

Combined skin prick and patch testing enhances identification of food allergy in infants with atopic dermatitis

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Background: Early recognition of dietary allergies in infants with atopic dermatitis is essential for avoidance of unnecessary elimination diets, amelioration of the skin disease, and secondary prevention of the development of multiple food allergies. Simple and accurate methods of identifying provocative foods are urgently needed.

Methods: The usefulness of skin prick and patch tests as indicators of cow milk allergy was evaluated in 183 patients ranging in age from 2 to 36 months with double-blind, placebo-controlled ($n = 118$) or open ($n = 65$) cow milk challenges.

Results: The oral cow milk challenges were interpreted as positive in 54% of both challenge types. Positive challenge rapidly elicited pruritus, urticaria, and/or exanthema in 49% of cases and delayed-onset eczematous lesions in 51%. The skin prick and patch tests gave markedly discrepant results; prick tests were positive in 67% of the cases with acute-onset reactions to milk challenge, whereas patch tests tended to be negative. Patch tests were positive in 89% of those with delayed-onset reactions, although prick tests were frequently negative.

Conclusions: The observations indicate that IgE and T cell-mediated responses to cow milk can be distinguished in atopic dermatitis. Parallel skin testing with combined prick and patch tests can significantly enhance the accuracy in diagnosis of specific dietary allergies in patients with atopic dermatitis. (*J ALLERGY CLIN IMMUNOL* 1996;97:9-15.)

Key words: atopic dermatitis, cow milk allergy, infants, patch test, skin prick test

Evidence for causal linkage between atopic dermatitis and food allergy stems from therapeutic benefit after exclusion of dietary allergens,¹ and the dermatologic outcome seems to be more favorable if the patient loses the food hypersensitivity.²⁻⁵ Demonstration that certain foods can cause or exacerbate atopic dermatitis is laborious, particularly in infancy, when these conditions are most prevalent.⁶

Most food allergies involve an IgE-mediated hypersensitivity reaction, and patients with atopic dermatitis very often show immediate reactions in skin tests with foods.⁷⁻⁹ Positive skin prick test responses with dietary allergens, however, do not necessarily imply a role for these allergens in the

Abbreviation used

CI: Confidence interval

pathogenesis of atopic dermatitis.¹⁰ Double-blind, placebo-controlled food challenges demonstrated acute-onset clinical reactions consisting of urticaria, pruritus, and erythema in a subset of patients with atopic dermatitis,^{7,8,11} whereas others had delayed-onset eczematous reactions.^{9,11-13} No relationship has been established between reactivity in skin prick tests and delayed-onset clinical reactions.⁶

The aim of this study was to delineate diagnostic methods of food allergy in infants. For this purpose patients with atopic dermatitis were randomized to double-blind, placebo-controlled, and open cow milk challenges to compare and characterize respective clinical reactions. Skin tests were performed before challenge. Specifically, we aimed to determine whether patch tests eliciting delayed-type hypersensitivity reaction¹⁴ in combination with skin prick tests giving IgE-mediated hypersen-

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sitivity reaction could provide an indicator of allergy to cow milk in infants with atopic dermatitis.

METHODS

Patients

The study comprised 183 children ranging in age from 2 to 36 months (mean 14 months) and fulfilling the Hanifin criteria¹⁵ of atopic dermatitis in children. They had been referred to a teaching pediatric or dermatologic clinic for evaluation of atopic dermatitis and were not selected on the basis of suspected allergy to cow milk. The mean age (95% confidence interval, [CI]) at onset of dermatitis was 4 months (95% CI, 3.5 to 5 months), and the duration of breast-feeding, exclusively and totally, was 3.5 months (95% CI, 3 to 4 months) and 6 months (95% CI, 5 to 7 months), respectively. Gastrointestinal disturbance such as loose stools, vomiting, or diarrhea and respiratory symptoms such as wheezing were seen in 61 and six patients, respectively. There was a family history of atopic disorders in 128 (70%) of the cases.

For 4 weeks before oral milk challenges the patients were given no cow milk. Instead they received breast milk (11%) or a tolerated formula (soy milk 39%, an extensively hydrolyzed whey protein formula 24%, or an amino acid-derived formula 26%). Based on the clinical history, previous skin testing, and/or RAST, the diet additionally excluded egg in most of the cases, cereals, wheat, barley, rye, and oat in 13%, and various fruits and vegetables in 19%. No patient was receiving systemic corticosteroid therapy.

Antihistamine medication was discontinued for periods of 3 days to 6 weeks before skin testing depending on the drug's duration of action. Skin tests were performed during the cow milk elimination period, always by the same person, and were scored (K. T.) before the result of oral milk challenge was known. The total IgE concentration in serum was measured, and RAST was performed to detect circulating cow milk-specific IgE antibodies before the challenge.

The patients were randomized to double-blind, placebo-controlled or open cow milk challenge to compare the respective rates of positive clinical reactions. The diet of the patients remained unaltered during the challenge period. Informed consent was obtained from the parents. The study was reviewed and approved by the Ethical Committee of Tampere University Hospital.

Double-blind, placebo-controlled, cow milk challenge protocol

On the first day of the challenge, rising doses of the allocated placebo or test formula (1, 5, 10, 50, and 100 ml) were given at approximate 30-minute intervals until milk intake appropriate for the age was reached. The placebo formula was the amino acid-derived Neocate (SHS Int. Ltd., Liverpool, U. K.), and the test formula consisted of Neocate and 10 gm skimmed cow milk powder/100 ml. The concentration of β -lactoglobulin in

the placebo preparation was 0.24 $\mu\text{g/L}$,¹⁶ and the β -lactoglobulin concentration in the test formula was comparable to that in cow milk, 4,000,000 $\mu\text{g/L}$.¹⁷ The placebo and the test formula preparations looked and smelled alike. A computerized randomization scheme was used to fix the sequence of the challenges so that the nursing staff, attending pediatricians, parents, and investigators were unaware of the administered formula's nature.

The challenge period was 1 week for both the placebo and the test formula. The challenge was begun in the hospital and after the first day was continued in the patient's home. The formulas were prepared under code in the hospital's milk bank for daily administration in age-appropriate doses. For 7 days after commencement of challenge with the placebo or the test formula, the parents recorded any clinical symptoms, specifically skin eruptions and pruritus, vomiting, and irritability. They also noted all stools, describing them as solid, loose, or watery. When a clinical reaction appeared, the challenge was stopped and the patients examined in the hospital. Onset of reaction was defined as the time between the latest dose and the specific symptom and from the start of the challenge. In like manner, the dose eliciting the symptom and the cumulated dose from the start of the challenge were determined. All patients were examined on day 7 and on day 14 of the challenge, and the code was thereafter opened.

Open cow milk challenge protocol

The open challenges were made with a ready-to-use infant formula containing 192,000 $\mu\text{g/L}$ β -lactoglobulin (Mäkinen-Kiljunen S, unpublished data). The challenge period was 1 week. The challenge was started in the hospital when rising doses of the infant formula were given in the same way as in the blinded challenges. The challenge then continued in the patient's home, and the parents recorded the symptoms of the child. The patients were reviewed in the hospital at the time of any adverse reaction. The challenge was discontinued when a clinical reaction was noticed. All patients were examined on day 7 of the challenge.

Cow milk allergy was defined as an unequivocal adverse reaction to challenge. To judge long-term tolerance and reveal any false-negative result of challenge, all patients negative to challenge continued to consume cow milk. All patients were seen 1 month after commencement of challenge, when the diagnosis was confirmed for both challenge types by the same investigator (E. I.).

Skin testing

Prick testing was done on the volar aspect of the forearm with a commercially available cow milk allergen, ALK (Allergologisk Laboratorium A/S, Horsholm, Denmark), and a milk powder diluted to normal feed concentration. A 1 mm, one-peak lancet with shoulder to prevent deeper penetration (ALK) was used, and 10 mg/ml histamine dihydrochloride (ALK) was used as positive control. Reactions were read at 15 minutes, and

TABLE I. Reaction onset time and dose, mean (95% CI), eliciting the specific reaction to double-blind, placebo-controlled and open cow milk challenge in patients with atopic dermatitis

	Challenge type	Acute reaction	Delayed reaction
Reaction onset (hr)			
From the last dose	Double-blind	0.1 (0.1-0.2)	5 (4-7)*
	Open	0.2 (0.1-0.2)	6 (4-9)*
From the start of challenge	Double-blind	1.0 (0.7-1.3)	34 (25-44)*
	Open	0.9 (0.6-1.2)	34 (21-46)*
Dose eliciting the symptom (ml)			
Last dose	Double-blind	11 (6-16)	87 (78-96)*
	Open	9 (3-14)	88 (77-99)*
Cumulated dose	Double-blind	21 (10-33)	250 (200-300)*
	Open	25 (5-45)	269 (201-337)*

*Differences statistically significant (Student's *t* test: $p = 0.0001$) compared with acute-onset reactions.

half of the histamine reaction size was recorded as positive (2+).

In patch testing 20 mg humidified skimmed cow milk powder containing 450 μ g β -lactoglobulin was applied on uninvolved skin to the patient's back with aluminium cups (Finn Chamber, Epitest Ltd., Hyrylä, Finland) and Scanpore tape. In the morning of the test day, 300 mg of the milk powder was mixed with 0.2 ml of isotonic saline solution to make a "porridge" that remains on the test cup. The same stock was used for several tests during the same day. The occlusion time was 48 hours, and the results were read 15 minutes after removal of the cups and then at 72 hours. Reactions were classified as negative, irritation (IR), significant erythema (?+), and erythema with edema or eczema (+). Microcrystalline cellulose was used as negative control. In a preliminary study patch testing with cow milk was performed on eight nonatopic infants ranging in age from 11 to 32 months with no sign of cow milk allergy. All these tests were negative.

In addition to skin testing with cow milk, prick and patch tests were done for a long list of dietary antigens.

Statistics

The results of the double-blind, placebo-controlled, and open cow milk challenges are given separately and also when the indexes of sensitivity, specificity, and likelihood ratios of positive and negative results for prick and patch tests are calculated alone or in combination (parallel and serial testing). Because of skewed distribution of serum total IgE concentration, logarithmic (ln) transformation was used. Student's two-tailed independent *t* test and chi square test were used in statistical comparisons. Concordance of prick and patch test results and the concordance of skin prick testing with the commercially available cow milk allergen (ALK) or a milk powder diluted to normal feed concentration were estimated by the Cohen's κ statistic. The McNemar test was used to evaluate the usefulness of parallel and serial

skin testing with prick and patch tests compared with that of either test alone.

RESULTS

Of the total 183 cow milk challenges, 99 (54%) gave results interpreted as positive. The mean (95% CI) age of the patients was 14 months (12 to 16 months) in the positive and 14 months (13 to 16 months) in the negative group; $t = 0.24$, $p = 0.81$. The mean serum IgE concentration was higher in the positive group than in the negative group, viz. 45 kU/L (95% CI, 30 to 67 kU/L) versus 23 kU/L (95% CI, 14 to 37 kU/L); $t = 2.16$, $p = 0.03$. Cow milk-specific IgE antibodies, RAST ≥ 0.4 kU/L, were detected in 55% and 25% of the respective groups; chi square test = 12.61, $p = 0.0004$.

Clinical reactions to cow milk challenge

There was a 54% positive reaction rate in the 118 patients with double-blind, placebo-controlled challenge and also in the 65 openly challenged patients. In eight cases (blinded challenge three cases and open challenge five cases) the challenge was repeated because of a concomitant viral infection. All placebo challenges were negative. In one case a negative blinded challenge was followed by clinical reactions to open cow milk feeding, making a 1% rate of false-negative challenge.

Of the 99 positive responses to challenge, 49 involved acute-onset pruritus, urticaria, and/or exanthema, and the other 50 involved delayed-onset reactions of eczematous type (Table I). The symptoms were confined to the skin in 68 of the 99 patients. Vomiting and diarrhea occurred in seven patients with acute-onset reactions, and loose stools or diarrhea occurred in 24 patients with

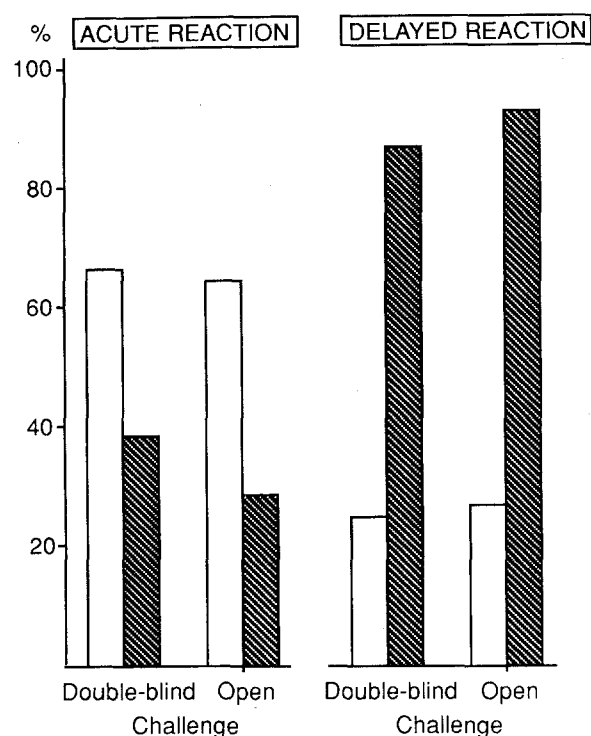


FIG. 1. Correlation of skin test reactivity to cow milk in patients with atopic dermatitis showing acute ($n = 49$) or delayed ($n = 50$) clinical reaction to cow milk challenge. Open bars denote percent of patients with positive prick test results and hatched bars denote percent with positive patch test results. Results are given separately for double-blind, placebo-controlled and open cow milk challenges.

delayed-onset reactions. Respiratory symptoms including wheezing and sneezing occurred in two patients with acute-onset reactions to the challenge.

Relationship between clinical response and skin test reactivity

Combined prick and patch testing could be performed in the scheduled elimination period in 143 patients, whereas in 40 of 183 cases persistent eczema did not allow skin testing. Of these patients 65 (45%) had multiple food allergies. There were no differences in the family history of atopy between multisensitized and monosensitized patients, but the patients with multiple food allergies showed atopic dermatitis earlier, at 2.8 (95% CI 2.3 to 3.3) months of age compared with the monosensitized patients, at 5.0 (4.1 to 5.9) months; $t = 3.87$, $p = 0.0002$. The patients with multiple food allergies were more frequently (69%) positive to the cow milk challenge than were the monosensitized patients (46%); chi square test = 9.30, $p = 0.002$. The clinical reaction to the cow

TABLE II. Skin prick and patch tests for cow milk allergy in relation to result of double-blind, placebo-controlled cow milk challenge (open challenge)* in patients with atopic dermatitis

Skin tests	Double-blind, placebo-controlled challenge (open challenge)	
	Percent positive $n = 64$ (35)	Percent negative $n = 54$ (30)
Prick		
Positive	48 (47)	14 (17)
Negative	52 (53)	86 (83)
Patch		
Positive	61 (59)	19 (17)
Negative	39 (41)	81 (83)

*The results are given separately for double-blind, placebo-controlled, cow milk challenge and (open challenge in parentheses).

milk challenge was more frequently confined to the skin (82%) in the multisensitized patients compared with the monosensitized patients (57%); chi square test = 7.03, $p = 0.008$, and a reaction of acute-onset type to low volumes of milk was seen in 64% and 37% of the respective subgroups; chi square test = 7.38, $p = 0.007$. Altogether 13 (15%) patients with negative reaction to cow milk challenges had clinically significant skin test reactivity to egg, cereals, wheat, barley, rye, or oat.

Prick testing with the commercially available cow milk allergen ALK and the milk powder diluted to normal feed concentration gave comparable results: Cohen's κ statistic for concordance was 0.86, with 95% CI 0.77 to 0.95.

Table II presents the skin test results in relation to the response to blinded and open cow milk challenge. Skin test reactivity was proportionally similar in double-blind, placebo-controlled, and open challenge. Positive prick and patch test responses were associated with negative oral challenge in 14% to 19% of cases (Table II). False-negative skin test results, however, were much more common, which was attributable to the high rate of inappropriately negative prick test results in patients with delayed-onset clinical reactions, whereas patch test results tended to be negative in patients with acute-onset clinical reactions (Fig. 1). On the other hand, when the results of both challenge types are considered together, prick test results were positive in 67% of the patients with acute reactions, and patch test results were positive

TABLE III. Sensitivity, specificity, and likelihood ratios of a positive and a negative result for skin prick and patch tests, separately and combined, in diagnosing cow milk allergy in patients with atopic dermatitis*

	Prick	Skin testing patch	Parallel†	Serial‡
Sensitivity	0.48 (0.47)	0.61 (0.59)	0.86 (0.81)	0.24 (0.25)
Specificity	0.86 (0.83)	0.81 (0.83)	0.72 (0.65)	0.94 (1.00)
Likelihood ratio of				
Positive test result	3.46 (2.70)	3.13 (3.41)	3.11 (2.34)	4.24 (undefined)
Negative test result	0.60 (0.64)	0.49 (0.49)	0.19 (0.29)	0.81 (0.75)

*The results are given separately for double-blind, placebo-controlled, cow milk challenge and (open challenge in parentheses).

†Prick or patch test positive results.

‡Prick and patch test positive results.

Sensitivity (Se) = probability of a true positive test result.

Specificity (Sp) = probability of a true negative test result.

Likelihood ratio of a positive test result = $Se/(1 - Sp)$.

Likelihood ratio of a negative test result = $(1 - Se)/Sp$.

in 89% of those with delayed-onset reactions. Parallel testing (prick or patch test positive results) results were positive in 78% of the patients with acute-onset reactions (sensitivity 0.78) and 92% of those with delayed-onset reactions (sensitivity 0.92). Serial testing (prick and patch test positive results) results were positive in 24% of the patients with acute-onset and delayed-onset reactions (sensitivity 0.24). The test responses were jointly positive or negative in only 15% and 38% of cases, respectively. Cohen's κ statistic for concordance of prick and patch test was 0.03, with 95% CI -0.13 to 0.19, indicating that no agreement better than chance exists between the two tests and that the two tests give discrepant values. In calculating values for sensitivity, specificity, and likelihood ratios of a positive and a negative test result for prick and patch testing singly or combined, we considered the results of the double-blind, placebo-controlled challenges and open challenges separately. The skin test reactivity was similar in both challenge types (Table III). Parallel prick and patch testing resulted in heightening of sensitivity with only marginal decrease of specificity when compared with use of either test alone. Serial prick and patch testing resulted in significantly decreased sensitivity and increased specificity compared with use of either test alone. In patients with atopic dermatitis, the probability of detecting cow milk allergy was significantly higher with parallel skin testing than with only prick test (McNemar test: chi square = 8.17, $p = 0.004$) or only patch test (McNemar test: chi square = 6.25, $p = 0.01$).

DISCUSSION

Atopic dermatitis is a common and complex, chronically relapsing skin disorder of infancy and childhood. Immunologically it is characterized by increased, mainly allergen-specific, IgE production. Because elevated specific IgE can be demonstrated by skin prick testing, these tests are widely used to disclose food allergy in patients with atopic dermatitis.⁷⁻⁹ The results in our study support previous findings that T cell-mediated reactions can be distinguished in atopic dermatitis¹⁸⁻²¹ and extend these observations to enhance the diagnostic accuracy of skin testing in infants with atopic dermatitis.

Hypersensitivity reactions to allergens have been contemplated in the pathogenesis of atopic dermatitis.³ In infancy, diet is probably the most important single environmental allergen. Hypersensitivity reactions to dietary allergens may induce dysfunction in the intestine's mucosal barrier,²² disturb the physiological protein transfer,²³ and consequently lead to excessive permeation of intact proteins.^{24,25} The way the antigen is transported across the small intestinal mucosa has a profound effect on the initiation of immune response.²³ Aberrant antigen absorption may lead to exaggeration of immune response, thereby broadening the sensitivity.^{23,26} Therefore early identification of patients who would profit from strict avoidance of dietary allergens is important for several reasons: (1) to avoid unnecessary elimination diets involving risk of growth retardation in early life, (2) to ameliorate the clinical course

of atopic dermatitis, and (3) as secondary prevention of the development of multiple food allergies.

Double-blind, placebo-controlled oral challenges have demonstrated clinical food hypersensitivity in 30% to 60% of patients with mild to severe atopic dermatitis, with egg and milk accounting for most of the positive clinical reactions.^{2, 7, 8, 11} In our study population of infants and young children with atopic dermatitis, the chance of having food allergy was 69%. The prevalence of cow milk allergy was 54%, which is significantly higher than in previous studies in older children.^{2, 7} This difference is most likely explained by the fact that cow milk allergy frequently is the first manifestation of an allergic diathesis because of the important nutritional role of milk and milk products and less varied diet in early childhood.^{2, 4, 5, 10} We therefore suggest that children in whom atopic dermatitis does not improve despite meticulous routine treatment with emollients and topical corticosteroids should be tested for allergy to foods, in particular cow milk.

Cow milk allergy is diagnosed by clinical response to withdrawal and subsequent challenge with cow milk. Children with positive clinical reaction to such oral cow milk challenges have been divided into subgroups according to the reaction onset time: immediate or delayed-onset reactions.^{5, 9, 12, 13} The diagnosis is seldom difficult for patients who manifest allergic reaction immediately after cow milk ingestion but may be complex in those with delayed reactions. We detected indistinguishable rates of positive reactions in double-blind, placebo-controlled, and open challenges. In like manner skin test reactivity was proportionally similar in both challenge types. These results would suggest that in this age group an open challenge and careful follow-up may be adequate for practical clinical purposes, also enabling diagnosis of delayed reactions. However, double-blind, placebo-controlled challenges clearly are the gold standard for objective diagnosis of specific food allergies in all age groups.

There is considerable discrepancy as to the estimated value of skin testing in diagnosing food allergy. We chose to investigate unselected patients with atopic dermatitis in an age group with peak prevalence of food allergy and reputedly poor diagnostic reliability of skin testing.^{6, 10} No direct comparison can be made with previous studies in older children or in patients with clinical history suggestive of food allergy.^{7-9, 11} However, our results lend support to observations in older children⁷ that skin prick testing is an acceptable means

of excluding immediate food allergy and of indicating clinical hypersensitivity. Our study demonstrated that many patients with negative prick test results but delayed-onset clinical reactions could be identified by patch testing. Such heterogeneity in response to skin prick and patch tests was previously observed in adults with atopic dermatitis with dust-mite allergen.¹⁹ Skin prick and patch tests thus may distinctly indicate cow milk allergy in infants with atopic dermatitis. However, the methods used for patch testing with foods is so far unstandardized. Also, the vehicle used for testing must be further standardized. With a casein used as allergen in patch testing, the sensitivity was only 0.33 in patients allergic to cow milk, which shows that crude milk powder might be preferable.²⁷ Regarding skin prick testing, the commercially available milk allergen (ALK) seems to give results comparable to those with milk powder used in normal feed concentration.

Taken together, our results indicate that parallel prick and patch testing will increase the probability of early detection of food allergy in infants with atopic dermatitis. Accurate and objective demonstration of a causal relationship between the dietary allergen and exacerbation of the infant's atopic dermatitis may be a prerequisite for the compliance of the family to the treatment. Confirmation of the diagnosis is essential in patients with positive skin test results, and for this purpose the most accurate method is double-blind, placebo-controlled food challenge followed by open challenge.

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