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Original article

The patch test, skin prick test, and serum milk-specific IgE as diagnostic tools in cow's milk allergy in infants

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We evaluated the value of the patch test, skin prick test, and milk-specific IgE by CAP RAST in 301 infants with suspected hypersensitivity to cow's milk. The patch test was carried out with milk powder, and the skin prick test with cow's milk-based formula. Hypersensitivity to cow's milk was determined with double-blind, placebo-controlled challenge. An immediate reaction to cow's milk challenge was observed in 100 infants (33%), a delayed reaction in 76 (25%), and a negative result in 125 (42%). Skin prick test wheals were significantly greater in infants with immediate reactions than in infants with delayed or negative reactions. Milk-specific IgE was correlated with the skin prick test ($r=0.78$, $P<0.001$, $n=268$) but did not contribute to further discrimination of immediate reactions from delayed or negative reactions compared to skin prick test alone. In our study population, the skin prick test (diameter ≥ 3 mm) showed a specificity and sensitivity of 91% and 69%; the results for milk-specific IgE (≥ 0.7 kU/l) were 88% and 58%, respectively. The patch test did not distinguish subjects with immediate or delayed reactions from those with negative reactions.

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Hypersensitivity to cow's milk occurs in 2–3% of infants in unselected cohorts (1). The recommended test in the diagnosis of food allergy is the double-blind, placebo-controlled food challenge (2). Because it may be laborious or its results difficult to interpret, new tools, such as the patch test, have been introduced for the screening and diagnosis of milk allergy (3, 4). An effective new tool is required for correct diagnosis of delayed reactions to cow's milk, because no laboratory tests have so far proven useful. Therefore, we conducted a study to evaluate the diagnostic

Table 1. Patient characteristics

No. of patients	301
Age (months; X±SD, range)	7.1±1.8, 2–11
Sex (male/female ratio)	176:125
Suspected symptoms from cow's milk	
Atopic eczema	66%
Urticaria/angioedema	28%
Gastrointestinal symptoms	34%
Respiratory symptoms	6%
Multiple (at least 2) symptoms	35%

sensitivity and specificity of the skin patch test and prick test, as well as that of milk-specific IgE antibody measurement in infants with suspected cow's milk allergy.

Material and methods

Subjects

The study included 305 infants (aged under 1 year) with suspected hypersensitivity to cow's milk referred to two pediatric university hospital clinics by well-baby clinics (n=124), general practitioners at health centers (n=87), or pediatricians or other specialists (n=94). Most of the children (74%) presented with atopic eczema (Table 1).

Milk challenge procedure

Milk allergy was diagnosed in all subjects by means of the double-blind, placebo-controlled milk challenge except in four patients with a history of anaphylactic reactions, who were excluded from the study. Before the challenge, milk products were eliminated from the infants' diet for at least 2 weeks. If a breast-fed infant was suspected of hypersensitivity to cow's milk products via breast milk, the mother was also requested to avoid cow's milk. When hypersensitivity to other food items was suspected, the suspected food or foods were eliminated from the diet. Egg, fish, citrus fruit, peanut, pea, tomato, strawberry, and chocolate products were eliminated. Instead of cow's milk-based formula, the infants were given a whey-based, extensively hydrolyzed formula (5) (PeptidiTutteli®, Valio, Finland). If the infant refused to drink the formula, or adverse reactions were suspected, the infant was given an amino-acid formula (Neocate®, SHS International, Liverpool, UK; used in seven cases) or a soy-based formula (Valio, Finland; used in 10 cases). The formula used during the elimination period was used as a placebo formula for challenge.

Milk challenge was carried out by mixing a cow's milk-based formula (Piltti®, Kuivamaito, Finland) with the placebo formula (1:1). Placebo and the milk formula were

given in a randomized order in the hospital outpatient department in gradually increasing amounts or until an adverse reaction occurred. First, a drop of milk was applied to the facial skin. Then, increasing formula doses (2, 20, 100, and 200 ml) were given. If no symptoms occurred, the formula was continued at home *ad libitum* for 5 days, and the parent(s) kept a diary of symptoms. The results of the challenges were evaluated and classified as negative, positive, or doubtful. Positive reactions occurring within 2 h after the last challenge dose in the hospital were classified as immediate reactions, and later reactions were classified as delayed reactions. Infants with doubtful results were rechallenged. Infants with negative reactions were openly fed cow's milk-based formula and considered nonallergic to cow's milk only if no adverse symptoms occurred during a 1-week period of open ingestion of cow's milk-based formula. If reactions occurred, the infant was rechallenged in a double-blind manner.

Positive delayed reactions were further classified into three categories: definite positive reactions (++), where previously suspected symptom(s) occurred during the milk challenge, but not during placebo challenge; positive reactions (+), where some symptoms were also recorded during placebo challenge, but significantly fewer than during milk challenge; and doubtful positive reactions (+?), where the difference between the challenges was less clear or symptoms were subjective (restlessness, abdominal pain, etc.). Because delayed reactions were sometimes difficult to interpret, the results were classified independently by three pediatric allergists (K.J.-B., T.K., and P.S. in Helsinki; A.K., E.V., and T.V. in Turku). If they disagreed, the classification shared by two of them was chosen.

Skin prick test

The skin prick test was carried out in all children on the volar aspect of the forearms (duplicate tests) with the cow's milk-based formula (milk powder diluted to normal feeding concentration), extensively hydrolyzed whey formula (PeptidiTutteli), negative control, and histamine hydrochloride 10 mg/ml. The results were expressed as the mean wheal diameter in mm.

Patch test

The patch test was carried out during the elimination diet or 2 weeks after milk challenge on healthy skin in the child's upper back. Approximately 20 µg of cow's milk powder containing 35 g of protein/100 g was mixed with saline,

Table 2. Wheal size in skin prick test with cow's milk and level of specific IgE in serum according to reaction to cow's milk challenge. Values are medians with ranges and number of observations in parenthesis

Challenge result	Skin prick test (mm)	Milk-specific IgE (kU/l)
Immediate positive	4.5* (0–11; n=100)	1.3* (<0.35–>100; n=90)
Delayed positive	0 (0–9; n=75)	<0.35 (<0.35–50; n=75)
Negative	0 (0–6; n=124)	<0.35 (<0.35–10; n=108)
* $P<0.05$ vs subgroups with delayed positive or negative challenge result.		

placed in aluminum test cups (Finn Chamber, Epitest, Finland), and attached to the patient's back with Scanpore tape (Norgesplaster A/S, Norway) (4, 6). Microcrystalline cellulose, petrolatum, and empty cups were used as negative controls. The patches were removed after 48 h. The final reading was done 72 h after attaching the patches. A basic comparison was carried out with the control cups. Those reactions were subtracted from the study sites to make the final evaluation. The reactions were considered negative, doubtful (?+), or positive (+, ++). In a negative reaction, there was neither visible nor palpable change in the skin. A doubtful reaction appeared as mild erythema in the contact area of the cup, with no induration. The positive reaction (+) indicated redness with induration. Redness with palpable induration occasionally extending beyond the cup area and vesicle was defined as ++.

Milk-specific IgE

A venous blood sample was obtained from the infant before challenge. IgE antibodies to cow's milk (code f2) were determined with the Pharmacia CAP SystemTM RAST^{RE} RIA (Pharmacia & Upjohn, Uppsala, Sweden), according to the manufacturer's instructions.

Statistical analyses

The evaluation of the sample size needed to assess the diagnostic utility of the milk patch test was based on the following premises. The proportion of correctly classified patients based on a diagnostic test of no discriminating power was assumed to be 50% (i.e., patients would be randomly distributed with regard to the test result). A diagnostic test of reasonable clinical utility should be able to

classify at least 70% of patients correctly. To obtain a chance of 90% of detecting such a true difference in discriminatory power, at a 5% risk of falsely recognizing a nonexisting difference, at least 61 patients would be needed in both groups of milk challenge outcome (immediate positive vs delayed positive or negative). The one-way analysis of variance and Tukey's studentized range test (HSD test; $P=0.05$ as the limit of significance) were used to assess the significance of differences in means between subgroups. The chi-square test was used for frequency analysis. Logistic regression analysis, using forward selection of independent variables, was used to determine the diagnostic tests that independently contributed to discrimination between infants with immediate, delayed positive, or negative response in the cow's milk challenge test. Pearson's correlation coefficients were used for correlation analyses. The data were log-transformed where appropriate to correct for skewness. SAS STATTM software (SAS Institute, Inc., Cary, NC, USA) was used for the statistical analyses. Receiver operator characteristic (ROC) analysis was used to measure the discrimination attained with the diagnostic tests (7, 8).

Ethical considerations

Written informed consent was obtained from all parents. The study was approved by the Joint Commission on Ethics of Turku University and the Turku University Central Hospital, and by the Commission on Ethics of the Skin and Allergy Hospital, Helsinki, Finland.

Results

An immediate positive reaction to cow's milk challenge was observed in 100 infants (33%), a delayed reaction in 76 infants (25%), and a negative challenge in 125 infants (42%). The amount of cow's milk formula eliciting immediate reactions varied from one drop to 161 ml (median 1 ml).

Serum milk-specific IgE levels and wheal size in the skin prick test using the cow's milk formula were significantly higher in infants with an immediate positive reaction to cow's milk challenge than in infants with a delayed positive reaction or a negative reaction (Table 2). No significant difference in serum milk-specific IgE levels or skin prick test results was found between infants with a negative reaction and those with a delayed positive reaction. A significant association was found between positive reactions in the skin prick and patch test ($P=0.02$; Table 3), whereas no associa-

Table 3. Relationship between patch test result and outcome of milk challenge test, results in skin prick test with cow's milk, and presence of serum milk-specific IgE. Numbers of cases are shown with respective percentages in parenthesis

	Patch test result		
	+ or ++	?+	Negative
Challenge result			
Immediate positive	14 (18%)	30 (38%)	35 (44%)
Delayed positive	10 (15%)	15 (23%)	40 (62%)
Negative	11 (11%)	34 (34%)	56 (55%)
Type of delayed challenge reaction			
+ or ++	7 (20%)	7 (20%)	21 (60%)
+?	3 (10%)	8 (27%)	19 (63%)
Skin prick test*			
≥3 mm	17 (24%)	22 (31%)	32 (45%)
<3 mm	18 (10%)	57 (33%)	98 (57%)
Serum milk-specific IgE			
≥0.7 kU/l	13 (22%)	16 (27%)	31 (52%)
<0.7 kU/l	18 (11%)	54 (33%)	93 (56%)

* $P=0.02$.

tion was found between the patch test result and the presence of serum milk-specific IgE (Table 3).

Wheal size in the skin prick test with cow's milk was the only significant independent variable retained at the end of the forward selection procedure in logistic regression analysis in discrimination between immediate positive, delayed positive, or negative reactions to cow's milk challenge. Milk-specific IgE provided no further contribution to such discrimination beyond that attained with the milk skin prick test. The level of milk-specific IgE was found to correlate significantly with wheal size in the skin prick test ($r=0.78$, $P<0.001$, $n=268$). Table 4 shows the diagnostic sensitivity, specificity, positive and negative predictive values, and overall agreement of the three diagnostic tests on immediate positive reactions. In the ROC analysis, the area under the ROC curve (AUC^{ROC} ;

Fig. 1) for wheal size in the skin prick test was slightly higher (0.829 ± 0.029) than the AUC^{ROC} for milk-specific IgE (0.756 ± 0.034).

The patch test showed a ++ reaction in five subjects (2%), a + reaction in 30 subjects (12%), a ?+ reaction in 79 subjects (32%), and a negative result in 131 subjects (53%). No significant association was found between the result of the patch test and the reaction to the cow's milk challenge. The patch test did not distinguish subjects with delayed or immediate reactions from those with negative reactions to cow's milk challenge, even when different categories of delayed reactions were considered (Table 5). Infants with atopic eczema did not have positive (+ or ++) patch test reactions to cow's milk more often than those without atopic eczema (15% and 10%, respectively).

Positive (≥ 3 mm) skin prick test reactions to the extensively hydrolyzed formula (PeptidiTutteli) were observed in three infants (3%) with immediate reactions to cow's milk challenge. Prick test reactions were small, 3–4 mm in diameter, and all three infants tolerated the formula both in the double-blind challenge and during the open introduction of this formula. None of the infants with delayed or negative challenge reactions to cow's milk had positive prick test reactions to hydrolyzed formula.

Discussion

Although our study was carried out in two university hospitals, the infants of the study were not a highly selected population. Referral to the study was made easy, and well-baby clinics were encouraged to contact us directly, if cow's milk allergy was suspected in infants. The type of referral also influenced the results: immediate reactions to cow's milk challenge were observed in 22% of infants referred by well-baby clinics, in contrast to 43% and 40% of referrals by a general practitioner or a specialist, respectively. Delayed

Table 4. Diagnostic sensitivity, specificity, positive and negative predictive values, and overall agreement of skin prick test, milk-specific IgE in serum, and patch test for immediate positive reaction to cow's milk challenge. Predictive values are calculated for prevalence of 33% of immediate positive reactions observed in study population. Overall agreement is total percentage of patients classified correctly with given individual diagnostic test

Test	Cutoff	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Overall agreement (%)
Wheal size in skin prick test	≥ 3 mm	69	91	79	85	84
Milk-specific IgE	≥ 0.7 kU/l	58	88	70	81	78
Patch test	$\geq +$	18	87	40	69	65

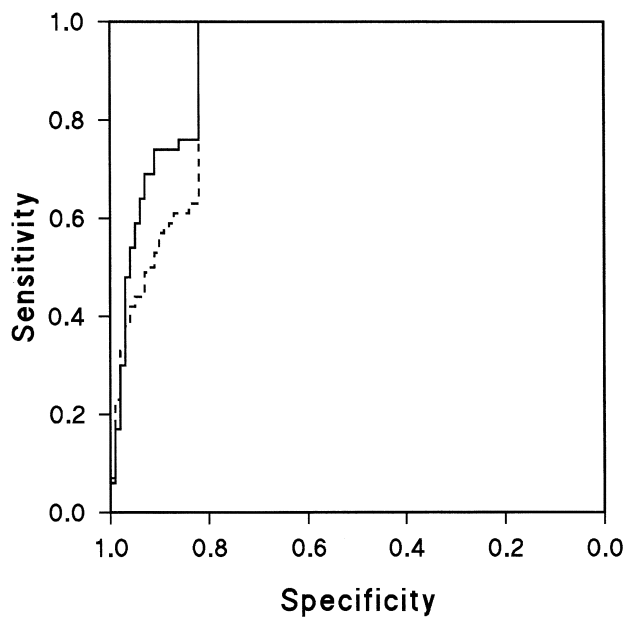


Figure 1. ROC curves for wheal size in skin prick test with cow's milk (solid line; $AUC^{ROC} 0.829 \pm 0.029$) and serum milk-specific IgE (dashed line; $AUC^{ROC} 0.756 \pm 0.034$) in discrimination of infants with immediate reaction to cow's milk challenge from those with delayed positive or negative reaction.

reactions to cow's milk showed a consistent distribution irrespective of the referring unit.

Double-blind, placebo-controlled milk challenge and its interpretation have some pitfalls. The challenge materials used in this study were easy to prepare and successfully blinded. In some infants, the palatability of whey hydrolysate was probably a problem, leading to irregular daily intake of challenge feed. However, the double-blind, placebo-controlled food challenge was usually well accepted by the parents, as reported previously (9), and compared to an open challenge, false labeling of subjects as milk-sensitive can be reduced even in infants (9). In our study, 25% of the infants had a delayed reaction during cow's milk challenge. Interpretation of delayed reactions is often difficult. The children were not so much improved during the elimination period that all infants with atopic eczema or gastrointestinal complaints would have been totally free of symptoms. In these subjects, differentiation of baseline symptoms from

additional symptoms observed during 5-day challenge was in some cases difficult. Therefore, delayed reactions were independently classified by three experienced pediatric allergists and categorized into either clearly positive or doubtful reactions. An orthodox trial design would have included three pairs of challenges with both active and placebo agents (10), but, in the diagnosis of delayed reactions, which require a follow-up of several days after challenge, such an approach would be demanding and rather unacceptable to the parents. None of the delayed reactions to cow's milk were severe or life-threatening. Infants with a delayed reaction improved during the elimination diet. However, we do not know the clinical importance of failing to diagnose delayed-type cow's milk hypersensitivity. A follow-up study is underway to investigate the natural history of delayed reactions to cow's milk. The combination of skin prick and patch testing has been suggested as an improvement of the diagnosis of food allergy in infants (4). Cell-mediated immunity is involved in the pathogenesis of atopic eczema, and some patients with atopic eczema have no detectable IgE-originating activity (11). In the study of Isolauri & Turjanmaa (4), food-sensitive patients with a delayed reaction to food challenge and negative skin prick tests results and low serum IgE concentrations showed patch test positivity and benefited from skin patch testing with dietary antigens. We could not confirm these results of patch testing in our study although we used the same patch test method: infants with immediate, delayed, or negative challenge reactions showed consistent distributions of patch test reactivity. We have no explanation of the conflicting results, but the more selected and slightly older patient population of the above study (4) and a different definition or interpretation of the delayed challenge reaction may partly explain the differences in results. Even the careful interpretation of delayed reactions in our study did not help make the patch test seem a useful diagnostic tool for cow's milk allergy in infants. The patch test was also slightly irritating to the skin, causing erythema also under control patches, itch in children, and sometimes long-lasting, toxic-like scaly lesions at the test site, in both challenge-negative and -positive subjects. Such a phenomenon was not men-

Table 5. Patch test results in relation to delayed challenge reactions with different degrees of severity (++ definite, + weak, +? doubtful, or negative challenge reaction). Challenge reactions were independently classified by three pediatric allergists

Patch test result	Delayed challenge + or ++ (n)	Delayed challenge +? (n)	Delayed challenge negative (n)	Total (N)
+ or ++	33% (7)	14% (3)	52% (11)	100% (21)
?+	15% (7)	17% (8)	67% (31)	100% (46)
– (negative)	22% (21)	20% (19)	58% (56)	100% (96)

tioned in the previous studies (4, 6), but it deserves observation, if the patch test method is used for diagnostic work in food allergy.

As reported previously (12), skin prick testing or serum milk-specific IgE antibodies were not useful for predicting delayed reactions to cow's milk. On the contrary, skin prick testing proved valuable in differentiating infants with immediate reactions to cow's milk from those with delayed or negative reactions. The sensitivity of the skin prick test in this study population was lower than that reported by Sampson & Ho (13), but its specificity was higher, probably because all our subjects were infants under 12 months of age. The sensitivity and specificity figures observed in our study cannot directly be extrapolated to an unselected population, where the prevalence of hypersensitivity to cow's milk is lower. The skin prick test was marginally better than the measurement of specific IgE. In the diagnosis of immediate allergy to cow's milk in infants, the determination of specific IgE gave no added advantage over the skin prick test alone. If the skin prick test is not available or cannot be carried out, the measurement of specific IgE in serum reacting to cow's milk offers a good diagnostic alternative.

In conclusion, the screening of infants with immediate-type hypersensitivity to cow's milk can be successfully

carried out in most cases by using the skin prick test, and, if it is not available, by the determination of specific IgE in serum. No similar screening method is available for delayed reactions. The patch test results with cow's milk do not seem to be related to acute or delayed challenge reactions.

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