

Diagnostic value of skin-prick and patch tests and serum eosinophil cationic protein and cow's milk-specific IgE in infants with cow's milk allergy

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Summary

Background The diagnosis of cow's milk allergy is based on a clinical response to an elimination-challenge test with cow's milk.

Objective We studied the usefulness of the skin-prick and patch tests and measurement of cow's milk-specific IgE and eosinophil cationic protein in serum as diagnostic tools for cow's milk allergy in a cohort of 6209 unselected infants followed from birth for the development of cow's milk allergy.

Results Of the 239 infants challenged with cow's milk, 118 showed a positive and 121 a negative response at a mean age of 6.9 months. A positive reaction to a skin-prick test with cow's milk (≥ 3 mm) was seen in 72 (61%) and 29 (24%) infants with positive and negative challenges, elevated serum cow's milk-specific IgE (≥ 0.7 kU/L) in 52 (45%) and 15 (13%) infants, a positive reaction to patch test with cow's milk protein fractions in 26 (26%) and eight (8%) infants, and elevated serum eosinophil cationic protein (≥ 20 μ g/L) in 22 (21%) and seven (13%) infants, respectively. Parallel use of the four tests with the above-mentioned cut-off values correctly classified 73% of the infants with a sensitivity of 0.76 and a specificity of 0.67. An immediate reaction to cow's milk challenge correlated with skin prick test positivity and elevated serum milk-specific IgE, and tended to correlate with patch test positivity.

Conclusions No single test or parallel use of the four tests could predict the challenge outcome acceptably in this prospectively followed, unselected cohort of 6209 infants. A positive reaction to one or more tests needs to be confirmed by a challenge test and a negative response to all four tests does not rule out the possibility of cow's milk allergy.

Keywords: cow's milk allergy, infant, skin-prick test, patch test, IgE, eosinophil cationic protein

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Introduction

The diagnosis of infantile cow's milk allergy (CMA) is based on a response to elimination of cow's milk (CM) from the diet, followed by a positive reaction to a clinical challenge with CM. A double-blind placebo-controlled

food challenge is regarded as the gold standard for diagnosis of food allergy [1], but in infants with CMA, most of whom are challenged during the first year of life, open challenges do not seem to cause bias [2]. Many laboratory tests have been introduced but their diagnostic value is limited by several weaknesses; no single test will probably identify all the patients with CMA. This is because several immunological mechanisms are involved [3], the age of the patient may influence the test result [4]

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and allergens of CM triggering the symptoms may vary between individuals [5,6]. Only the IgE-mediated reaction, usually associated with an immediate-type reaction, is well characterized; the immunological mechanisms associated with a delayed type reaction are still poorly understood [7]. Of the recently identified markers of allergic inflammation, an increase in serum eosinophil cationic protein (ECP) has been associated with cutaneous symptoms in infants with CMA [8], persistent asthma symptoms [9], and active atopic dermatitis [10]. Recently a positive response to the skin-patch test was associated with a delayed-type reaction to CM [2] and, when used parallel with a skin-prick test (SPT), increased the detection rate.

Our aim was to study the usefulness of four different tests, i.e. SPT and a patch test with CM and the presence of serum IgE CM antibodies and ECP for the diagnosis of CMA as compared with the result of CM challenge in a population-based, unselected group of infants.

Subjects and methods

Study subjects

A cohort of 6209 healthy, full-term newborn infants were recruited to a study in which we examined the effects of different supplementary milks given soon after delivery on the subsequent incidence of CMA [11]. Details of the diagnostic procedure of CMA were described earlier [11,12]. In brief, the mothers were given written information about symptoms suggestive of CMA. If an infant had any symptoms compatible with CMA, CM was eliminated from the infant's diet and supplemented with extensively hydrolysed whey formula when needed. After a successful elimination diet, an open CM challenge was performed at the out-patient clinic. If an infant had a positive SPT to CM (≥ 3 mm) the challenge was started with drops of CM placed on the volar side of the wrist, the cheek and the lips, followed by CM formula given orally in quantities of 1, 10, 50 and 100 mL at intervals of 30–60 min. The next day, those infants without symptoms continued to receive the formula at home. All the infants were examined for delayed symptoms on the fifth day of the challenge. The test was considered positive if one or more of the following symptoms had appeared in the interim: urticaria, exanthema, atopic dermatitis, vomiting, diarrhoea, wheezing or allergic rhinitis. Infants reacting adversely to the challenge were given a milk-free diet and those with no symptoms received CM formula. One month later, all infants were re-examined to confirm the challenge result.

A total of 622/6209 (10%) mothers contacted the authors. On elimination of CM from the diet, the symptoms subsided in 247 infants. Of these, 118 (48%) reacted adversely to the CM challenge and 129 (52%) had a

negative challenge. According to the national organization, Social Insurance Institution, CMA was diagnosed elsewhere in 81 infants [11], including eight infants who were challenged by us and diagnosed as not having CMA. Because the classification of these eight infants was uncertain, they were excluded from the present study and the results of 121 infants with negative challenge are reported.

Skin tests

Before the challenge, SPT with liquid CM formula was performed on the volar surface of the forearm [11,12] according to a standard technique [13].

In skin-patch tests, we used CM formula powder, bovine serum albumin (Sigma, St Louis, MO, USA), crystallized bovine β -lactoglobulin (Sigma) and bovine casein (Sigma). Each test substance was mixed with 0.9% saline solution in a separate tube in which a filter-paper disc was placed for an hour to become saturated with the test substance; the filter-paper was then attached to the patient's back under an aluminium cup (Finn Chamber, Epitest Ltd, Hyrylä, Finland) fixed with a Scanpore tape. A filter paper moistened with 0.9% saline solution was used as a negative control. The occlusion time was 48 h, and the results were read 48 h after removal of the cups. Marked erythema (over half the size of the cup) and erythema with induration were considered to be positive responses.

Serum studies

Shortly before the challenge and on day 4 after the beginning of the challenge a blood sample was drawn from the infant. Serum samples were stored frozen at -20°C until analysed. CM-specific IgE were measured in 233 available single samples and serum ECP in 156 available paired samples by the Pharmacia CAP system (Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). The following cut-off values were selected for serum ECP measured on challenge day 4: $\geq 15\text{ }\mu\text{g/L}$ (suggested by the manufacturer) [14], $\geq 20\text{ }\mu\text{g/L}$ [15] and $\geq 24.7\text{ }\mu\text{g/L}$ [16].

Statistical methods

The data are presented with means and 95% confidence intervals (CI) or with medians and ranges. Pearson's chi-square test and Student's two-tailed *t*-test were used in statistical comparisons. For comparisons between the paired measurements of serum ECP we used the Wilcoxon signed-rank test. Correlations were calculated by the Spearman rank correlation test. The data with a skewed distribution were analysed after logarithmic transformation. The following formulas were used for calculations: sensitivity

= number of infants with a positive challenge identified as correct by the test(s)/total number of infants with a positive challenge. Specificity = number of infants with a negative challenge identified as correct by the test(s)/total number of infants with a negative challenge. Positive predictive value = number of positive challenges identified as correct by the test(s)/total number of positive test results. Negative predictive value = number of negative challenges identified as correct by the test(s)/total number of negative test results. Overall agreement = total number of infants correctly classified by the test(s)/total number of infants tested. Analyses were performed using a SPSS (version 7.5 for Windows, SPSS, Chicago, IL, USA) software package.

The ethics committee of the Hospital for Children and Adolescents, University of Helsinki, approved the study protocol. Written informed consent was obtained from the mother of each infant entering the study.

Results

The first symptoms suggestive of CMA developed earlier in the 118 infants subsequently diagnosed as having CMA than in the 121 infants with a negative challenge: mean (95% CI) 2.9 (2.5–3.2) months vs. 3.6 (3.2–4.0) months ($P = 0.008$). According to the parents' reports, the infants with CMA had had skin symptoms more often than the infants with a negative challenge (Table 1).

At the time of the CM challenge, the mean ages of the infants with a positive and with a negative challenge were 6.7 (6.3–7.2) and 7.1 (6.6–7.5) months, respectively. The challenge was started with a drop of milk on the skin given to the 95 infants with a positive SPT. A positive reaction (urticaria and/or erythema) developed in 31/68 (46%) infants with CMA and in 4/27 (15%) infants with a

negative challenge ($P = 0.005$). Six infants with positive reactions to dermal CM challenge and SPT were not given milk orally at challenge because their symptoms had been severe. The symptoms elicited by the challenge are shown in Table 1. A positive reaction developed in 58 (49%) infants within 2 h, in 33 (28%) infants between 2 h and 24 h, and in 27 (23%) infants more than 24 h after the challenge.

Specific IgE CM antibodies measured in the skin and the circulation were detected more often in the infants with CMA (Table 2). The median (range) diameters of the SPT were 4 (0–12) mm and 0 (0–9) mm in infants with a positive and with a negative challenge ($P < 0.001$), and the mean (95% CI) concentrations of CM-specific IgE were 1.01 (0.75–1.37) kU/L and 0.34 (0.29–0.40) kU/L ($P < 0.001$), respectively. When the cut-off values were raised, the sensitivity of both tests decreased and the specificity increased.

The skin-patch test was also more often positive in the infants with CMA, both whole CM and CM protein fractions eliciting a positive response more frequently in infants with a positive challenge (Table 3). However, only 43% of the infants with CMA had a positive response to the patch test, and 29/72 (40%) of the positive responses were related to a negative challenge.

The mean (95% CI) concentrations of ECP in serum taken before the challenge were 7.2 (6.0–8.7) $\mu\text{g/L}$ and 9.8 (8.1–11.8) $\mu\text{g/L}$ in infants with a positive and a negative challenge ($P = 0.04$), and 8.6 (7.1–10.5) $\mu\text{g/L}$ and 11.3 (9.8–12.9) $\mu\text{g/L}$ on challenge day 4 ($P = 0.07$), respectively. During the challenge, serum ECP increased significantly in the infants with CMA ($P = 0.02$) but not in those with a negative challenge ($P = 0.12$). Similar percentages of infants in the two groups had moderately

Table 1. Symptoms suggestive of CMA reported by parents in 239 infants challenged with CM and symptoms at challenge in 118 infants with a positive challenge.

Symptom	Number (%) with symptoms suggestive of CMA		Sensitivity	Specificity	Number (%) with symptoms at challenge at challenge $n = 118$
	Positive challenge $n = 118$	Negative challenge $n = 121$			
Urticaria and/or exanthema	54 (46)**	28 (23)	0.46	0.77	61 (52)
Atopic dermatitis	106 (90)*	94 (78)	0.90	0.22	52 (44)
Gastrointestinal symptoms	61 (52)	73 (60)	0.52	0.40	24 (20)
Respiratory symptoms	42 (36)	31 (26)	0.36	0.74	18 (15)
Allergic conjunctivitis	6 (5)	4 (3)	0.05	0.97	5 (4)
Anaphylactic reaction	3 (3)	0	0.03	1.00	1 (1)
Multiple symptoms	76 (64)	74 (61)	—	—	27 (23)

* $P < 0.05$, ** $P < 0.001$ between infants positive and negative to the challenge.

Table 2. Cutaneous and circulatory IgE CM antibody responses in relation to the result of the CM challenge test.

Test	Number (%) with positive test		Sensitivity	Specificity	Positive predictive value	Negative predictive values	Overall agreement
	Positive challenge	Negative challenge					
<i>SPT to CM formula†</i>	<i>n</i> tested = 118	<i>n</i> tested = 121					
≥ 3 mm	72 (61)**	29 (24)	0.61	0.76	0.71	0.67	0.69
≥ 6 mm	44 (37)**	9 (7)	0.37	0.93	0.83	0.60	0.65
≥ 8 mm	22 (19)**	2 (2)	0.19	0.98	0.92	0.55	0.59
<i>Serum IgE CM antibodies</i>	<i>n</i> tested = 116	<i>n</i> tested = 117					
≥ 0.35 kU/L	84 (72)**	60 (51)	0.72	0.49	0.58	0.64	0.61
≥ 0.7 kU/L	52 (45)**	15 (13)	0.45	0.87	0.78	0.61	0.66
≥ 3.5 kU/L	29 (25)**	2 (2)	0.25	0.98	0.94	0.57	0.62

†Greater than the negative control.

** $P \geq 0.001$ between infants with a positive and with a negative challenge.

elevated serum ECP measured on challenge day 4, but the highest values were confined to the infants with a positive challenge (Table 3).

Sensitivity, specificity, positive and negative predictive values and overall agreement for the four tests combined at different cut-off points are presented in Table 4. A positive reaction to one or more tests in a combination was considered to be a positive response and a negative reaction to all four tests was regarded as a negative response. For a combination of SPT ≥ 3 mm, CM-specific IgE ≥ 0.7 kU/L, a positive reaction to CM fractions in the patch test and ECP ≥ 20 μ g/L, we selected the cut-off values of each test

that gave the best overall agreement. The combination that produced the best overall agreement (0.73) had a sensitivity of 0.76 and a specificity of 0.67. The infants with a positive challenge not detected by this combination more often had a reaction of a delayed type with gastrointestinal symptoms (data not shown).

Of the infants with positive patch test, 58% had a positive SPT (≥ 3 mm) and, of those with a negative patch test, 64% had a negative SPT ($P = 0.002$). A positive correlation was found between the diameter of the weal in SPT and the amount of circulatory CM-specific IgE (Spearman's $\rho = 0.724$, $P < 0.001$). Both SPT and CM-specific IgE

Table 3. Number of infants with a positive patch test to whole CM and its protein fractions and with elevated serum ECP measured on challenge day 4 in relation to the result of the CM challenge test.

Test	Number (%) with a positive test		Sensitivity	Specificity	Positive predictive value	Negative predictive value	Overall agreement
	Positive challenge	Negative challenge					
<i>Patch test positive</i>	<i>n</i> tested = 100	<i>n</i> tested = 104					
Whole CM and/or CM protein fractions†§	43 (43)*	29 (28)	0.43	0.72	0.60	0.57	0.58
Whole CM	37 (37)*	24 (23)	0.37	0.77	0.61	0.56	0.57
CM protein fractions§	26 (26)**	8 (8)	0.26	0.92	0.77	0.57	0.60
<i>Serum ECP</i>	<i>n</i> tested = 103	<i>n</i> tested = 53					
≥ 15.0 μ g/L	28 (27)	14 (26)	0.27	0.74	0.67	0.34	0.43
≥ 20.0 μ g/L	22 (21)	7 (13)	0.21	0.87	0.76	0.36	0.44
≥ 24.7 μ g/L	13 (13)*	1 (2)	0.13	0.98	0.93	0.37	0.42

†Response to whole CM ($n = 17$) or to CM protein fraction(s) ($n = 6$) or to both whole CM and to CM protein fraction(s) ($n = 20$).§CM protein fractions = β -lactoglobulin, bovine serum albumin, casein. * $P < 0.05$, ** $P < 0.001$ between infants with positive and negative challenge.

Table 4. Sensitivity, specificity, and predictive indices at different cut-off levels for the four tests derived from the responses of 137 infants† challenged with CM.

Test and cut-off value						Pos. pred. value	Neg. pred. value	Overall agreement
SPT (mm)	IgE (kU/L)		ECP	Sensitivity	Specificity			
≥ 3	≥ 0.35	Whole CM and/or CM protein fractions	≥ 15	0.85	0.23	0.67	0.46	0.64
≥ 6	≥ 0.7	Whole CM	≥ 20	0.66	0.65	0.78	0.51	0.66
≥ 8	≥ 3.5	CM protein fractions	≥ 24.7	0.54	0.94	0.94	0.52	0.68
≥ 3	≥ 0.7	CM protein fractions	≥ 20	0.76	0.67	0.81	0.60	0.73

Positive response: a positive reaction to one or more tests. Negative response: negative reaction to all four tests. †Number of infants on whom all four tests were done.

were more often positive in the infants showing an immediate reaction (≤ 2 h) at challenge (Table 5). Furthermore, the patch test tended to be positive more frequently in infants with an immediate reaction ($P = 0.10$), whereas the amount of serum ECP did not correlate with the reaction type at challenge or with any other tests used.

Discussion

In the present prospective study of 6209 unselected infants followed from birth for the development of CMA, we evaluated the performance of four immunological tests as diagnostic tools for CMA in 239 infants who were challenged with CM at a mean age of 7 months. Of the challenges, 118 (49%) were positive, a reaction rate that is comparable to those of earlier studies [2,17,18].

None of the four tests alone was able to predict CMA satisfactorily. Serum CM-specific IgE at low levels (≥ 0.35 kU/L) gave the best value for sensitivity, detecting

72% of the challenge-positive infants, but 51% of the responses were false-positive (poor specificity) like in an earlier study [19]. Transient low-level IgE responses to food antigens are commonly seen during the first year of life and do not always signify a clinical disease [20]. The rate of false-positive reactions (1-specificity) of an individual test varied from 2% to 51%, depending on the test and cut-off level used. The overall agreement is the total proportion of patients correctly classified by a given test or combination of tests. Of the individual tests, SPT at a cut-off value ≥ 3 mm produced the best value for overall agreement (0.69) with sensitivity of 0.61 and a specificity of 0.76. When all four tests were used in parallel (a positive reaction to at least one test was considered as a positive response), 73% of the patients were correctly classified with a combination of SPT ≥ 3 mm, CM-specific IgE ≥ 0.7 kU/L, a positive reaction to CM fractions in the patch test and ECP ≥ 20 μ g/L, with a sensitivity of 0.76 and specificity of 0.67. At the highest cut-off values, the parallel use of the four tests detected

Table 5. Relationship between the reaction time at challenge and SPT, CM-specific IgE, the patch test and serum ECP in infants with CMA.

Test (<i>n</i> tested)	Number with a positive test/ total number within a group (%)	
	Reaction time at challenge < 2 h	≥ 2 h
SPT to CM formula ≥ 3 mm (118)	49/58 (85)**	23/60 (38)
S-IgE CM antibodies ≥ 0.7 kU/L (116)	39/58 (67)**	13/58 (22)
Patch test positive to whole CM and/or CM protein fractions (100)	26/51 (51)	17/49 (35)
S-ECP ≥ 20 μ g/L (103)	11/51 (22)	11/52 (21)

** $P < 0.001$ between infants with immediate- and delayed-type reactions.

54% of the challenge-positive infants with a high specificity of 0.94. The parallel use of several tests improves the detection rate, probably because several immunological mechanisms are involved in CMA [7]. However, as also shown earlier [7,21], the tests most frequently failed to detect those challenge-positive infants who had the delayed type of reaction with gastrointestinal symptoms.

The sensitivity of skin-patch test ranged from 0.26 to 0.43 and the specificity from 0.72 to 0.92, depending on the antigens used, with whole CM being of the same magnitude as previously reported [22]. A positive reaction in patch test tended to correlate with the immediate-type reaction at challenge, whereas, in other studies, positive patch-test responses have been associated with delayed-type reactions to CM [2,23] or no associations have been found [21]. Differences in reading time and criteria for a positive reaction may explain this discrepancy. Using CM protein fractions as antigens, good specificity (0.92) was achieved with a low sensitivity of 0.26, as reported earlier [23]. When patch test was used in parallel with other tests the detection rate of CMA increased, as previously suggested [2,23]. However, until the methodology is standardized, patch test should be used only for research purposes.

Serum ECP measured on challenge day 4 distinguished poorly between the challenge-positive and -negative infants. Although the increase in serum ECP during the challenge was significant in infants with CMA and not in those with a negative challenge, the test did not discriminate between the challenge-positive and -negative infants at low cut-off values. However, the small number of high values ($\geq 24.7 \mu\text{g/L}$) [16] were mostly confined to the infants with CMA. Others have measured the post-challenge ECP earlier than we did in the present study [8,24]. In the study by Niggemann et al. on infants with food-sensitive atopic dermatitis, serum ECP reached a maximum 24 h after a positive food challenge and 48 h after a clinical reaction had returned to the baseline value [24]. We measured the post-challenge ECP 96 h after the start of the challenge. Therefore, the difference in pre- and post-challenge ECP values might have been greater if the second samples had been taken earlier. The late second visit in our study was selected to ensure that we found all the patients with a delayed reaction. In fact, in the present study the slowest symptoms appeared 90 h after the beginning of the challenge.

The challenge outcome could not be predicted from the symptoms suggestive of CMA reported by the parents, as shown earlier [18]. The occurrence of urticaria produced the best value for overall agreement (0.62) with a sensitivity of 0.46 and a specificity of 0.77. Among the infants with a positive SPT, dermal CM challenge elicited a positive reaction in 46% of the infants with a positive challenge, with a specificity of 0.85. Earlier, in older

infants with a positive SPT and suspicion of CMA not confirmed by a challenge test, a reaction rate of 69% to dermal CM challenge has been reported [25].

In summary, we found that in a group of 239 infants suspected to have CMA who were challenged with CM at a mean age of 7 months, no single test or combination of tests satisfactorily detected the infants with CMA. The parallel use of SPT, CM-specific IgE, patch test and ECP at low cut-off values detected 85% of the infants with CMA with a poor specificity of 0.23; at high cut-off values respective figures were 54% and 0.94. Thus, a clinical CM elimination-challenge test is essential for confirmation of the diagnosis of CMA.

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