

# Reducing the need for food allergen challenges in young children: a comparison of *in vitro* with *in vivo* tests

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## Summary

**Background** Double-blind placebo-controlled food challenges (DBPCFC), the gold standard for the diagnosis of food hypersensitivity, are time-consuming and not without risk. We have recently reported skin prick test (SPT) weal diameters to cow's milk, egg and peanut above which infants and young children referred for investigation of suspected food allergy showed an adverse reaction on food challenge. We have termed these the '100% diagnostic SPT levels'. In this study, we compare *in vivo* with *in vitro* measurement of IgE antibody levels to three common food allergens — cow's milk, egg and peanut — in infants and young children with suspected food allergy, in order to reduce the need for food challenges.

**Methods** SPT and Enzyme Allergo-sorbent Test (EAST) (from 1992 to 1998) and CAP values (from 1999 to 2000) were performed in 820 children < 2 years of age with suspected allergy to cow's milk and/or egg and/or peanut. SPT levels previously shown to be diagnostic of challenge-proven allergy to cow's milk, egg and peanut were used as the '100% diagnostic SPT levels' and compared with EAST and CAP values associated with IgE food allergy according to the manufacturer's definition.

**Results** McNemar's test showed a significant difference between the '100% diagnostic SPT levels' and positive EAST in identifying patients who did not require food challenge for cow's milk ( $P = 0.01$ ), egg ( $P < 10^{-6}$ ) and peanut ( $P < 10^{-6}$ ), and a significant difference between the '100% diagnostic SPT levels' and positive CAP ( $P < 10^{-6}$ ) for egg and peanut but not cow's milk. Twenty-three per cent of food challenges which, based on the results of EAST and CAP, would have been necessary to confirm the diagnosis of food allergy were avoided by the use of the '100% diagnostic SPT levels'.

**Conclusion** The use of the '100% diagnostic SPT levels' compared with *in vitro* measurement of IgE antibody to cow's milk, egg and peanut reduces the need for food challenge in young children with suspected food allergy.

**Keywords:** food allergy, infancy and childhood, acute hypersensitivity skin tests, food challenge, *in vitro* tests of IgE hypersensitivity

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## Introduction

The gold standard for the diagnosis of food hypersensitivity is the double-blind placebo-controlled food challenge (DBPCFC). DBPCFC is time-consuming and is not without

risk [1]. In an attempt to reduce the need for DBPCFC, Sampson and Albergo [2] identified positive and negative predictive values of different levels of circulating IgE antibodies to milk, egg and peanut using RAST. They found these values comparable to skin prick test results when a RAST score  $> 3$  was used. Subsequently, Sampson and Ho [1] found CAP-FEIA for cow's milk, egg and peanut comparable to skin tests. In addition, they

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determined serum levels of IgE to cow's milk, egg and peanut, which identified patients likely to experience clinical reactions to these foods on challenge. A skin prick test (SPT) weal diameter of  $>3$  mm was used as a dichotomous marker of food allergy.

We have previously reported skin prick test weal diameters  $>6$  mm to be more specific and of greater use than RAST in identifying children with challenge-proven cow's milk allergy (CMA) [3]. We have recently reported SPT weal diameters to cow's milk, egg and peanut above which infants and young children referred for investigation of suspected food allergy showed an adverse reaction on food challenge [4]. We have termed these levels the '100% diagnostic SPT levels' for diagnosing (but not excluding) IgE food allergy to cow's milk, egg and peanut.

These studies have highlighted the potential value of markers of IgE food sensitization to identify children with IgE-associated food allergy, thereby avoiding unnecessary food challenges. In this study we explore the association between the level of serum IgE antibodies with the '100% diagnostic SPT levels' for cow's milk, egg and peanut in infants and young children with suspected food allergy.

## Materials and methods

### Subjects

The subjects were 820 children, median age 13.1 months (range 0.72–24 months) (531 males) presenting between 1992 and 1998 with suspected allergy to cow's milk, egg and/or peanut. They presented with clinical features attributable to food allergy and similar to those patients in our previous studies of food allergy, i.e. manifesting as cutaneous, respiratory and/or gastrointestinal symptoms occurring immediately or evolving over time following the ingestion of cow's milk, egg or peanut [5]. Skin prick tests were completed and blood samples taken on the day of initial assessment.

### Skin tests

Allergen skin prick tests were performed with a lancet technique using extracts of the relevant foods, cow's milk, egg white and peanut (Dome-Hollister-Stier, Spokane, WA, USA) [6]. Histamine (1 mg per ml) and a negative control solution were also used. Skin tests were performed on the patients' backs. The skin weal diameter (mm) was measured after 10–15 min for histamine and after 15–20 min for allergens. The tests were performed by a specialist nurse.

### Serum IgE food antibody levels

Between 1992 and 1998 serum-specific IgE antibody levels for cow's milk, egg and peanut were measured by an ELISA with the Allercoat microplate Enzyme Allergo-sorbent Test (EAST: Kallestad Diagnostics Inc., Cheska, MN, USA). There were 667 children, median age 13 months (range 0.72–24 months) (435 males). EAST results were interpreted according to the manufacturers directions as:

- negative, 0,  $<0.35$  AEU/mL;
- borderline, 1,  $0.35$ – $0.69$  AEU/mL;
- positive, 2,  $0.70$ – $3.49$  AEU/mL;
- strong positive, 3,  $3.5$ – $17.5$  AEU/mL;
- highly positive, 4,  $>17.5$  AEU/mL.

From early 1999, IgE antibodies to these foods have been measured by the ImmunoCAP fluoroenzymeimmunoassay (Pharmacia Upjohn AB, Uppsala, Sweden). There were 153 children investigated (median age 13.1 months, range 2.9–23.6, 96 males). Results were interpreted according to the manufacturers directions as:

- negative, 0,  $<0.35$  kUA/L;
- borderline, 1,  $0.35$ – $0.69$  kUA/L;
- positive, 2,  $0.70$ – $3.49$  kUA/L;
- strong positive, 3,  $3.5$ – $17.49$  kUA/L;
- highly positive, 4,  $17.5$ – $49$  kUA/L;
- highly positive, 5,  $50$ – $99$  kUA/L;
- highly positive, 6,  $>99$  kUA/L.

### Definition of IgE food allergy

Based on our previous studies of children less than 2 years of age with challenge-proven food allergy we designated the following SPT weal diameters to define the '100% diagnostic SPT levels' for IgE food allergy. Above these values, all children less than 2 years of age presenting with a history of food allergy react to the appropriate food on challenge, that is the diameter at which the positive predictive value (PPV) and specificity are 100%. These were: cow's milk  $>6$  mm, egg  $>5$  mm and peanut  $>4$  mm [4]. In those studies SPTs were performed within the 4 weeks prior to food challenge.

### Statistics

McNemar's test was used to compare the results of SPTs with EAST and CAP.

## Results

In Table 1, the frequency with which IgE food allergy to cow's milk, egg and peanut was diagnosed by SPT, the '100% diagnostic SPT level' (milk  $>6$  mm, egg  $>5$  mm,

**Table 1.** Frequency with which IgE food allergy to cow's milk, egg and peanut was diagnosed by SPT

| EAST (AEU/mL)                |            | Cow's milk*<br>allergy | Egg*<br>allergy | Peanut*<br>allergy |
|------------------------------|------------|------------------------|-----------------|--------------------|
| Score                        | Value      |                        |                 |                    |
| 0                            | 0–0.34     | 20                     | 92              | 37                 |
| 1                            | 0.35–0.690 | 14                     | 51              | 33                 |
| 2                            | 0.7–3.49   | 29                     | 93              | 85                 |
| 3                            | 3.5–17.5   | 61                     | 150             | 108                |
| 4                            | > 17.5–50  | 1                      | 10              | 7                  |
| Total                        |            | 125                    | 396             | 270                |
| Total no. of tests performed |            | 640                    | 665             | 643                |

\*Allergy diagnosed by SPT [4].

peanut > 4 mm), is shown with corresponding serum IgE antibody levels scored by EAST.

From Table 1, if the EAST results are dichotomized to grade 0+ , 1+ , 2+ vs. grade 3+ , 4+, and grades 3+ and 4+ are regarded as diagnostic of food allergy [4], 63 children had IgE milk allergy by SPT but not by EAST and thereby avoided formal cow's milk challenge. Conversely, 37 patients with positive EAST scores (3+ , 4+) had SPT < 6 mm and should have been challenged. For egg, 236 challenges that were indicated by EAST were avoided by using SPT results and four were 'positive' by EAST and 'negative' by SPT. Finally for peanut, 155 challenges were avoided by using SPT; seven patients were negative by SPT but positive by EAST.

Overall, 454 challenges were avoided in patients who were at or above the '100% diagnostic SPT levels' but had negative EAST. Conversely, 48 challenges would have been required based on the 'negative' SPT but 'positive' EAST.

McNemar's test showed that there was a significant difference between the '100% diagnostic SPT levels' and 'positive' EAST in diagnosing food allergy, i.e. identifying patients who did not require a food challenge, for cow's milk ( $P = 0.01$ ), egg ( $P < 10^{-6}$ ) and peanut ( $P < 10^{-6}$ ).

In Table 2, the relationship between different levels of IgE antibodies to cow's milk, egg and peanut measured by CAP is shown together with the frequency of IgE food allergy to these foods defined by SPTs. From Table 2, if CAP results are dichotomized as grade 4, 5, 6 'positive', and 0, 1, 2, 3 'negative', the '100% diagnostic SPT level' identified 11 children with CMA, 63 with egg allergy and 37 with peanut allergy who, based on a 'negative' CAP, would have required challenges to confirm food allergy.

However, CAP kUA/L levels, with a positive predictive

**Table 2.** The relationship between different levels of IgE antibodies to cow's milk, egg and peanut measured by CAP

| CAP (kUA/L)           |           | Cow's milk*<br>allergy | Egg*<br>allergy | Peanut*<br>allergy |
|-----------------------|-----------|------------------------|-----------------|--------------------|
| Score                 | Value     |                        |                 |                    |
| 0                     | 0–0.34    | 0                      | 2               | 5                  |
| 1                     | 0.35–0.70 | 0                      | 7               | 3                  |
| 2                     | 0.7–3.5   | 5                      | 26              | 15                 |
| 3                     | 3.5–17.5  | 6                      | 28              | 14                 |
| 4                     | 17.5–50   | 2                      | 15              | 11                 |
| 5                     | 50–99     | 1                      | 2               | 2                  |
| 6                     | > 99      | 1                      | 2               | 4                  |
| Total                 |           | 15                     | 82              | 54                 |
| Total patients tested |           | 127                    | 150             | 137                |

\*Food allergy based on SPT [4].

value of 95% (95%PPV) for children with challenge-proven allergy to cow's milk, egg and/or peanut have been identified by Sampson [1]. In Table 3, the frequency of IgE food allergy identified by SPT and 95%PPV CAP is compared.

From Table 3, using the 100% diagnostic SPT levels for IgE food allergy, 12 children had cow's milk allergy, 47 egg allergy and 37 peanut allergy, but had CAP levels of < 95%PPV, i.e. a total of 96 food challenges were avoided by using these SPT levels. Conversely, 15 challenges could have been avoided by use of the 95%PPV CAP data in patients who showed 'negative' SPT, i.e. less than the 100% diagnostic SPT levels.

Statistical analysis (McNemar's test) showed there was a difference between the 100% diagnostic SPT levels and the 95%PPV CAP [1] in diagnosing food allergy, i.e. identifying those patients who did not need to be challenged, for egg ( $P < 10^{-6}$ ) and peanut ( $P < 10^{-6}$ ) but not for cow's milk ( $P > 0.05$ ).

## Discussion

The results showed that the '100% diagnostic SPT levels' to cow's milk, egg and peanut have different management implications compared with *in vitro* tests in infants and young children with suspected food allergy. Thus, 23% of food challenges based on the results of EAST and CAP that would have been necessary to confirm the diagnosis of food allergy were avoided by the use of the 100% diagnostic SPT levels. The CAP 95%PPV [1] values (23% requiring challenge) marginally improved the overall performance of CAP compared with the manufacturers suggested cut-off levels (35% requiring challenge), but gave significantly different results from the 100% diagnostic SPT levels for

**Table 3.** The frequency of IgE food allergy identified by SPT and 95%PPV CAP is compared

|            | CAP value < 95%<br>+ ve PPV | Food allergy by positive<br>SPT but 'negative CAP' | Food allergy by positive<br>CAP but 'negative SPT' | Total<br>tested |
|------------|-----------------------------|----------------------------------------------------|----------------------------------------------------|-----------------|
| Cow's milk | < 32 kUA/L                  | 12                                                 | 9                                                  | 127             |
| Egg        | < 6 kUA/L                   | 47                                                 | 4                                                  | 150             |
| Peanut     | < 15 kUA/L                  | 37                                                 | 2                                                  | 137             |
| Total      |                             | 96                                                 | 15                                                 | 414             |

egg and peanut but not for milk. Small patient numbers may have obscured the significance of the milk results.

Why should tissue-fixed IgE antibodies be of greater clinical value in the diagnosis of IgE food allergy than circulating IgE antibody levels? IgE antibodies can be considered to be in two pools. The first, a circulating pool of IgE, and the second, a tissue-fixed IgE pool bound to basophils and mast cells [7,8]. Because of the high affinity for IgE of the Fcε receptors on mast cells and basophils with affinity constants ( $K_a$ ) ranging from  $10^9$  to  $10^{10} \text{ M}^{-1}$ , the IgE equilibrium will favour the tissue pool until tissue saturation is achieved, relatively depleting circulating IgE antibody levels [7]. During this period there will be a dissociation between the skin and serum markers of IgE sensitization. Tissue-fixed IgE antibodies will be available to respond to organ challenge either clinically or via SPTs, despite low or undetectable levels of specific IgE circulating antibodies. Thus, children with severe IgE food allergy would be expected to have high tissue-bound and high serum IgE antibody levels, whereas those with less severe food allergy would be expected to have low levels of serum IgE food antibody levels but high levels of skin test reactivity. These findings are consistent with results of our previous studies of the natural history of infants with cow's milk allergy [3]. Patients with severe cow's milk allergy had persistent disease and had high SPT and serum IgE antibodies to cow's milk, whereas those with less severe disease which rapidly remitted had high SPT levels but low levels of circulating IgE antibodies to cow's milk.

Eigenmann *et al.* have recently defined positive SPTs to food allergens where the mean weal diameter exceeded the 95% confidence interval of SPTs in food-tolerant children with a history of atopic dermatitis [9]. These cut-off values were not superior to assigning a positive skin test as  $> 3$  mm diameter for the prediction of food allergy by a double-blind placebo-controlled trial. However, Sporik *et al.* have described the poor specificity of this 3 mm cut-off level in the identification of children with allergy to cow's milk, egg or peanut [4].

Sampson *et al.*, using a similar approach to ourselves, have defined the 95%PPV CAP values above which

children with food allergy will react by DBPC [1]. Thus, both the CAP 95% PPVs and our own '100% diagnostic SPT levels' SPTs have used a similar approach to redefine test cut-off levels in order to minimize the number of food challenges which need to be undertaken, i.e. both groups have identified levels that have practical clinical management implications. Thus, children with SPT diameter above these 'cut-off' levels do not require challenge but those below these levels require challenge as they may or may not have clinical food allergy.

Despite a similar approach, why do our results seem to differ so markedly from the results of Sampson and Ho [1]? Firstly, our patients were less than two years of age and presented with a variety of symptoms, whereas those of Sampson and Ho were older (median age 5.2 years) and presented with atopic dermatitis, thus they would be expected to have higher serum IgE levels and higher PPV cut-off values than our population. Secondly, we utilized the range of SPT weal diameters to determine the point where specificity equalled one in this population. Sampson and Ho used a 3-mm cut-off when comparing CAP with SPT, thus not utilizing all the potential information available from a skin prick test. It would be interesting to determine the association between CAP results and graded SPT results for Sampson and Ho's patients. Thirdly, our allergen extracts and those used by Sampson were from different manufacturers.

In our studies the 100% diagnostic SPT levels were obtained using allergen extracts from one manufacturer and studies were performed by specialist trained staff. Although we utilized arbitrary serum-specific IgE cut-off values advised by the manufacturers, and Sampson's CAP findings are from a different population, our findings suggest that if allergen extracts and skin test methodology can be standardized, skin prick tests to food allergens could be made more clinically relevant and used in epidemiological studies [10].

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