

The diagnostic value of skin prick testing in children with food allergy

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The diagnostic accuracy of the skin prick test (SPT) in food allergy is controversial. We have developed diagnostic cut-off levels for SPT in children with allergy to cow milk, egg and peanut. Based on 555 open food challenges in 467 children (median age 3.0 yr) we defined food-specific SPT weal diameters that were '100% diagnostic' for allergy to cow milk (≥ 8 mm), egg (≥ 7 mm) and peanut (≥ 8 mm). In children < 2 yr of age, the corresponding weal diameters were ≥ 6 mm, ≥ 5 mm and ≥ 4 mm, respectively. These SPT cut-off levels were prospectively validated in 90 consecutive children ≤ 2 yr with challenge-proven food allergy. In young infants under 6 months of age who have not previously been exposed to a particular food item, the SPT were often negative or below the diagnostic cut-off but reached the diagnostic cut-off at the time of challenge in the second year of life. We assessed the diagnostic agreement between food-specific immunoglobulin E (IgE) antibody levels and SPT in a cohort of 820 infants and children under 2 yr of age (median age 13.1 months) with suspected allergy to cow milk, egg or peanut. When applying published 95%-positive predictive CAP values, the diagnostic accuracy of SPT and IgE antibody levels was similar for cow milk, but SPT was more sensitive in diagnosing allergy to egg ($p < 0.0001$) and peanut ($p < 0.0001$). Further studies are required to define age-specific diagnostic IgE antibody and SPT cut-off levels use in infants under 2 yr of age with suspected food allergies.

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The diagnostic accuracy of the allergen skin prick test (SPT) in patients with suspected immunoglobulin E (IgE)-mediated food allergy is controversial. A positive SPT indicates food sensitization, i.e. the presence of food-specific IgE antibodies (1). However, a positive SPT may occur in tolerant individuals and does not necessarily represent food allergy. Measurement of food-specific IgE antibodies can be achieved *in vivo* by SPT (2), or *in vitro* using the PharmaciaTM CAP System IgE fluoroimmunoassay (CAP) (Pharmacia Diagnostics AB, Uppsala, Sweden) (3). When SPT is performed, minute amounts of allergen are introduced into the superficial layers of the skin with a lancet (4). The allergen diffuses into the surrounding tissue. In sensitized patients, specific IgE antibodies on the surface of mast cells recognize the antigen,

which is followed by the release of histamine and other mediators (5). This mediator release and axonal reflex lead to the development of the weal-and-flare reaction typical of a positive SPT.

In our studies we defined diagnostic weal diameters to common food allergens which would invariably be associated with adverse clinical reactions on challenge (2). We were seeking a simple diagnostic tool to use in clinical practice that could reliably identify children with food allergy without the need for resource-consuming and potentially hazardous food challenges (6). Sampson and Ho have published 95%-positive predictive CAP values (95%-PPV CAP) for cow milk, egg, peanut and fish (3, 7). Others have demonstrated that by combining CAP with the atopy patch test the need for food challenges was significantly

reduced (8). In the following review, the results of studies on the diagnostic value of SPT and food-specific IgE antibodies, conducted at the Royal Children's Hospital, Melbourne, will be presented.

Diagnostic value of SPT and RAST in children with cow milk allergy

Cow milk is usually one of the first food proteins that infants are exposed to, either directly or indirectly through breast milk (9). The Melbourne Milk Allergy Study (MMAS) (10) described a group of adverse clinical symptoms and syndromes which could be elicited by dietary challenge. These ranged from immediate reactions occurring within minutes (anaphylaxis, urticaria), to intermediate reactions within hours (vomiting, diarrhoea, distress) and late reactions within 24 h (cutaneous, gastrointestinal or respiratory symptoms). The immediate reactors had high levels of cow milk-specific IgE antibodies, as detected *in vitro* by radioallergosorbent test (RAST), or *in vivo* by SPT. From a clinical perspective, IgE hypersensitivity to cow milk was likely if adverse reactions occurred within minutes of ingesting cow milk.

In our initial study on cow milk allergy (CMA) in childhood (9), 135 consecutive challenges were conducted in 75 boys and 45 girls (median age 24 months; range: 5 months to 13 yr), who had been referred for investigation of suspected CMA. Our standard challenge protocol was followed (2, 11). SPT was performed with whole cow milk allergen extract (Dome-Hollister-Stier, Spokane, WA, USA). The size of the skin weal elicited by the milk extract was compared with that of histamine 1 mg/ml and a control solution (12). We found that if a child presented with a history suggestive of CMA and we selected an SPT with '100% specificity', i.e. 4+ histamine equivalent (HE) units, SPT identified 47% of children with CMA (sensitivity). The 4+ HE to cow milk extract corresponded with a weal diameter of 6 mm. By contrast, using the RAST score with 100% specificity (RAST class 4 and above), this identified only 10% of children with CMA. Based on this observation, we considered the RAST less sensitive than SPT and therefore clinically less useful as a screening tool for CMA.

Usefulness of SPT in predicting positive open food challenges

Over the next decade, a prospective study of children with suspected hypersensitivity to cow milk, egg and peanut was undertaken (2). These

children were referred from a high-risk population for the investigation of food allergy. The results of 555 food challenges performed in 467 children (median age 3.0 yr) were available (2). Challenge outcomes were categorized into three groups: positive, negative or inconclusive. The sensitivity and specificity of each weal diameter as a predictor of a positive food challenge were calculated and data from inconclusive challenges excluded. Fig. 1a–c summarize the relationship between SPT weal diameters to cow milk, egg and peanut, and challenge outcomes. Challenges were analysed separately for children aged 0–2 yr, and older children. For the whole group (median age 3 yr), an SPT weal diameter to cow milk ≥ 8 mm, egg ≥ 7 mm and peanut ≥ 8 mm was invariably associated with an adverse reaction on formal open challenge. Of major importance was the finding that for children 2 yr of age or younger '100% specificity' was achieved with lower SPT weal diameters: ≥ 6 mm for cow milk, ≥ 5 mm for egg and ≥ 4 mm for peanut. This may in part be attributable to lower intrinsic skin reactivity in this age group (13). It may also reflect a lower threshold of reactivity to food allergens in young children with IgE food hypersensitivity.

Limitation of using a 3 mm cut-off weal diameter in the diagnosis of food allergy

In the past, international best practice has been to consider an SPT weal cut-off diameters of ≥ 3 mm as a positive reaction (14). Below this level non-specific and poorly reproducible reactions may be seen (1, 6, 15). Cutaneous reactivity varies with age to both histamine and non-specific mast cell degranulators (16). Therefore, many centres use a 1% or 10% histamine solution-induced SPT as a reference, and some report the size of the allergen-induced weal as HE units (12, 16). Our data have shown that these SPT cut-off levels will vary depending on the clinical context, the allergen extract used and the age of the child.

In Tables 1 and 2, the diagnostic accuracy of different SPT weal diameters is shown for cow milk, egg and peanut, in children 0–2 yr, and children 2 yr and older (2). It should be noted that a positive allergen SPT of ≥ 3 mm was a poor predictor of clinically relevant food allergy. For the entire cohort, regardless of age group, an SPT ≥ 3 mm had a sensitivity for detecting allergy to cow milk of 74%, 84% to egg, and 96% to peanut, but the specificity was only 79%, 70% and 71%, respectively.

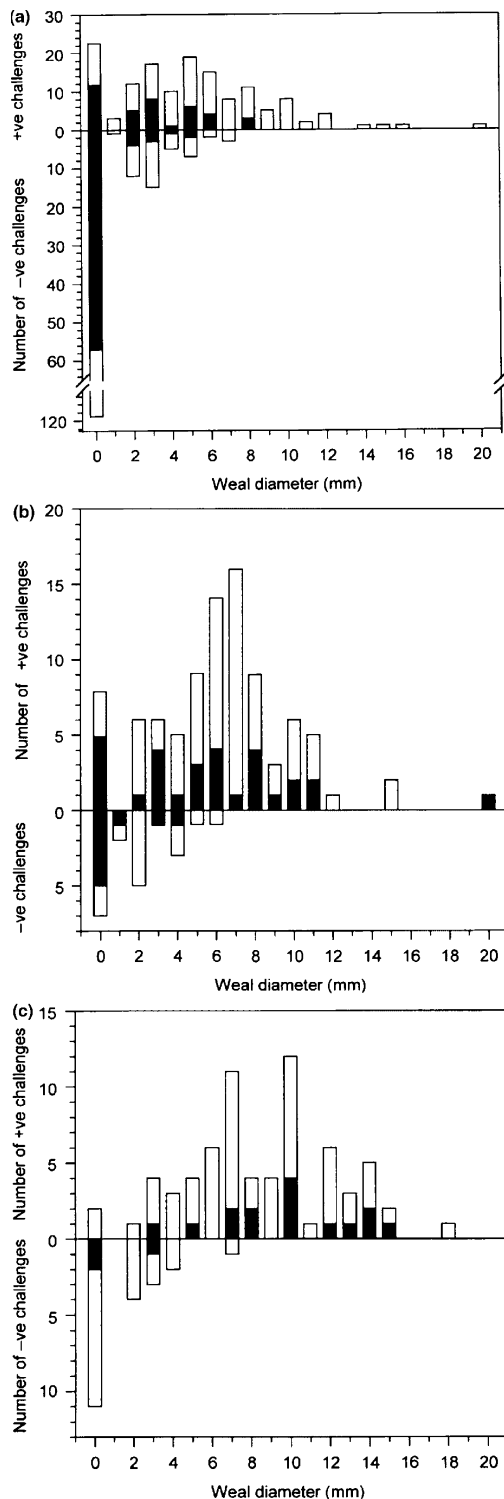


Fig. 1. (a) The outcome of cow milk challenge and cow milk skin test weal diameter (mm). The solid bars represent the subgroup of children 0–2 yr. Reproduced with permission from Sporik et al. (2). (b) The outcome of egg challenge in egg skin test weal diameter (mm). The solid bars represent the subgroup of children 0–2 yr. Reproduced with permission from Sporik et al. (2). (c) The outcome of peanut challenge and peanut skin test weal diameter (mm). The solid bars represent the subgroup of children 0–2 yr. Reproduced with permission from Sporik et al. (2).

Diagnostic SPT weal diameters in infants and young children not previously exposed to suspected food allergens

We investigated the diagnostic accuracy of SPT in children who had never directly ingested a particular food item. Infants were identified from the Melbourne Atopy Cohort Study (MACS) (17), a birth cohort of 620 atopy-prone infants. Mothers were encouraged to breastfeed, and infants commenced solids from 6 months of age. Introduction of egg, peanut and tree nuts into the diet were delayed until after 12 months. All infants were skin tested at 6 months of age, and 1 yr thereafter. Infants with any SPT reactivity to cow milk, egg or peanut underwent open food challenges (2).

We identified 37 infants who had never directly been exposed to cow milk, egg or peanut, but who reacted one of these food items on challenge before 2 yr of age. In these patients, SPT at 6 months were often below our recently defined age-specific 100%-diagnostic cut-off (2), but SPT weal diameters reached the diagnostic cut-off at the time of challenge in the second year of life. As expected, some, but not all children with levels below the '100% diagnostic level' reacted on challenge. However, all children above that level had a positive challenge. Fig. 2a,b shows the SPT weal diameter to egg and peanut at 6 months of age, and at the time of challenge in the second year of life. SPT levels to egg ($p = 0.0004$) and peanut ($p = 0.01$) were significantly lower at 6 months of age than at the time of challenge about 12 months later.

At 6 months of age, 13 of the infants had a negative SPT (0 mm) to egg, but 12 months later, at the time of open challenge, 10 of these infants had developed positive SPT (≥ 5 mm) above the diagnostic SPT threshold. Similarly, of six infants who were SPT-negative (0 mm) to peanut at 6 months, four had developed positive SPT of ≥ 4 mm at the time of challenge 12 months later (Fig. 2a,b). All of these children with an SPT above the age-specific diagnostic cut-off level subsequently reacted to open challenge with egg and peanut. All but one of the children had never had egg or peanut during this period (one had accidental exposure to egg at 7 months of age that elicited perioral erythema). Thus, negative skin tests to these foods in early infancy do not preclude the subsequent development of IgE hypersensitivity in later childhood.

Table 1. Diagnostic accuracy of age-specific skin prick weal diameters in children aged 2 yr and above (2)

Food item	Weal diameter (mm)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Cow milk	0	0.11	0.36	0.15	0.28
	≥3	0.79	0.73	0.75	0.77
	≥6	0.49	0.95	0.91	0.64
	≥8	0.30	1.00	1.00	0.58
Egg	0	0.05	0.83	0.60	0.14
	≥3	0.87	0.67	0.93	0.50
	≥6	0.68	0.92	0.98	0.35
	≥7	0.52	1.00	1.00	0.29
Peanut	0	0.04	0.50	0.18	0.15
	≥3	0.95	0.72	0.91	0.81
	≥6	0.78	0.94	0.98	0.59
	≥8	0.51	1.00	1.00	0.40

Table 2. Diagnostic accuracy of age-specific skin prick weal diameters in children under 2 yr of age (2)

Food item	Weal diameter (mm)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Cow milk	0	0.30	0.15	0.17	0.26
	≥3	0.58	0.91	0.79	0.78
	≥6	0.20	1.00	1.00	0.68
Egg	0	0.17	0.38	0.50	0.11
	≥3	0.79	0.75	0.92	0.50
	≥5	0.62	1.00	1.00	0.42
Peanut	0	0.00	0.33	0.00	0.06
	≥3	1.00	0.67	0.94	1.00
	≥4	0.93	1.00	1.00	0.75

Prospective validation of 100%-diagnostic SPT levels in children ≤ 2 yr of age

We prospectively validated the diagnostic accuracy of the age-specific SPT cut-off levels in 90 consecutive infants ≤ 2 yr of age with IgE-mediated allergy to cow milk, egg or peanut (Table 3). All infants had presented with a convincing history of immediate-onset urticaria or angioedema after ingestion of small amounts of allergen. SPT were performed in all infants within a mean of 1.5 ± 0.1 months after the initial allergic reaction. SPT diameters (median, 95% CI: minimum, maximum) for each food item are summarized in Table 3. None of the infants presented with SPT diameters below the published diagnostic cut-off levels (2), suggesting that all infants would have been correctly diagnosed as allergic to cow milk, egg or peanut.

Comparison of *in vitro* and *in vivo* tests in young children with food allergy

As mentioned above, SPT appeared more sensitive than RAST in identifying children with food allergy (11). In that study, we explored the agreement between CAP-RAST levels with the 95%-PPV CAP, as defined by Sampson and Ho

(3), and the '100%-diagnostic' SPT levels for milk, egg and peanut in young children with suspected food allergy. We studied 820 children, median age 13.1 months (range: 0.7–24 months; 531 male), who had presented with suspected allergy to cow milk, egg and/or peanut. The clinical features attributed to food allergy were similar to patients in our previous studies. SPT were performed and blood samples taken on the day of the initial assessment. In Table 4, the frequency of IgE food allergy identified by the '100%-diagnostic SPT' and the 95%-PPV CAP is shown.

From the data presented in Table 4, McNemar's test showed a significant difference between the 100%-diagnostic SPT levels and 95%-PPV CAP in diagnosing food allergy to egg ($p < 10^{-6}$) and peanut ($p < 10^{-6}$), but not to cow milk ($p > 0.05$). Children (23.2%) with IgE food allergy were identified by the '100%-diagnostic SPT' but not by 95%-PPV CAP. On the contrary, only 3.6% of the patients were identified by 95%-PPV CAP but not by SPT. These results suggest that the use of the '100%-diagnostic SPT' levels, compared with *in vitro* measurement of IgE antibodies to cow milk, egg and peanut, reduce the need for food challenges in young children with suspected food allergy.

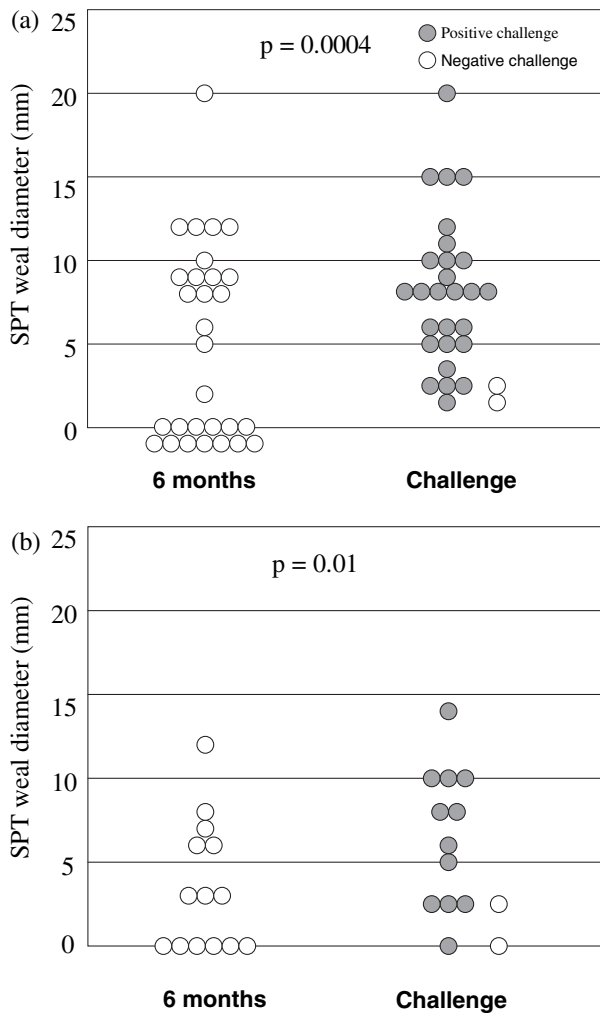


Fig. 2. (a) Skin prick test weal diameter at 6 months and at the time of egg challenge in 29 children under 2 yr of age not previously exposed to egg. (b) Skin prick test weal diameter at 6 months and at the time of peanut challenge in 14 children under 2 yr of age not previously exposed to peanut.

Table 3. Skin prick test (SPT) results in 90 children <2 yr of age with food allergic reactions (18)

	Cow milk	Egg	Peanut
Number of infants	27	30	33
Mean age at first reaction (months, s.d.)	10.7 (4.6)	11.5 (2.4)	12.6 (4.4)
Mean age at SPT (months, s.d.)	11.9 (4.4)	13.1 (2.8)	14.3 (4.3)
Median weal diameter (mm, 95% CI)	6.0 (6–8)	8.0 (6–13)	7.0 (5–13)
Minimum weal diameter (mm)	6.0	5.0	4.0
Maximum weal diameter (mm)	12.0	22.0	17.0

CI, confidence interval.

A major limitation of comparisons between positive SPT and CAP cut-off values were performed between children from different age groups. The median age of our patients in our study was 13 months, whereas patients in the

cohort of Sampson and Ho (3) were up to 16 yr of age (median age 5.2 yr). Two more recent Spanish studies have defined 95%-PPV CAP for cow milk and egg in infants and young children (18, 19). In a study of children under 2 yr of age with egg allergy, Boyano-Martínez et al. (18) found that CAP levels ≥ 0.35 kUA/l were diagnostic of egg allergy; these diagnostic levels were lower than the 95%-PPV CAP level of 6 kUA/l found by Sampson and Ho (3). In addition, García-Ara et al. (19) found a 95%-PPV CAP of 5 kUA/l for cow in infants with challenge-proven CMA, which was again lower than the cut-off level of 32 kUA/l defined by Sampson and Ho (3).

In the light of the Spanish studies (18, 19), we have re-examined some of our early data comparing the '100%-diagnostic SPT' weal diameters for cow milk and egg allergy with the 95%-PPV CAP from the Spanish cohorts (Table 5). Generally, there was good agreement between both parameters, but as shown in Table 5, disagreement was noted in 16.6% of cases for cow milk, and in 8.0% for egg. Further, prospective studies are required to re-evaluate these findings.

Limitations of '100%-diagnostic SPT' in the diagnosis of IgE food allergy

Use of the '100% diagnostic SPT' cut-off levels, i.e. levels representing 100% specificity, has the advantage of making an accurate diagnosis of food allergy without subjecting children to inappropriate dietary restriction. However, it does have the disadvantage of increasing the need for formal food challenge in order to determine the status of children who fell below the diagnostic cut-off. Our cut-off values were derived from a referred population with a suspected diagnosis of food allergy or from an atopy-prone birth cohort (MACS). These cut-off levels have not been validated on a population-based sample of unselected children. As the level of an SPT is affected by a number of factors, including the potency of the extract being used (20), the method by which the SPT is performed, the intrinsic skin reactivity, the age of the child and variations in technique (13), it will be essential for individual centres to develop their own *in vivo* and *in vitro* markers of food allergy in relation to the outcome of food challenges.

Concluding remarks

Our studies have demonstrated that SPT is a useful tool in the diagnosis of food allergy. Using

Table 4. Diagnostic agreement between '100%-diagnostic skin prick test (SPT)' wheal diameter (2) and or 95%-positive predictive CAP-IEA level in children with challenge-proven allergy to cow milk, egg or peanut (3)

	≥95% positive predictive CAP level (kU _A /l)	Agreement between SPT and CAP, n (%)	Positive SPT, but negative CAP, n (%)	Positive CAP, but negative SPT, n (%)	Total number of positive challenges, n (%)
Cow milk	≥32	106 (83.5)	12 (9.4)	9 (7.1)	127 (100)
Egg	≥6	99 (66.0)	47 (31.3)	4 (2.7)	150 (100)
Peanut	≥15	98 (71.5)	37 (27.0)	2 (1.5)	137 (100)
Total		303 (73.2)	96 (23.2)	15 (3.6)	414 (100)

Table 5. Diagnostic agreement between '100%-diagnostic' skin prick test (SPT) wheal diameter (2) and or '95%-predictive CAP-IEA levels' (19, 20) in children ≤ 2 yr of age with challenge-proven allergy to cow milk or egg

	≥95% positive predictive CAP level (kU _A /l)	Agreement between SPT and CAP, n (%)	Positive SPT, but negative CAP, n (%)	Positive CAP, but negative SPT, n (%)	Total number of positive challenges, n (%)
Cow milk	≥5	105 (83.3)	11 (8.7)	10 (7.9)	126 (100)
Egg	≥0.35	138 (92.0)	5 (3.3)	7 (4.7)	150 (100)
Total		243 (88.0)	16 (5.8)	17 (6.2)	276 (100)

'100%-diagnostic SPT' cut-off values, a proportion of children with symptoms of food allergy do not need to be challenged, which reduces costs and hazards. In our early studies, SPT appeared to be more sensitive than food-specific serum IgE antibody testing in the diagnosis of food allergy. However, when age-specific cut-offs were developed, this difference was less significant. Based on these findings, the indications for food challenges in our unit have changed. If on initial assessment the SPT to cow milk, egg or peanut exceeds the '100%-diagnostic' cut-off, the child is considered allergic and will not undergo food challenge at that time. Children are re-evaluated annually and challenged according to the SPT results. Children with adverse reactions to other foods (such as soy and wheat) where the predictive SPT values have not been established, may still require food challenges. Further studies are required to define the diagnostic CAP and SPT cut-off levels for common food allergens in infants and young children.

References

- BOCK SA, BUCKLEY J, HOLST A, MAY CD. Proper use of skin tests with food extracts in diagnosis of hypersensitivity to food in children. *Clin Allergy* 1977; 7: 375–83.
- 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.
- SAMPSON HA, HO DG. Relationship between food-specific IgE concentrations and the risk of positive food challenges in children and adolescents. *J Allergy Clin Immunol* 1997; 100: 444–51.
- ØSTERBALLE O, WEEKE B. A new lancet for skin prick testing. *Allergy* 1979; 34: 209–12.
- KAY AB. Allergy and allergic diseases. First of two parts. *New Engl J Med* 2001; 344: 30–7.
- 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.
- SAMPSON HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001; 107: 891–6.
- ROEHR CC, REIBEL S, ZIEGERT M, SOMMERFELD C, WAHN U, NIGGEMANN B. Atopy patch tests, together with determination of specific IgE levels, reduce the need for oral food challenges in children with atopic dermatitis. *J Allergy Clin Immunol* 2001; 107: 548–53.
- HILL DJ, DUKE AM, HOSKING CS, HUDSON IL. Clinical manifestations of cows' milk allergy in childhood: II. The diagnostic value of skin tests and RAST. *Clin Allergy* 1988; 18: 481–90.
- HILL DJ, HOSKING CS, HEINE RG. Clinical spectrum of food allergy in children in Australia and South-East Asia: identification and targets for treatment. *Ann Med* 1999; 31: 272–81.
- 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.
- AAS K, BELIN L. Standardization of diagnostic work in allergy. *Int Arch Allergy Appl Immunol* 1973; 45: 57–60.
- DREBORG S. Histamine reactivity of the skin. *Allergy* 2001; 56: 359–64.
- EIGENMANN PA, SAMPSON HA. Interpreting skin prick tests in the evaluation of food allergy in children. *Pediatr Allergy Immunol* 1998; 9: 186–91.
- DREBORG S. Skin test in diagnosis of food allergy. *Allergy Proc* 1991; 12: 251–4.
- DREBORG S, HOLGERSSON M, NILSSON G, ZETTERSTRÖM O. Dose response relationship of allergen, histamine, and

- histamine releasers in skin prick test and precision of the skin prick test method. *Allergy* 1987; 42: 117–25.
17. HILL DJ, SPORIK R, THORBURN J, HOSKING CS. The association of atopic dermatitis in infancy with immunoglobulin E food sensitization. *J Pediatr* 2000; 137: 475–9.
18. BOYANO-MARTÍNEZ T, GARCÍA-ARA C, DÍAZ-PENA JM, MARTÍN-MUÑOZ F, GARCÍA SG, MARTÍN-ESTEBAN M. Validity of specific IgE antibodies in children with egg allergy. *Clin Exp Allergy* 2001; 31: 1464–9.
19. GARCÍA-ARA C, BOYANO-MARTÍNEZ T, DÍAZ-PENA JM, MARTÍN-MUÑOZ F, RECHE-FRUTOS M, MARTÍN-ESTEBAN M. Specific IgE levels in the diagnosis of immediate hypersensitivity to cows' milk protein in the infant. *J Allergy Clin Immunol* 2001; 107: 185–90.
20. AAS K, BACKMAN A, BELIN L, WEEKE B. Standardization of allergen extracts with appropriate methods. The combined use of skin prick testing and radio-allergo-sorbent tests. *Allergy* 1978; 33: 130–7.