

PREDICTION OF THE DEVELOPMENT OF TOLERANCE TO MILK IN CHILDREN WITH COW'S MILK HYPERSENSITIVITY

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Objectives To investigate whether the development of tolerance to cow's milk (CM) by aged 4 years can be predicted with a skin prick test (SPT) and measurements of total or specific immunoglobulin E (IgE) in the serum, taken at the time of diagnosis of cow's milk hypersensitivity (CMH).

Study design Infants with immediate (n = 95) or delayed (n = 67) challenge reactions to CM were prospectively followed to aged 4 years. CMH status was assessed annually by CM challenges.

Results By aged 2, 3, and 4 years, children with delayed reactions developed tolerance to CM faster than those with immediate reactions: 64%, 92%, and 96% versus 31%, 53%, and 63%, respectively. A wheal size of <5 mm in SPT correctly identified 83% of 124 infants who developed tolerance to CM by aged 4 years, and a wheal size of ≥5 mm in SPT correctly identified 71% of 39 infants with persistent CMH. Milk-specific IgE <2 kU/L correctly identified 82% of infants who developed tolerance to CM, and milk-specific IgE ≥2 kU/L correctly identified 71% of infants with persistent CMH.

Conclusion SPT and milk-specific IgE in the serum are useful prognostic indicators of the development of tolerance to CM in infants with CMH. (*J Pediatr* 2004;144:218-22)

Cow's milk hypersensitivity (CMH) occurs in about 2% to 3% of infants in unselected cohorts.¹⁻⁴ It has been reported to be immunoglobulin E (IgE)-mediated in 53% to 64% of these cases. However, in these studies, different diagnostic criteria, IgE cutoff levels, and different methods of IgE measurement were used. In prospective follow-up trials, CMH has been reported to persist to aged 3 years in 13% to 49% of the children.^{2,5,6}

There are conflicting reports of the usefulness of the skin prick test (SPT) or of specific IgE antibody levels in predicting the persistence of cow's milk (CM) allergy.^{2,5,7} In older children with atopic eczema, the SPT wheal size did not correspond to the disappearance of clinical milk hypersensitivity.⁸ Sicherer and Sampson found a correlation between IgE levels to casein and the development of CM tolerance; children who became tolerant had lower IgE levels in early childhood than those who remained sensitive.⁹

The purpose of this study was to determine whether SPT results, total serum IgE, or IgE antibodies to CM or to casein measured by CAP fluorescent enzyme immunoassay (Pharmacia, Uppsala, Sweden) in infancy could predict the development of clinical tolerance in children with CMH. The children were followed prospectively to aged 4 years.

The diagnosis of CMH was based on a double-blind, placebo-controlled food challenge (DBPCFC), and specific IgE-levels were measured by the Pharmacia UniCAP 100 System (Pharmacia, Uppsala, Sweden).

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CM	Cow's milk	IgE	Immunoglobulin E
CMH	Cow's milk hypersensitivity	ROC	Receiver operator characteristic
DBPCFC	Double-blind, placebo-controlled food challenge	SPT	Skin prick test

Table. Characteristics of the initial group of patients with an immediate and a delayed reaction in the DBPCFC to milk at the age of < 1 y

	Immediate	Delayed
Sex (male/female)	51/46	48/25
Age in months (mean; SD)	7.1; 2.2	6.8; 2.3
SPT with CM (≥ 3 mm; %)	68%	13%
IgE antibodies to CM (≥ 0.35 kU/L; %)	66%	31%
Subjects with atopic dermatitis (%)	82%	79%
Subjects with gastrointestinal symptoms (%)	22%	40%
Subjects with urticaria (%)	45%	22%

MATERIAL AND METHODS

Patients

The study originally included 180 infants with CMH who had been diagnosed before aged 12 months.¹⁰ The study was conducted in two university hospitals, and the subjects were recruited from well-baby clinics (33%), from general practitioners (33%), and from specialist doctors (34%). Hypersensitivity to CM was determined by means of the DBPCFC, except in the case of 4 infants with a history of anaphylactic reactions who also had positive SPTs and elevated IgE antibodies to CM. After confirmation of CMH, the families were offered the possibility of participation in a study in which the infants were randomized to receive, when they needed a supplement to breast-feeding, either a soy-based infant formula or a whey-based, extensively hydrolyzed infant formula.¹¹ The parent(s) of 170 subjects agreed to participate in the study, and 162 subjects were followed to aged 4 years. Patient characteristics are shown in the Table.

Milk Challenge

Hypersensitivity to CM was diagnosed at the beginning of the study by means of the DBPCFC as described by Vanto et al.¹⁰ The challenge was started with a drop of milk that was placed in the child's mouth inside the lower lip. If no adverse reactions occurred, increasing doses of milk (1, 15, 50, 100, and 200 mL) were given at ≥ 30 -minute intervals. If no immediate reactions occurred within 2 hours, the challenge was continued at home for 5 days by giving the child the placebo powder diluted to normal feeding concentration (an extensively hydrolyzed formula; Peptidi-Tutteli, Valio Ltd, Finland), or the placebo powder mixed 1:1 with a CM-based infant formula (Piltti, Kuivamaito, Finland) diluted to normal feeding concentration. The placebo and the CM formulas were given during separate weeks in random order. The child was considered tolerant if no symptoms occurred during the 5 days. Subjects with an immediate ($n = 95$) or a delayed ($n = 67$) challenge reaction were prospectively followed at aged 2, 3, and 4 years with a clinical examination and with an open challenge to assess the development of tolerance to CM.

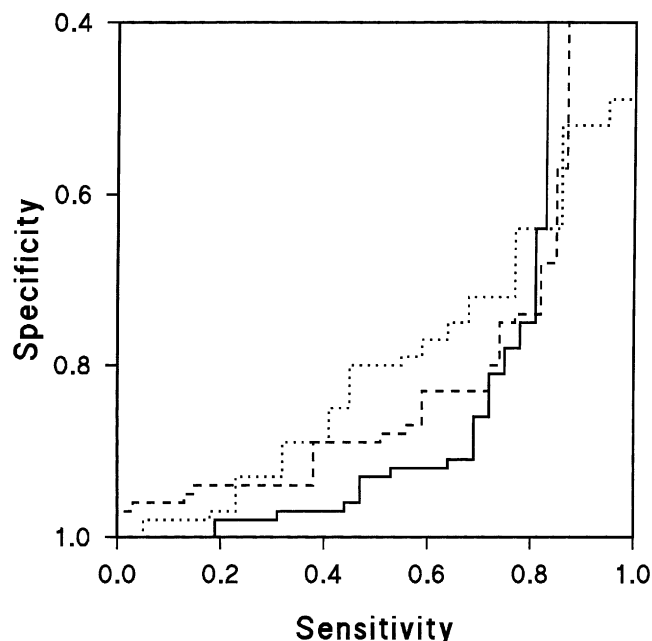


Fig 1. ROC curves of CM-specific IgE in serum (solid line), wheal size in SPT (dashed line), and total IgE in serum (dotted line) measured at the time of diagnosis for prediction of persistence of hypersensitivity to CM until aged 4 years. The areas under the ROC curves were 0.934 ± 0.027 , 0.891 ± 0.029 , and 0.807 ± 0.047 for CM-specific IgE in serum, SPT, and total IgE in serum, respectively. The ROC curves show the relationship between the levels of diagnostic sensitivity and specificity at various cutoff levels of the diagnostic test being evaluated.

Use of Soy-based Formula and Hydrolyzed Formula

CM products were eliminated from the infants' diets until tolerance to CM was confirmed in the challenge test. During the first 2 years of the infants' lives, a soy-based formula (Soija-Tutteli, Valio Ltd, Finland) or an extensively hydrolyzed formula (Peptidi-Tutteli, Valio Ltd, Finland) was given instead of CM products. Other food items that, on the grounds of clinical history and/or SPT, might cause hypersensitivity reactions were also avoided.

Skin Prick Test

The SPT was carried out at the same time that the initial milk challenge test was performed (± 2 weeks). The test was performed by trained nurses on the volar aspect of both arms (duplicate tests), with a CM-based formula (milk powder diluted to normal feeding concentration), histamine-dihydrochloride (10 mg/mL), and a negative control solution (glycerol-phosphate diluent, ALK, Hørsholm, Denmark). The results were expressed as mean wheal diameter in millimeters. The SPT was considered positive if the wheal diameter was at least 3 mm.

Total IgE and Allergen-specific IgE Antibody Measurements

Serum was collected at the time of the initial milk challenge and stored at -20°C until analysis. A UniCAP 100

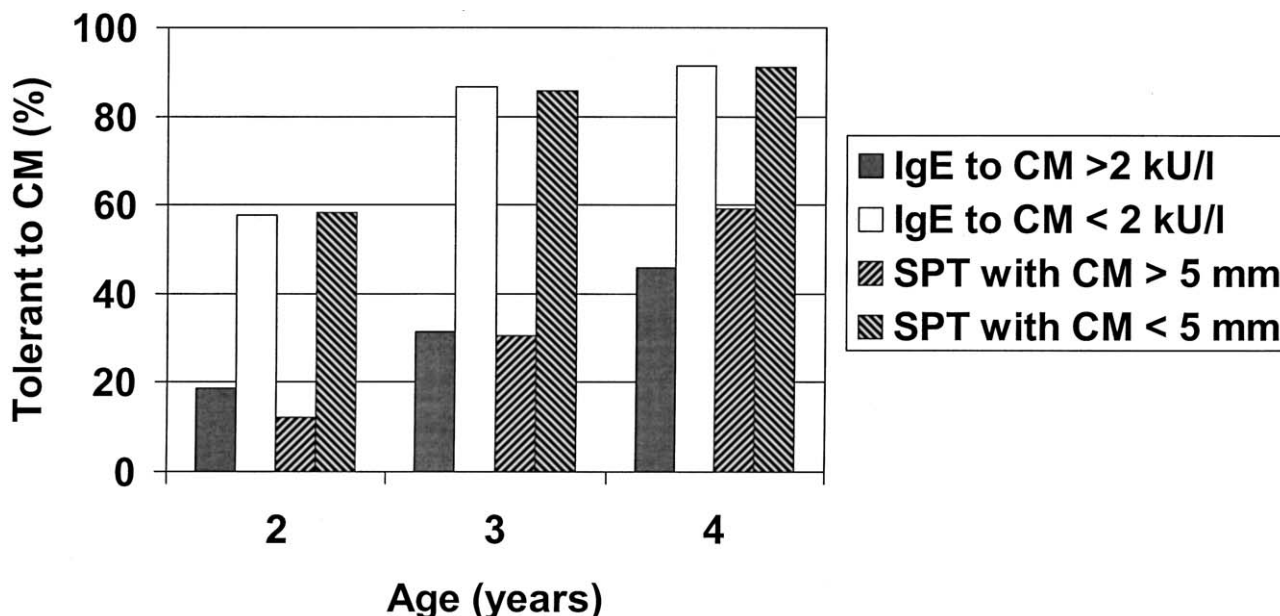


Fig 2. Development of tolerance to CM by aged 2, 3, and 4 years according to cutoff values that in the ROC analysis were shown to separate best those children who became tolerant and those who remained hypersensitive (5 mm for SPT and 2 kU/L for the specific IgE level, respectively).

automatic analyzer (Pharmacia, Uppsala, Sweden) was used for the measurement of total IgE and IgE specific to milk, to α -lactalbumin, to β -lactoglobulin, and to casein in the serum.

Statistical Analyses

Either Student's *t* test or Wilcoxon sign-rank test was used to assess the significance of differences in the means between subgroups. Analysis of frequencies was carried out by χ^2 analysis and by Fisher's exact test. Logistic regression analysis with backward elimination of the independent variables was used to evaluate the redundancy of the variables used to classify the infants into those who developed tolerance and those with persistent CMH. The degree of discrimination between the two subgroups of infants was assessed by receiver operator characteristic (ROC) analysis.^{12,13} Probability curves for the prediction of outgrowing CMH were generated with logistic regression analysis as done by Sampson.¹⁴ SAS Release 8.02 software (SAS Institute Inc, Cary, NC) was used in the statistical analyses.

Ethical Considerations

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

RESULTS

Initially, 170 families agreed to participate in the study, but during the follow-up, 5 families moved out of the area, and 3 families declined to participate in the study for other reasons. Three families refused to allow their children to participate in

the milk challenge at aged 4 years because the children had previously experienced severe symptoms in a milk challenge and/or accidental exposure to CM and because they all had high (>15 kU/L) levels of IgE to CM. These 3 children were included in the study and were classified as intolerant to CM at aged 4 years. The families of 12 children who had become tolerant to CM refused to attend the follow-up visit at aged 4 years, but these 12 children were nevertheless included in the study and were classified as tolerant to CM. Thus, we had data available from 162 children regarding their tolerance to CM at aged 4 years.

Tolerance to CM had developed in 44% of the children (72/162) by aged 2 years, in 69% (111/162) by aged 3 years, and in 77% (124/162) by aged 4 years. If the infants were divided into two groups according to the IgE level specific to CM, non-IgE-mediated (<0.35 kU/L at the time of diagnosis of CMH) and IgE-mediated (≥ 0.35 kU/L) groups, there was already a significant ($P < .001$) difference between these groups in recovery rate by aged 2 years (59% [47/80] versus 30% [23/76], respectively). At aged 4 years, 93% (74/80) of the subjects in the non-IgE-mediated group and 59% (44/74) of the subjects in the IgE-mediated group tolerated CM ($P < .001$). In the ROC analysis, the cutoff level of 2 kU/L was shown to be useful in discriminating between children who remained hypersensitive and those who became tolerant to CM at aged 4 years. Milk-specific IgE < 2 kU/L correctly identified 82% (95/116) of the infants who developed tolerance to CM, and the level of ≥ 2 kU/L identified 71% (25/35) of infants with persistent CMH (Figs 1 and 2). In the ROC analysis, a wheal size of < 5 mm in SPT correctly identified 83% (103/124) of the infants who developed tolerance to CM by aged 4 years, and a wheal size of ≥ 5 mm correctly identified 74% (28/38) of the infants with persistent

CMH (Figs 1 and 2). Probability curves of the development of tolerance to CM were calculated as determined by the levels of wheal size in SPT and IgE specific to CM (Fig 3). Children who became tolerant to CM displayed at the time of the CMH diagnosis significantly lower levels of IgE antibodies specific to CM, to casein, to β -lactoglobulin, and to α -lactalbumin than those with persistent CMH. They also had lower total IgE levels. However, according to the logistic regression analysis, measurement of total IgE or milk-protein-specific IgE levels at that time point could not be shown to give more prognostic information than that obtained by SPT with CM or by the measurement of IgE specific for CM. It was seen that no additional diagnostic benefit would be achieved by combining the results of SPT and IgE specific to CM. Similarly, no additional utility was obtained by combining the results of total IgE measurements with those of SPT or IgE specific to CM.

The type of reaction in the initial DBPCFC with milk was clearly prognostic of the development of tolerance to CM. By aged 2, 3, and 4 years, children with delayed reactions developed tolerance to CM faster than those with immediate reactions: 64%, 92%, and 96% versus 31%, 53%, and 63%, respectively. The children with immediate reactions had, for the most part, positive SPT and specific IgE results, whereas the children with delayed reactions usually had negative results (Table). Only 3 children in the group of delayed reactors were still hypersensitive to milk at aged 4 years. Two of these 3 children were SPT negative and their milk-specific IgE levels were <0.35 kU/L at the time of the initial diagnosis of CMH. Both children had had increasingly positive SPT reactions and increasing amounts of milk-specific IgE antibodies since aged 1 year, and all 3 children had had an immediate reaction to the CM challenge at aged 4 years.

The infants in our study were randomized to receive either a soy-based formula or an extensively hydrolyzed infant formula. The development of tolerance to CM did not differ between the groups. Children with persistent CMH were breast-fed longer than those who became tolerant to CM (mean; SD 10.3; 9.0 versus 6.5; 4.1 months, respectively; $P = .014$). Infants with an atopic family history were breast-fed for the same length of time as those with no atopic family history (mean; SD 6.7; 4.2 versus 7.9; 6.9 months, respectively; NS).

DISCUSSION

This was a prospective follow-up study of CMH in which the initial diagnosis was based on a DBPCFC and in which infants with delayed reactions to CM were also included for the follow-up. Infants with IgE-mediated, often immediate, reactions were shown to have a more persistent CMH than those with non-IgE-mediated, often delayed-type, CMH who nearly always became tolerant to CM by aged 4 years.

The results are similar to those reported earlier^{7,9,15} and are comparable to those of a Danish cohort of children who were prospectively followed to aged 3 years.² In that study, the

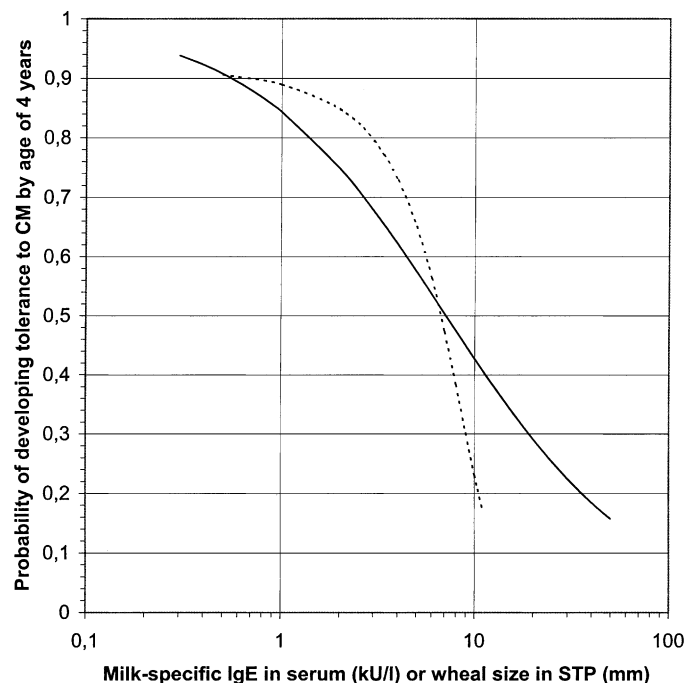


Fig 3. Logistic relationship between the probability of development of tolerance to CM by aged 4 years and the level of milk-specific IgE in serum (*solid line*) or wheal size in the SPT (*dashed line*), measured at the time of diagnosis of CMH. The following equations define the probability curves: probability = $1 / (\exp[-1.6826 + 1.9783 \times \log(\text{milk-specific IgE in serum [kU/L]})] + 1)$ probability = $1 / (\exp[-2.4469 + 0.3655 \times \text{wheal size in SPT (mm)}] + 1)$.

majority of the 39 infants with CMH had no IgE antibodies when Al-RAST (ALK, Hørsholm, Denmark) was used instead of the CAP system for measuring the IgE antibodies specific to CM. All the children with non-IgE-mediated allergy became tolerant to CM by aged 3 years, compared with only 76% of the infants with IgE antibodies. Similarly, Hill et al reported a lower rate of remission at aged 3 years for infants with IgE-mediated compared with non-IgE-mediated reactions (57% and 78%, respectively) at a tertiary-level university hospital where it is probable that infants with more severe symptoms were enrolled.¹⁶

According to a Dutch study,⁵ infants who initially appeared with gastrointestinal symptoms and with low serum total IgE levels at the time of diagnosis had a better recovery rate, as in our study. The measurement of total IgE did not give any more practical information than that obtained by SPT or milk-specific IgE, as we found earlier.¹⁰

The predictive value of SPT with CM was similar to that of milk-specific IgE.¹⁰ Combining both methods did not add further to the predictive value of these tests. SPT has some practical advantages over the measurement of specific IgE antibodies: It is cheaper and faster, but it is difficult to standardize.¹⁷

It has been documented that in patients who become clinically tolerant to CM, the IgE-specific antibody concentrations for CM and specific milk proteins are initially

lower and decrease significantly with time, in comparison with those patients who retain their clinical sensitivity.^{7,9,15} In our study, the IgE concentrations specific to casein and to β -lactoglobulin were lower at the time of diagnosis in tolerant children compared with children who remained allergic. However, the IgE specific to casein and to β -lactoglobulin could not be shown to be more predictive than the IgE specific to CM.

In our study, the SPT wheal size and milk-specific IgE levels were almost identical to the levels shown to predict best the development of tolerance to egg¹⁸ by ROC analysis (5 mm versus 6 mm in wheal size and 2 kU/L versus 1.98 kU/L in specific IgE, respectively). There are no similar longitudinal studies concerning other food allergies. In peanut allergy, all the subjects with a resolving allergy had SPT wheals of <6 mm at the time of the peanut challenge, whereas only 3 of 14 subjects with persistent peanut allergy had wheals <6 mm.¹⁹

One unexpected finding in our study was that children with persistent CMH were breast-fed longer than those who became tolerant to milk. This phenomenon was not explained by a longer period of breast-feeding by the atopic mothers because there was no significant difference in the duration of breast-feeding between the atopic and non-atopic mothers. One explanation could be that there are cellular, cytokine, or other components of breast milk that enhance the persistence of CMH.²⁰⁻²²

In conclusion, this prospective study shows that persistent CMH is related to IgE-mediated allergy with immediate symptoms in infancy. Delayed, often gastrointestinal, symptoms in a milk challenge, a negative or a small (<5 mm) wheal reaction in the SPT, and low specific (<2 kU/L) IgE levels at the onset of CMH favor the development of tolerance by aged 4 years.

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