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Cow's milk allergy: diagnostic accuracy of skin prick and patch tests and specific IgE

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Key words: cow's milk allergy; food challenge; patch test; prick test.

Background: The objective of the present study was to evaluate the relevance of skin tests and the concentration of cow's milk-specific IgE antibodies in correlation with oral cow's milk challenge in infants with suspected cow's milk allergy.

Methods: The study material comprised 143 infants under the age of 2 years who had undergone a diagnostic elimination challenge because of suspected cow's milk allergy in 1996. Cow's milk-specific IgE was measured, and skin prick and patch tests were performed.

Results: Of the 143 oral cow's milk challenges performed, 72 (50%) were positive. Of the positive reactions, 22 involved immediate-type reactions. In 50 patients, delayed-onset reactions of eczematous or gastrointestinal type appeared. Of the infants with challenge-proven cow's milk allergy, 26% showed elevated IgE concentrations to cow's milk, 14% had a positive skin prick test, and 44% had a positive patch test for cow's milk. Interestingly, in most patch test-positive patients, the prick test for cow's milk was negative.

Conclusions: Our study demonstrated that many patients with a negative prick test result had a positive patch test to cow's milk. The patch test was a more sensitive method than the prick test or RAST to detect cow's milk allergy in this study population. Our results indicate that patch testing will significantly increase the probability of early detection of cow's milk allergy. Confirmation of the diagnosis is essential in patients with negative test results but a clinical suspicion of food allergy, and in patch test-positive patients. For this purpose, the most reliable method is the elimination-challenge procedure.

Food allergy is a common problem in infants and small children. Despite advances in the understanding of the

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mechanisms, the diagnostic accuracy of the methods for early detection of clinically significant food allergy is poor (1). The classical allergist's tool for diagnosing allergies is skin prick testing, also applied to aid in the diagnosis of food allergy (2). In addition, specific IgE can be measured, and, more recently, skin patch testing has been shown to be a useful addition in suspected delayed reactions (3).

Despite various skin and laboratory testing, the current clinically valid method to demonstrate significant food allergy is elimination and challenge with the suspected food (4). There is, however, no universally accepted standard for performing the elimination-challenge test (5). A careful diagnostic protocol is essential, especially when a basic food such as cow's milk is suspected, since the treatment of food allergy is elimination of the food in question from the diet.

We wanted to evaluate the clinical practice in diagnosing food allergy over 1 year, and to assess the usefulness of skin testing and measuring specific IgE. Cow's milk was chosen as the model food, since the protocol to identify cow's milk allergy is relatively well standardized in this hospital. Due to increasing numbers of infants in need of the diagnostic work-up, it has become acceptable to diagnose cow's milk allergy in infants less than 1 year old on the basis of a positive skin prick test or specific IgE, and abatement of symptoms on elimination diet. In 1996, a large number of infants were diagnosed in this manner as having cow's milk allergy. Consequently, they were not all included in the present report.

Material and methods

Patients

The study material comprised 143 infants (51 girls, 92 boys) less than 2 years of age (mean 0.9 years). In 1996, they underwent a diagnostic elimination challenge because of suspected cow's milk allergy at the Department of Paediatrics of Tampere University Hospital (Finland). The patients were suffering from skin symptoms ($n=68$), gastrointestinal symptoms ($n=21$), or both skin and gastrointestinal symptoms ($n=54$). Skin symptoms included pruritus, urticaria, exanthema, and atopic eczema; gastrointestinal symptoms were vomiting, loose stools, and diarrhea.

The mean (SD) ages at onset of skin and gastrointestinal symptoms were 2.7 (2.2) and 4.3 (4.1) months, respectively. There was a positive family history of atopic disorders in 108 (76%) cases. The mean (SD) durations of exclusive and total breast-feeding were 2.4 months (1.7) and 5.3 months (3.2),

respectively. In 112 patients (78%), the symptoms had appeared already during exclusive breast-feeding. The mean (SD) time from the onset of symptoms to the clinical challenge was 0.7 (0.6) years.

In Finland, the Social Insurance Institution reimburses part of the costs of medication prescribed by physicians, including the special formulas used during cow's milk elimination diets. A prerequisite to receive the reimbursement is a diagnostic procedure agreed on by representatives of the Social Insurance Institution and pediatric allergists. Due to increasing numbers of infants in need of the diagnostic work-up, it has become acceptable to diagnose cow's milk allergy in infants less than 1 year old on the basis of a positive skin prick test or specific IgE, and abatement of symptoms on elimination diet. In 1996, a large number of infants were diagnosed in this manner as having cow's milk allergy; therefore, they were not referred to Tampere University Hospital. Consequently, they were not all included in the present report.

Determination of serum IgE, skin prick and patch tests, and cow's milk challenge

The serum total IgE concentration and cow's milk-specific IgE (CAP RAST, Pharmacia, Uppsala, Sweden) were measured, and skin prick and patch tests for cow's milk and various other foods (e.g., soy, egg, cereals) were performed. Prick and patch testing were performed as described previously (6). Antihistamine medication was discontinued for periods of 3 days to 6 weeks before skin testing, depending on the drug's duration of action. Prick testing was done on the volar aspect of the forearm with a commercially available cow's milk allergen of ALK (Allergologisk Laboratorium A/S, Hørsholm, Denmark). Histamine dihydrochloride 10 mg/ml (ALK) was used as a positive control. Reactions were read after 15 min, and a response was recorded as positive if the mean diameter of the wheal was at least 3 mm and the negative control was 0 mm at the same time. The median size of the histamine wheal was 5 mm.

On 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". In patch testing, approximately 20 mg of porridge (containing 450 µg β -lactoglobulin) and blotting paper were applied to uninvolved skin on the patient's back with aluminum cups (Finn Chamber, Epitest Ltd., Hyrylä, Finland) attached with Scanpore tape. Microcrystalline cellulose was used as a negative control. The occlusion time of patch testing was 48 h. The results were read for the

Table 1. Sensitivity, specificity, and positive and negative predictive values of RAST, skin prick, and patch tests for cow's milk in relation to clinical response to oral cow's milk challenge in infants with suspected cow's milk allergy

Test	Sensitivity (95% CI)	Specificity (95% CI)	Predictive value	
			Positive (95% CI)	Negative (95% CI)
RAST	0.26 (0.16–0.38)	0.94 (0.85–0.98)	0.82 (0.60–0.95)	0.54 (0.44–0.63)
Prick	0.14 (0.07–0.25)	0.98 (0.92–1.0)	0.91 (0.59–1.0)	0.51 (0.42–0.60)
Patch	0.44 (0.32–0.56)	0.71 (0.57–0.82)	0.63 (0.47–0.76)	0.52 (0.41–0.63)

first time 15 min after removal of the cups. The second reading was done 72 h after attaching the patches (day 3). In a negative reaction, there were neither visible nor palpable changes on the skin. Clear redness with palpable infiltration was defined as a positive reaction. This type of reaction tended to grow stronger by the next inspection. Irritation reaction included redness with no infiltration. The reaction tended to become weaker by the next inspection.

The patients were subjected to double-blind, placebo-controlled cow's milk challenge ($n=37$) or open ($n=95$) cow's milk challenge, as described previously (3). Before challenge, the patients had been on a milk elimination diet for at least 3–4 weeks. During this time, they were consuming either an extensively hydrolyzed whey-protein formula or an amino acid-derived formula. In a double-blind, placebo-controlled challenge, the placebo formula was the amino acid-derived formula (Neocate, SHS Int. Ltd, Liverpool, UK), and the test formula comprised 10 g cow's milk powder in 100 ml of placebo formula. The open challenges were made with a ready-to-use infant formula containing 192 000 µg/l β -lactoglobulin. The challenge period was 1 week. The challenge was started in the hospital when rising doses (1, 5, 10, 20, 50, and 100 ml) of the formula were given at 30-min intervals. If no reaction occurred within 3 h in hospital, the challenge was continued at the patient's home. When a clinical reaction appeared, the patient was examined in hospital, and the challenge was discontinued. All patients were examined on day 7 of the challenge. To judge long-term tolerance and reveal any false-negative result of challenge, all patients negative to challenge continued to consume cow's milk. The patients positive to cow's milk challenge consumed a tolerated substitute formula.

Statistics

The descriptive values of variables were expressed as means and standard deviations (SD) or percentages. Sensitivity,

specificity, and positive and negative predictive values of the tests are presented with 95% confidence intervals (CI).

Ethics

The study protocol was approved by the ethical review committee of Tampere University Hospital.

Results

Of the total 143 cow's milk challenges, 72 (50%) were positive. Of the positive reactions, 22 (31%) involved immediate-type reactions during 2 h from the commencement of the challenge. Skin eruptions included pruritus, urticaria, and exanthema. In three patients, the first reaction to the challenge was vomiting. The mean (SD) dose and time until reaction were 15 (27) ml and 27 (41) min, respectively.

In 50 (69%) patients, delayed-onset reactions of eczematous or gastrointestinal type appeared. The mean (SD) dose and time until reaction were 163 (117) ml and 25.2 (25.7) h, respectively.

Of the infants with challenge-proven cow's milk allergy, 26% (18/70) showed elevated IgE concentration to cow's milk. Of these 18 patients, eight manifested immediate-type reactions and 10 delayed-type reactions to cow's milk challenge. Of these 18 patients with positive cow's milk specific RAST, eight had a positive skin prick test to cow's milk.

In patients positive to challenge, 14% (10/70) had a positive skin prick test to cow's milk. Eight patients showing prick test positivity reacted during the first 2 h to challenge. In two patients, prick test positivity was associated with a delayed-type reaction.

False-positive prick and RAST tests were very uncommon. Positive RAST and prick tests were associated with a negative oral challenge in 6% and 2% of the cases,

respectively. False-negative prick and RAST test results were much more common.

The skin patch test for cow's milk was positive in 43% (30/69) of patients positive to cow's milk challenge. A positive patch test for cow's milk was associated in seven cases with an immediate-type reaction and in 23 cases with a delayed-type reaction. In 27 patients with patch test positivity, the prick test for cow's milk was negative. Thus, both the prick test and the patch test were positive simultaneously only in three patients. An irritation reaction to cow's milk was noted in 14 patients in our study population.

The mean serum total IgE was 66 (range, 5–2151) kU/l. In 33 infants, the concentration of total IgE was below 5 kU/l. Altogether, 27 (38%) infants with challenge-proven cow's milk allergy did not show skin test or RAST positivity to cow's milk. Table 1 presents the sensitivity, specificity, and predictive values of RAST and skin test results in relation to the clinical response to oral cow's milk challenge.

Prick test and RAST results for egg were available from 108 and 80 patients, respectively. They were positive in 22% and 42% of the infants, respectively. Prick test and RAST results for wheat were available from 106 and 80 patients, respectively. They were positive in 19% and 25% of the cases, respectively.

Discussion

In this study, we evaluated the correlation of skin tests and RAST with the clinical reaction to cow's milk challenge in infants with suspected cow's milk allergy. Cow's milk allergy, frequently manifested in infancy as skin or gastrointestinal symptoms, is considered to be due to several pathogenetic mechanisms operating separately or together, depending on the expression of clinical symptoms (7–9). Infants with cow's milk allergy may show more than one symptom or show symptoms in more than one organ system. Patients with food allergy have also been classified on the basis of the reaction onset time (7). IgE-mediated hypersensitivity reactions are responsible for immediate-type reactions. Delayed reactions in food allergy are poorly understood. Cell-mediated reactions may be responsible for delayed-type symptoms (10, 11). It has been suggested that the delayed-type allergy to foods may also be an IgE-mediated allergy, even in cases with low total IgE and no detectable specific IgE to foods (12).

In the diagnosis of adverse reactions to foods, the clinical history is often unreliable, and to date there are no skin tests

or other laboratory tests that can confirm or exclude food allergy with certainty (13, 14). The age of the child must be taken into account. Allergen-specific IgE and skin prick tests may not be positive early in life (15). Moreover, these tests detect only IgE-mediated reactions, and a negative test does not imply that the individual is not sensitized. In gastrointestinal hypersensitivity disorders, prick tests with the responsible food allergen are often negative, since a non-IgE-mediated hypersensitivity reaction is thought to be responsible (16). The oral food-challenge testing procedure is so far the only reliable method to verify a causal relationship between ingested food and the development of symptoms (4).

On the other hand, previous studies have shown that only 30–40% of patients (aged 3 months to 19 years) with food-specific IgE antibodies experience clinical symptoms when the food is ingested (17, 18). In a recent study, elevated cow's milk-specific IgE concentration was detected in 25% of children (aged up to 36 months) not reacting to cow's milk challenge (3). Meglio et al. (19) studied diagnostic procedures in children (aged 6 months to 10 years) with atopic dermatitis and food allergy. They found high skin prick test sensitivity (0.88) to cow's milk for immediate reactions. However, the specificity was low (0.25). This may be due to the older age of patients in previous studies as compared to this study. Older patients may have become tolerant.

Demonstration of food-specific IgE antibodies either with RAST or skin prick tests refers to IgE-mediated food allergy. In infants under the age of 1 year, the tests suggest a relevant food allergy, because at this age it is more difficult to detect specific IgE, and the secondary development of tolerance has hardly taken place yet (20). False-positive prick and RAST tests were very uncommon in our study population. Our study demonstrated that in children less than 2 years, a positive skin prick test or RAST is associated with positive clinical response to cow's milk challenge. In these patients, the diagnosis can be made on the basis of a positive test result and a favorable response to the elimination diet. In older children, the relevance of RAST and skin test has to be confirmed by oral food challenge.

Disclosing delayed-type reactions to dietary antigens is particularly difficult. Assessment of lymphocyte stimulation and cytokine generation has shown some promise in detecting delayed-type reactions to food (9–11, 21–23). We have previously shown that the concentration of TNF- α in feces was elevated, particularly in patients manifesting delayed-type reactions to cow's milk challenge and in cow's milk-allergic infants before elimination diet (22, 23). Recently, skin patch testing has been shown to be a useful

addition in suspected delayed reactions (3). Previous studies in children with atopic eczema have shown that immediate-type clinical reactions are more often associated with skin prick test positivity, and delayed reactions with patch test positivity (3, 6, 21).

In the present study, the skin patch test for cow's milk was positive in 44% of patients positive to cow's milk challenge. Interestingly, in most of these patients, the prick test for cow's milk was negative. A positive patch test for cow's milk was highly associated with delayed-type reactions to oral challenge. This and other studies have shown that false-negative test results are common (3, 6) in pediatric populations. In the present study, only 26% of infants with challenge-proven cow's milk allergy showed an elevated IgE concentration to cow's milk, and 14% had a positive skin prick test for cow's milk. Altogether, 38% of the patients with challenge-proven cow's milk allergy did not react in skin testing or by RAST to cow's milk. Instead, false-positive prick and RAST tests were very uncommon.

These results may be due to the following reasons. Firstly, the selection criteria were different in the present and other studies. In this study, the patients had been referred to Tampere University Hospital on the basis of suspected cow's milk allergy and were selected by challenge test in 1996. The patients of Isolauri & Turjanmaa were selected on the basis of atopic dermatitis (3). Secondly, our study population was younger than in many previous studies. False-positive test results were uncommon, confirming the previous finding that in infants under the age of 1 year a positive test result suggests a relevant food allergy (20). Thirdly, in 1996, when this study was performed, those infants with clearly positive skin prick or patch test for cow's milk, together with a clear clinical history of adverse

reactions, were not subjected to cow's milk challenge, and only more complicated cases were referred to challenge procedures. Fourthly, in this study population, a large number of patients manifested gastrointestinal symptoms. In gastrointestinal hypersensitivity disorders, prick tests and RAST to the responsible food allergen are usually negative, since a non-IgE-mediated hypersensitivity reaction is thought to be responsible (16).

The diagnostic reliability of skin tests for food allergy depends on several factors including patient selection, symptoms considered, standardization of the allergen extract, and kind of food involved (2, 24). Rancé et al. (25) have shown that the skin prick test with a standardized commercial cow's milk extract is a sensitive method to identify IgE-mediated cow's milk allergy, but the methods used for patch testing with foods are so far not standardized. In the present study, immediate-type reactions were highly associated with skin prick test positivity and delayed-type reactions with patch test positivity. The patch test was the most sensitive method to detect cow's milk allergy in this study population.

Our results indicate that patch testing will increase significantly the probability of early detection of cow's milk allergy in infants. Confirmation of the diagnosis is essential in patients with negative test results, but who are clinically suspected of food allergy, and in patients with patch test positivity. For this purpose, the most reliable method is the elimination-challenge procedure.

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