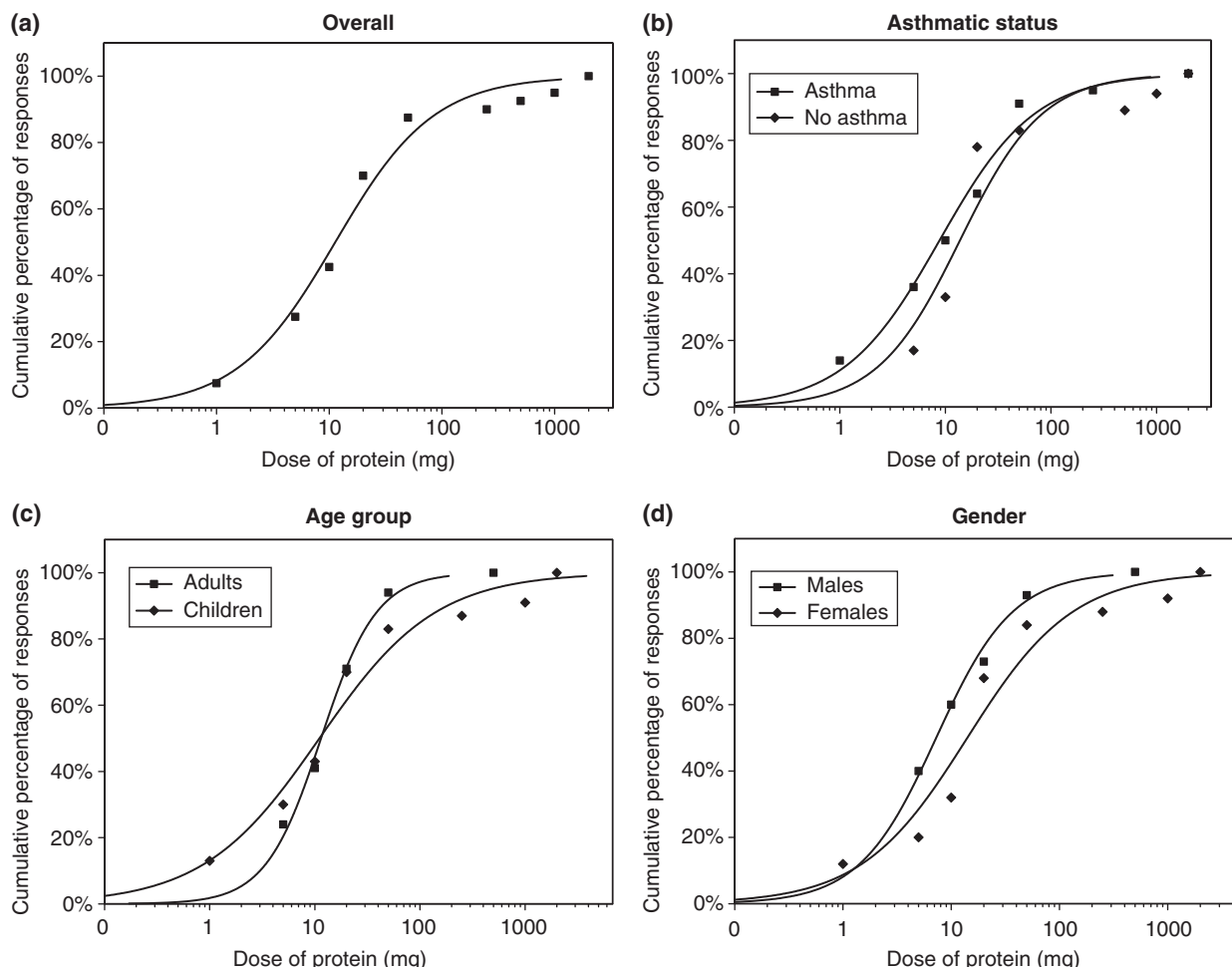


Fig. 1. Cumulative eliciting dose in low-dose peanut double-blind, placebo-controlled food challenge is not affected by asthma, gender or age group (log-logistic modelling).

cumulative eliciting dose (Mann–Whitney U , $P > 0.05$, Fig. 1b), or the challenge score (Mann–Whitney U , $P = 0.90$). Age group and gender had no effect on cumulative eliciting dose (Figs 1c and d) respectively).

Challenge scores in 40 volunteers studied

The median challenge score was 10 (range 2–17). There was no difference in the challenge score between age groups, gender and asthma status using the Mann–Whitney U -test (Figs 1b, c and d). Categorizing the challenge score into three severity groups resulted in nine mild, 22 moderate and four severe cases. As the severe group was too small for meaningful analysis, the moderate and severe groups were combined for some tests to assess the predictive value of PsIgE.

Serum-specific immunoglobulin E as a predictor of grade of clinical reactivity in double-blind, placebo-controlled food challenge

Concentrations of PsIgE and challenge score correlated significantly in the whole group (Fig. 2), although the association was stronger in adults than in children, despite the median values of PsIgE being similar. In adults Spear-

man's r -value increased to 0.766 ($P < 0.001$), compared with children ($r = 0.49$, $P = 0.018$).

Effect of asthma on the association of challenge score and peanut-specific immunoglobulin E

The absence of asthma (as assessed by questionnaire) strengthened the positive correlation of PsIgE and challenge score (Table 3). There was a significant difference in the PsIgE in the three increasing grade groups (mild, moderate, severe) using the Jonckheere–Terpstra test to compare three independent groups ($P = 0.006$, $n = 40$). The usefulness of PsIgE for predicting those who have a mild reaction in DBPCFC compared with those who have a moderate or severe grade was explored. The PsIgE concentration of these two groups overlapped. Using a cut-off value of 15 kU/L, the sensitivity of detecting those with moderate or severe challenge score in a peanut-allergic population was only 54%, whereas the specificity was 92%. Using a lower cut-off value of 4 kU/L, the sensitivity was 72% and the specificity was 33%.

Correlation between challenge and community scores

The score determined by challenge was compared with the community scores using a Spearman's rank correlation (Table 4).

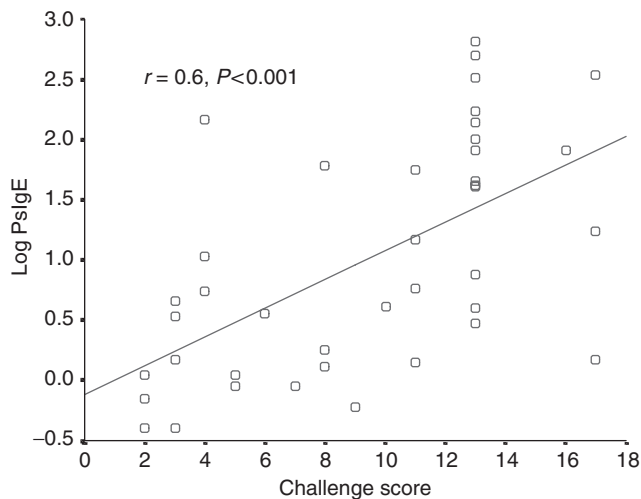


Fig. 2. Correlation of log peanut-specific IgE (PsIgE) and challenge score.

Table 3. Relationship between peanut-specific IgE and DBPCFC challenge score in the whole population and subgroups. Spearman's rank correlation

Group	Number	Correlation coefficient	Significance
Whole group	40	0.62	<0.001
Asthma	23	0.48	0.02
No asthma	17	0.72	0.001
Children	23	0.49	0.02
Adults	17	0.77	<0.001

DBPCFC, double-blind, placebo-controlled food challenge.

Table 4. Relationship between scores using the challenge and reported history of accidental exposures

Comparison	Number	Correlation coefficient	Significance
Challenge score vs. most recent	Total: 35	0.37	<0.03
	Adults: 14	0.44	0.12
community score	Children: 21	0.38	0.09
Challenge score vs. second most recent	Total: 21	0.25	0.14
	Adults: 14	0.06	0.845
community score	Children: 13	0.19	0.54
Challenge score vs. the most severe	Total: 31	0.12	0.54
	Adults: 12	0.17	0.61
community score	Children: 19	0.07	0.77

Discussion

The primary aims of this study were achieved in that only a weak association can be demonstrated between the severity of reactions reported in the community with reactivity during low-dose DBPCFC. This is an important finding as it has an impact on the assessment of future risk and clinical management. We also found that PsIgE concentrations can predict severity of reaction in DBPCFC, if the dose of allergen is considered. The secondary aim was not fully achieved as all asthmatic volunteers had only mild asthma. Nonetheless, asthma's critical role as a covariable for clinical severity has

been confirmed indirectly, as its absence strengthened the association of PsIgE and clinical reactivity.

Relationship of clinical history with severity of reactions induced by low-dose double-blind, placebo-controlled food challenge

There was a poor correlation between the community- and challenge-based reactions, with only the report of the most recent reaction correlating (weakly) with the challenge-based reaction. Clinical history of past reactions may be an unreliable indicator of low-dose challenge reactions. As a corollary, subjects with a history of severe reactions should not be automatically excluded from carefully supervised low-dose challenge studies, making such studies more representative of peanut-allergic subjects in general. However, low-dose challenges are not yet standardized [16, 17], and their usefulness in prediction of future risk is uncertain, as the dose involved in community reactions may be much higher or variations in the food matrix may affect allergen recognition [28].

Comparability of low-dose double-blind, placebo-controlled food challenge studies

The cumulative challenge doses appear to be within acceptable limits reported in the literature and compare favourably with those from the Netherlands [14] and France [29]. Absolute differences may be accounted for in part by differences in study design and challenge material [17], and future, international, single-design studies are awaited.

Consistency of response in low-dose double-blind, placebo-controlled food challenges depends on identical study design

Since their low-dose peanut challenge study was published [14] Knulst's group have reported that there is reasonable inter-challenge consistency when people have a second, identical challenge 1–2 years after their first challenge [30]. Small changes in challenge vehicle can have clinically significant effects. We have shown that re-challenged subjects had their more "usual", less severe, reactions when re-challenged with a lower-fat recipe [28]. So prediction of future risk in the community from the isolated result of a low-dose DBPCFC may not be appropriate.

Impact of asthma and airway reactivity on clinical reactivity to peanut in community and low-dose challenge reactions

A history of asthma was only supported by a demonstrable fall in FEV₁ during histamine challenge in four out of 40 subjects, presumably because of subjects' self-selection bias. Even this unsupported history of asthma did not correlate with community score or severity of community reaction, however, assessed. This is an important negative finding, contradicting the literature regarding fatal food-allergic reactions. It is likely that peanut allergies with unstable asthma did not respond to the advertisements. We cannot use our findings to reject the apparent effect of more severe grades of asthma on reactions in a community setting, where fatal reactions are strongly associated with asthma [18–22].

Research volunteers recruited from an allergy clinic or from the membership of the Anaphylaxis Campaign may be highly motivated, self-aware individuals. The take-up rate of food challenge was only 27% of those initially contacted, so our findings may not be directly applicable to the broader community of food-allergic individuals, particularly young children (not studied in this series) and severe asthmatics. Details of the 40 volunteers' rates of carriage of adrenaline kits, and their use thereof, suggest that they are a well-supported and pro-active group of peanut-allergic subjects.

The asthma reported by the subjects who came for a low-dose DBPCFC study and BHR testing appeared to be so well controlled that we were unable to identify any effect of asthma (as assessed by BHR) on challenge outcome. This is a self-fulfilling prophecy in that standard practice in performing food challenges is that asthma must be stable on the day of challenge [2, 27].

Interaction of a history of asthma and level of peanut-specific immunoglobulin E

Asthma's impact on challenge reactivity, in this series, is mediated through PsIgE; a history of asthma *decreased* the strength of association of PsIgE and challenge score or, to put it simply, people without asthma have a stronger association between their PsIgE concentration and their challenge score than subjects with asthma. This may be very important clinically. Emphasis on asthma care to prevent fatal outcomes may deflect attention away from subjects without asthma who may have high levels of PsIgE and therefore have an independent risk factor for severe outcome. Alternatively, asthma may be such a dominant risk factor that other factors such as PsIgE only become more important (relatively) in the absence of asthma.

The role of asthma will remain to be examined *post hoc*, in a series of reported fatalities and of subjects who survive severe reactions. The inclusion in research trials of subjects with unstable asthma would require very careful evaluation. It is likely to remain more fruitful – and ethically and publicly more acceptable – to prospectively assess the severity of asthma in food-allergic subjects who are admitted to hospital having survived a severe reaction. Recent UK data regarding survived allergic reactions are difficult to interpret because of the highly restrictive entry criteria used [21].

We could not identify any difference in PsIgE concentrations between adults and children but there was a significant difference in the strength of association between their PsIgE and challenge scores: adults showed a much stronger correlation. This may reflect changes in the immune response over time, in response to predictable/unavoidable exposures to peanut. It might explain, in part, the well-established higher risk of severe or fatal reactions in adults, compared with children.

Relationship of peanut skin prick test and specific immunoglobulin E

We did not find a direct relationship between mean weal diameter of SPT and levels of PsIgE, although end-point titration skin prick testing and measurements of weal area were not performed. Furthermore, the two test systems do

not share a common source of allergen. It has been shown in other studies based around measurement of specific IgE, without challenge, that people who report the worst reactions have higher levels of specific IgE [9]. We have been unable to confirm this in this study, which was based upon the gold standard of double-blind challenge (but designed to identify threshold or eliciting doses of peanut).

The correlation between PsIgE and challenge score is an original and important finding. While allergen-specific IgE concentrations have been identified in the past as predicting reactivity [3], this is the first occasion on which it has been shown to be prospectively and positively associated with severity. The inclusion of dose in the scoring system appears to be critical. The fundamental tenet of the grid system is that differences between contiguous dose–reaction pairs may not be as important as serial progression, recognizing increasing severity from one score through others to the highest scores. The association held even when the novel challenge score was converted into the familiar clinical grading of mild and moderate–severe reactions.

In summary, this study, based on low-dose DBPCFC of a highly selected group of peanut allergic subjects, has highlighted that PsIgE level is strongly associated with severity of reaction induced during peanut DBPCFC, as assessed by a scoring system that takes account of dose of allergen. This effect is stronger in non-asthmatics, thereby adding PsIgE concentration to the currently limited list of risk factors for severe reactions in the future. PsIgE levels may have more significance in non-asthmatic subjects but it remains the case that no single parameter should ever be used in isolation to predict future risk of severe reactions.

Subjects with a history of severe reactions need not be automatically excluded from carefully controlled low-dose challenge-based studies. The use of low-dose DBPCFC can assist in the prediction of future reactivity but account must always be taken of the dose of allergen ingested and the many other factors that are controlled in challenge-based research studies.

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References

- 1 Sampson HA. Update on food allergy. *J Allergy Clin Immunol* 2004; 113:805–19.
- 2 Bindslev-Jensen C, Ballmer-Weber BK, Bengtsson U et al. Standardization of food challenges in patients with immediate reactions to foods – position paper from the European Academy of Allergology and Clinical Immunology. *Allergy* 2004; 59:690–7.
- 3 Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; 107:891–6.
- 4 Eigenmann PA, Sampson HA. Interpreting skin prick tests in the evaluation of food allergy in children. *Pediatr Allergy Immunol* 1998; 9:186–91.

- 5 Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy* 2000; 30:1540–6.
- 6 Hill DJ, Hosking CS, Reyes-Benito LV. Reducing the need for food allergen challenges in young children: a comparison of in vitro with in vivo tests. *Clin Exp Allergy* 2001; 31:1031–5.
- 7 Hourihane JO, Kilburn SA, Dean P, Warner JO. Clinical characteristics of peanut allergy. *Clin Exp Allergy* 1997; 27:634–9.
- 8 Hourihane JO'B, Kilburn SA, Nordlee JA, Hefle SL, Taylor SL, Warner JO. An evaluation of the sensitivity of peanut allergic subjects to very low doses of peanut protein: a randomised, double-blind, placebo-controlled food challenge study. *J Allergy Clin Immunol* 1997; 100:596–600.
- 9 van Odijk J, Ahlstedt S, Bengtsson U, Hulthen L, Borres MP. Specific IgE antibodies to peanut in western Sweden - has the occurrence of peanut allergy increased without an increase in consumption? *Allergy* 2001; 56:573–7.
- 10 Clark AT, Ewan PE. Interpretation of tests for nut allergy in one thousand patients, in relation to allergy or tolerance. *Clin Exp Allergy* 2003; 33:1041–5.
- 11 Vander Leek TK, Liu AH, Stefanski K, Blacker B, Bock SA. The natural history of peanut allergy in young children and its association with serum peanut-specific IgE. *J Pediatr* 2000; 137:749–55.
- 12 Skolnick HS, Conover-Walker MK, Barnes Koerner C, Sampson HA, Burks AW, Wood RA. The natural history of peanut allergy. *J Allergy Clin Immunol* 2001; 107:367–74.
- 13 Fleischer DM, Conover-Walker MK, Christie L, Burks AW, Wood RA. The natural progression of peanut allergy: resolution and the possibility of recurrence. *J Allergy Clin Immunol* 2003; 112:183–9.
- 14 Wensing M, Penninks AH, Hefle SL, Koppelman SJ, Bruijnzeel-Koomen CA, Knulst AC. The distribution of individual threshold doses eliciting allergic reactions in a population with peanut allergy. *J Allergy Clin Immunol* 2002; 110:915–20.
- 15 Osterballe M, Bindslev-Jensen C. Threshold levels in food challenge and specific IgE in patients with egg allergy: is there a relationship? *J Allergy Clin Immunol* 2003; 112:196–201.
- 16 Taylor SL, Hefle SL, Bindslev Jensen C et al. Factors affecting the determination of threshold doses for allergenic foods: how much is too much? *J Allergy Clin Immunol* 2002; 109:24–30.
- 17 Taylor SL, Hefle SL, Bindslev Jensen C et al. A consensus protocol for the determination of the threshold doses for allergenic foods: how much is too much? *Clin Exp Allergy* 2004; 34:689–95.
- 18 Furlong TJ, DeSimone J, Sicherer SH. Peanut and tree nut allergic reactions in restaurants and other food establishments. *J Allergy Clin Immunol* 2001; 108:867–70.
- 19 Pumphrey RS. Lessons for management of anaphylaxis from a study of fatal reactions. *Clin Exp Allergy* 2000; 30:1144–50.
- 20 Bock SA, Munoz-Furlong A, Sampson HA. Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 2001; 107:191–3.
- 21 Sampson HA, Mendelson L, Rosen J. Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992; 327:380–4.
- 22 MacDougall CF, Cant AJ, Colver AF. How dangerous is food allergy in childhood? The incidence of severe and fatal allergic reactions across the UK and Ireland. *Arch Dis Child* 2002; 86:236–9.
- 23 Roberts G, Patel N, Levi-Schaffer F, Habibi P, Lack G. Food allergy as a risk factor for life-threatening asthma in childhood: a case-controlled study. *J Allergy Clin Immunol* 2003; 112:168–74.
- 24 Muller UR. Insect sting allergy, clinical diagnosis and treatment. Stuttgart, Gustav Fischer, 1990.
- 25 Ewan PW, Clark AT. Long-term prospective observational study of patients with peanut and nut allergy after participation in a management plan. *Lancet* 2001; 357:111–5.
- 26 Hourihane JO'B, Bedwani SJ, Dean TP, Warner JO. Randomised, double-blind, crossover challenge study of allergenicity of peanut oils in subjects allergic to peanuts. *Br Med J* 1997; 314:1084–8.
- 27 Bock SA, Sampson HA, Atkins FM et al. Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol* 1988; 82:986–97.
- 28 Grimshaw KEC, King RM, Nordlee JA, Hefle SL, Warner JO, Hourihane JO'B. Presentation of allergen in different food preparations affects the severity of the allergic reaction – a case series. *Clin Exp Allergy* 2003; 33:1581–5.
- 29 Morisset M, Moneret-Vautrin DA, Kanny G et al. Thresholds of clinical reactivity to milk, egg, peanut and sesame in immunoglobulin E-dependent allergies: evaluation by double-blind or single-blind placebo-controlled oral challenges. *Clin Exp Allergy* 2003; 33:1046–51.
- 30 van der Zee JA, Wensing M, Penninks AH, Bruijnzeel-Koomen CAF, Knulst AC. Threshold levels in peanut and hazel nut allergic patients do not change over time. *Proceedings of XXII Congress of European Academy of Allergology and Clinical Immunology*, Paris, 2003.