

Specific IgE levels in the diagnosis of immediate hypersensitivity to cows' milk protein in the infant

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Background: A milk-free diet with substitute formula should be established when immediate symptomatic hypersensitivity to cows' milk protein (CMP) is diagnosed, and therefore an accurate diagnosis is very important.

Objective: This study aims to find the optimal cutoff values for specific IgE antibody levels that discriminate between allergic and tolerant infants by using cows' milk and its principal proteins as allergens.

Methods: A prospective study was carried out on 170 patients under 1 year old (mean, 4.8 months). These patients were seen consecutively over a 4-year period in our outpatient clinic and for the first time because of a reaction suggesting immediate hypersensitivity after ingestion of cows' milk formula. A clinical history, prick test with cows' milk and its proteins (α -lactalbumin, β -lactoglobulin, and casein), determination of specific IgE antibodies with the CAP system FEIA for the same allergens as for the prick test, and a challenge test according to the diagnostic protocol were performed in all of the children. A study of validity of the prick test (cutoff point, 3 mm) and CAP system by using different cutoff points in the specific IgE values for cows' milk and its proteins were also analyzed.

Results: Prevalence of immediate symptomatic hypersensitivity to CMP in this study was 44%. When both the whole milk and its principal milk proteins were used in the prick test, the negative predictive value was very high, and a negative value excluded allergy in 97% of the patients. When the different cutoff points of the specific IgE for milk were analyzed, 2.5 KU_A/L had a positive predictive value of 90% and 5 KU_A/L had a positive predictive value of 95%.

Conclusions: When diagnosing immediate hypersensitivity to CMP in infants, negative skin test responses exclude allergy in most of the patients. If the prick test response is positive, specific IgE levels for cows' milk may be helpful. If these values are 2.5 KU_A/L or greater, the challenge test should not be performed because of its high positive predictive value (90%). (*J Allergy Clin Immunol* 2001;107:185-90.)

Key words: Cows' milk hypersensitivity, infant, specific IgE, skin prick test, predictive values, challenge test

Allergy to cows' milk proteins (CMPs) occurs principally in the first half year of life, coinciding with its introduction into the infant's diet. IgE-mediated reactions are the best known and characterized of all food allergy reactions.¹

Diagnosis of immediate hypersensitivity to CMP is based on the clinical background and on the demonstration of specific IgE antibodies for CMP; however, the challenge test will either confirm or reject the presence of clinical symptoms.² Once the presence of clinical reactivity is verified, the only treatment is a diet excluding this protein and the administration of a substitute formula.³ Thus it is very important to use the challenge test to verify the diagnosis; however, it must be remembered that these tests are uncomfortable for the patient, take time, and are not free from undesirable effects. Therefore it is necessary to find methods that will make it possible to avoid using those challenges that have a high probability of having positive responses.

The skin prick test is the first choice to investigate immediate hypersensitivity reactions because it has great sensitivity and negative predictive value (NPV).^{4,5} The quantification of the specific IgE antibodies in serum with the CAP system FEIA is reported to have improved sensitivity compared with the standard RAST.⁶ Some studies have found an association between higher levels of specific IgE and clinical reactivity.^{7,8} A position statement by the American Academy of Allergy and Immunology⁹ mentions the need to perform studies that relate the amount of IgE antibody and the symptoms. This work aimed to find the optimal cutoff values for specific IgE antibody levels that discriminate between allergic infants and tolerant infants by using cows' milk and its principal proteins as allergens.

METHODS

Patients

A total of 170 infants (90 boys and 80 girls) who had come to the Allergy Service of the "Hospital Infantil La Paz" in Madrid for the first time and who were selected consecutively over a 4-year period

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TABLE I. Clinical symptoms

	Clinical symptoms after milk ingestion (n = 170)	Clinical symptoms to challenge test (n = 67)
Cutaneous symptoms	168 (99%)	
Urticaria, angioedema, or both	161/168 (96%)	56 (85%)
Erythema	7/168 (4%)	
Gastrointestinal symptoms (vomiting, acute diarrhea, or both)	59 (35%)	22 (33%)
Respiratory symptoms (bronchospasm, rhinitis, or both)	10 (6%)	8 (12%)
Associated symptoms	78 (45%)	17 (25%)

Abbreviations used

ALA:	α -Lactalbumin
BLG:	β -Lactoglobulin
CMP:	Cows' milk protein
CPMA:	Cows' milk protein allergy
NPV:	Negative predictive value
PPV:	Positive predictive value
ROC:	Receiver operating characteristic

because of symptoms suggesting immediate hypersensitivity to CMP were included in this study. Those infants in whom late digestive symptoms and atopic dermatitis were the only manifestations and whose symptoms had no clear relationship to the ingestion of CMP were excluded. Fifty percent had a positive family background of atopy, and 23% had atopic dermatitis. The mean age of the patients on presentation to the outpatient clinic was 4.8 months (range, 1-12 months), and the age at which the reaction to CMP had occurred was 3.3 months (range, 1-8 months). Immediate cutaneous symptoms with cows' milk were reported in 99% of the patients, digestive symptoms in 35%, and respiratory symptoms in 6%. Symptoms associated with 2 or more shock organs were found in 45% of patients (Table I). The symptoms occurred during the first 30 minutes after the ingestion of the cows' milk formula in 77% and during the first 2 hours in the other patients. The symptomatology was present with the first ingestion of the milk in 55%, during the first 7 days of its introduction in 28%, and after 7 days in the rest of the patients.

Skin prick tests

Skin prick tests were performed in all patients with whole cows' milk extract (5% wt/vol; Alergia e Inmunología Abelló, Madrid, Spain) and with isolated CMPs: 10 mg/mL α -lactalbumin (ALA), β -lactoglobulin (BLG), and casein (Sigma Chemical, St Louis, Mo) in PBS. Histamine dihydrochloride diluted 1:100 was used as a positive control, and glycerosaline was used as a negative control. Reactions were read at 15 minutes. A net wheal diameter 3 mm larger than that produced by the negative control was considered positive. These extracts have been used in our clinical practice for a long time.

In vitro test

Serum samples from all patients were analyzed for total serum IgE and specific IgE antibodies to milk, ALA, BLG, and casein by using the CAP system FEIA (Pharmacia Diagnostics, Uppsala, Sweden). The test was considered positive when a result of 0.35 KU_A/L was obtained.

Challenge test

Open controlled challenge tests with cows' milk were carried out in all but the 9 infants who had a clear history of a severe allergic

reaction to CMP and evidence of CMP-specific IgE antibodies. All of the challenges were performed in the allergy unit of the hospital, where appropriate medication and resuscitation equipment was directly available. Informed consent was previously obtained from the parents. Each patient came early in the morning to the allergy unit, where he or she remained for 4 hours under observation after the last milk dose intake before going back home.

On the first day of the challenge, increasing doses of cows' milk infant formula (2, 5, and 10 mL) were given at 90-minute intervals. In the following days, 25, 50, and 100 mL was administered. When 100 mL was reached with good tolerance, this feeding was continued in the patient's home, and tolerance was confirmed at 15 days by means of telephone survey. If a clinical reaction appeared, the challenge was discontinued, and treatment was provided if necessary. The challenge was considered to be positive when there were skin (urticaria, angioedema, or erythematous rash), gastrointestinal (vomiting or diarrhea), respiratory (rhinoconjunctivitis or bronchospasms), or generalized (anaphylactic shock) manifestations in the 2 hours after the intake of the food. Allergy to cows' milk was confirmed by means of positive challenge results and proof of specific IgE to cows' milk or its proteins (skin prick test, CAP system, or both). The challenge was not performed on 9 infants because of the high risk of a severe anaphylactic reaction, contraindicating a food challenge. The patients with negative challenge results were considered as the control group.

Data analysis

A study on the validity of the diagnostic tests, skin prick tests, and CAP system, with the challenge test used as the gold standard, was carried out. The sensitivity, specificity, positive predictive value (PPV), and NPV, together with 95% confidence intervals, were calculated. The statistical calculations were carried out with the SPSS 7.5 statistical program. The cutoff levels of specific IgE for cows' milk and its proteins were determined by analysis with the receiver operating characteristic (ROC) curve. The analysis was performed with the computer program Graphroc (Veli Kairisto, Turku University, Finland). Differences in the levels of specific IgE among the allergic and tolerant subjects were analyzed with the Mann-Whitney *U* test. The Fisher exact test was used to compare the qualitative variables.

RESULTS

The challenge test was performed in 161 of the 170 patients (mean age, 6.5 months), with results being positive in 67 and negative in 94 patients. On the basis of the clinical criteria of the protocol, it was not performed in 9 patients. The allergic group was made up of 76 (44%) children, and the control group consisted of 94 infants who tolerated the food. The clinical history showed little difference between both groups, except for the predomi-

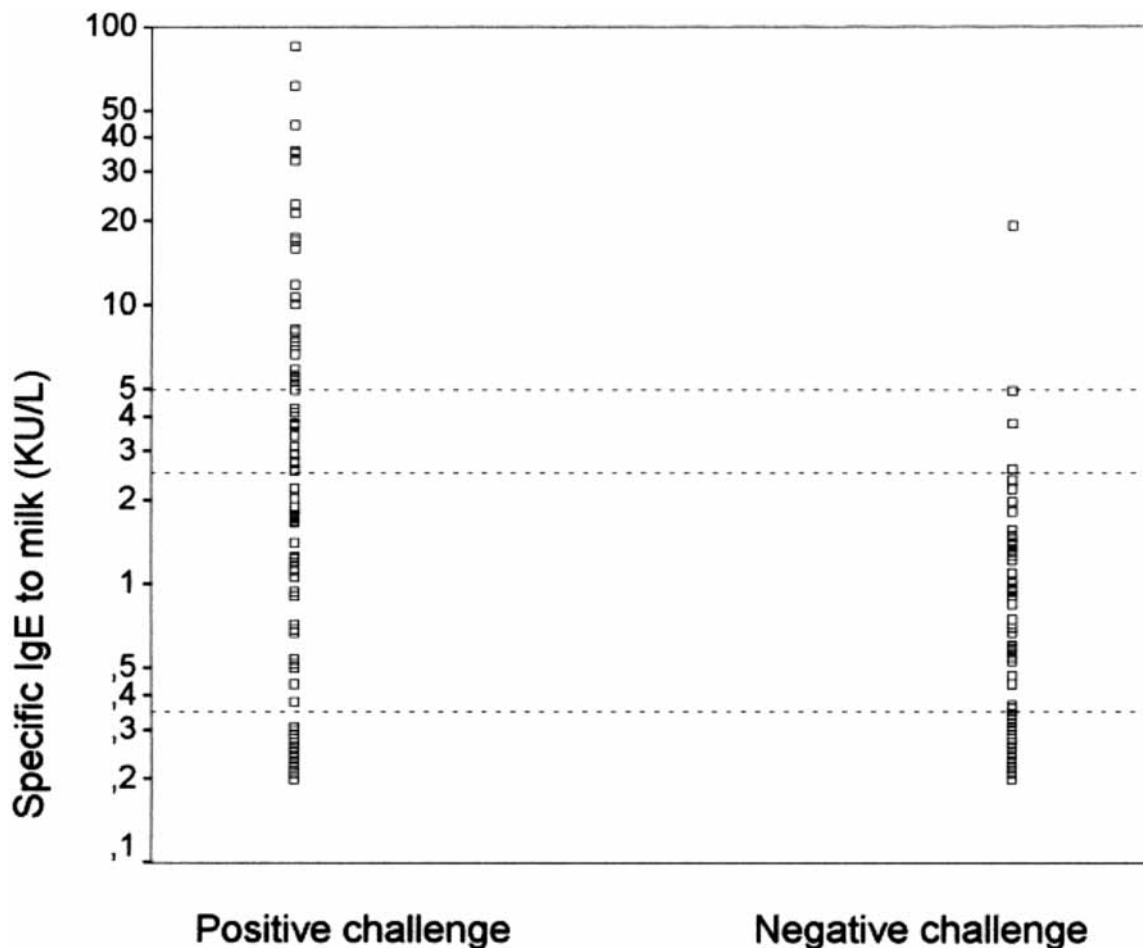


FIG 1. Specific IgE levels to milk in patients with positive and negative challenge test responses. Cutoff values of 2.5 and 5 KU_A/L resulted in PPVs of 90% and 95%, respectively, and 0.35 KU_A/L corresponded to the highest NPV (81%).

nance of angioedema, with the ingestion of milk in the allergic compared with the tolerant patients (42% vs 25%, $P = .01$), in the presence of associated symptoms in 2 or more shock organs (54% vs 38%, $P = .02$), and in the appearance of symptoms with the first apparent intake of milk (55% vs 38%, $P = .01$).

Table I shows the results of the challenge. Of the 76 infants evaluated, 44 had symptoms with amounts varying from 2 to 25 mL, 10 with 50 mL, and 7 with 100 mL, and 6 infants tolerating 100 mL at the outpatient clinic had symptoms when the amount was increased at home. All the reactions were assessed and controlled by a physician and disappeared within 1 or 2 hours. No treatment was required in 39 of 67 infants, oral antihistamines were administered to 23 infants, and several drugs were administered to 5 infants.

Table II shows the performance characteristics of the skin prick tests (cutoff level, 3 mm) for each one of the

allergens used. The skin tests had greater NPV than PPV. The BLG prick test had the greatest sensitivity, and that of casein had the greatest specificity. The greatest sensitivity was obtained when the positivity of at least one skin test was evaluated. The negativity of all the skin tests had a high NPV (97%). Table III shows the performance characteristic of the CAP system for the different allergens used, considering 0.35 KU_A/L as the positive cutoff point. It was observed that the assessment of at least one positive CAP result shows little difference in regard to the CAP result for milk, which has the greatest sensitivity and the best NPV. The CAP for the other allergens analyzed had a greater specificity and a greater PPV but lower sensitivity. It can be deduced from the analysis of the different specific IgE levels for milk in the ROC curve that the optimal decision point would be 0.7 KU_A/L . The cutoff point of 2.5 KU_A/L is a point at which specificity

TABLE II. Performance characteristics of skin prick tests at the cutoff level of 3 mm

Allergen	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
Milk	0.72 (0.61-0.82)	0.62 (0.51-0.72)	0.60	0.73
ALA	0.66 (0.54-0.76)	0.64 (0.53-0.73)	0.59	0.70
BLG	0.84 (0.74-0.91)	0.53 (0.42-0.63)	0.59	0.81
Casein	0.55 (0.43-0.67)	0.87 (0.79-0.93)	0.78	0.71
At least one	0.99 (0.93-1)	0.38 (0.28-0.49)	0.56	0.97

TABLE III. Performance characteristics of CAP system FEIA at the cutoff level of 0.35 KU_A/L

Allergen	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
Milk	0.84 (0.74-0.92)	0.56 (0.46-0.67)	0.61	0.81
ALA	0.55 (0.43-0.67)	0.84 (0.75-0.91)	0.74	0.70
BLG	0.59 (0.47-0.70)	0.80 (0.70-0.87)	0.70	0.71
Casein	0.71 (0.59-0.81)	0.75 (0.75-0.84)	0.70	0.76
At least one	0.85 (0.75-0.92)	0.56 (0.45-0.66)	0.61	0.83

TABLE IV. Performance characteristics of CAP system FEIA to milk at different cutoff levels (KU_A/L)

	≥0.35	≥0.7	≥2.5	≥5
Sensitivity	0.84	0.74	0.48	0.30
Specificity	0.56	0.71	0.95	0.99
PPV	0.61	0.67	0.90	0.95
NPV	0.81	0.77	0.69	0.64

increases, although sensitivity decreases. Its PPV is 90%. As can be seen in Fig 1, the challenge results above 2.5 KU_A/L were almost always positive. Almost all of the tolerant patients had positive results below this value, although there were also positive challenge results in one third of all the patients with CAP values below 2.5 KU_A/L. Table IV shows cutoff values of specific IgE to milk that provide 90% and 95% PPVs. The negative predictive accuracies never reached 90%, with the cutoff value of 0.35 KU_A/L showing the highest NPV (81%). Analyzing the ROC curves of the different milk proteins, the optimal decision points would be 0.35 for casein, and less than this value (detection limit of the technique) for the other proteins. The PPV of 90% or greater for these proteins corresponded to very low sensitivities: 2.5 KU_A/L to ALA (sensitivity, 0.21%), 3 KU_A/L to BLG (sensitivity, 0.17%), and 5 KU_A/L to casein (sensitivity, 25%).

The medians of specific IgE for milk and its proteins were all greater in the allergic group than in the tolerant

group (2.12 KU_A/L vs <0.35 KU_A/L to milk, 0.46 KU_A/L vs <0.35 KU_A/L to ALA, 0.46 KU_A/L vs <0.35 KU_A/L to BLG, and 1.41 KU_A/L vs <0.35 KU_A/L to casein). When the nonparameter Mann-Whitney *U* test was applied to compare the specific serum IgE levels between both groups, the differences were statistically significant (*P* < .001).

DISCUSSION

Immediate symptomatic hypersensitivity to CMP was diagnosed in 44% of the children who came to the outpatient clinic because of immediate symptoms with CMP. The clinical history usually suggests the presence of CMP allergy, but this is only verified with challenge tests in 30% to 60% of patients.^{10,11} These results support the usefulness of the challenge test in the diagnosis of immediate hypersensitivity to CMP in the infant.

The group of infants in whom allergy was not verified (tolerant) was similar in regard to age and sex to the allergic group; however, in the latter group, among the clinical manifestations of CMP allergy, angioedema symptoms predominated over the urticaria symptoms, and there was also predominance of associated symptoms in 2 or more shock organs, showing more clinical severity in the allergic group than in the tolerant group in general. Thus in the group of infants who received a diagnosis of allergy, the specific serum IgE levels for milk and its proteins were significantly higher than in the group in which allergy was not verified. This agrees with those studies in which high specific IgE levels have been associated with clinical reactivity.^{7,8}

The skin prick test is the first test that should be performed to diagnose immediate hypersensitivity to food. When the validity of the skin prick test was analyzed in the diagnosis of immediate hypersensitivity to CMPs in the infant compared with the challenge test, the diagnostic advantage was greater when the principal proteins were used together with whole milk extract. The negativity of all the skin test responses excludes allergy in most of the cases because of its high NPV. The PPV of the skin tests is low, and therefore its positivity should be followed by the assessment of the specific IgE antibodies in serum. When the positivity limit was considered to be 0.35 KU_A/L, the CAP had greater NPV than PPV, as did the skin prick test, but when other proteins in addition to whole milk were analyzed, their predictive values showed little improvement. Thus the CAP for milk would be sufficient in the diagnosis of immediate symptomatic hypersensitivity to CMPs.

The ROC curve analysis demonstrates the variation of sensitivity and specificity of a test as a function of threshold values and helps to determine a positive threshold value.¹² Although it can be deduced from the ROC curve for milk that 0.7 KU_A/L is the optimal decision point (ie, the point at which the sensitivity weight is equal to that of the specificity), in clinical practice the most adequate decisional level of specific IgE to indicate whether the challenge test should be performed depends on the number of patients clinicians agree are overdiagnosed or missed. If the challenge test could be dangerous for the patient, a low cutoff point would be of interest to avoid risk challenges, even at the cost of erroneously labeling many children who could have had a negative challenge test response as having allergy. This work aimed to find that cutoff point that would adequately separate those infants who have a high probability of having a positive challenge result (allergic) and thus avoid the need to carry it out for those who have a high probability of having a negative result (tolerant). Even though we have not found a good cutoff point that would totally separate the allergic from the tolerant patient, in regard to the optimal decision point of the ROC curve, 2.5 KU_A/L greatly improves the PPV (90%) and slightly lowers the NPV, whereas 5 KU_A/L reaches a PPV of 95% but shows drastic reduction in its sensitivity. Thus in a population with a prevalence similar to ours, the challenge test should not be carried out with a cutoff value of specific IgE to milk of 2.5 KU_A/L or greater because of the high probability of a positive challenge result (PPV, 90%), but below this value almost one third of challenge results will be positive (NPV, 69%).

In the work of Sampson and Ho,⁸ with a 50% prevalence for milk, a cutoff point of 23 KU_A/L for milk had a PPV of greater than 90%. In our study, with a 44% prevalence, this value would be at 2.5 KU_A/L. The reason that our study found a lower value could be related to the population studied (ie, infants under 1 year). The IgE values vary with the age of the patients. On the other hand, in the work of Sampson and Ho, all of the patients had atopic dermatitis, which is associated with higher

levels of total and specific serum IgE. Because prevalence influences rates of positive and negative predictive accuracy, it must be taken into account in the population being studied.

Although double-blind challenges with placebo are considered as the gold standard¹³ in the diagnosis of immediate hypersensitivity to food, open challenges are used in infants because of the minimal risk of overestimating them with the psychogenic reactions¹⁴ and also because the type of immediate symptoms of the challenge are easily assessed. Although other authors use shorter challenge protocols, ours is more expansive in time, but it facilitates the outpatient clinical treatment of the infant and does not interfere with his habits. This protocol has proved to be safe because the reactions were easily controlled by the responsible physician performing the challenges. As in 6 infants, the challenge test result was positive when doses exceeding 100 mL were administered at home. We recommend increasing the challenge dose in the hospital until reaching the amount the infant needs in accordance with his or her age.

The cows' milk protein allergen (CMPA) fundamentally occurs during the first half year of life, coinciding with its introduction into the infant's diet. The CMPA studies should consider the age of the patients at the time of the diagnosis in order to obtain results that can then be compared because the IgE values vary according to the patient's age. Because the natural history in CMPA is its evolution to tolerance,¹⁵⁻¹⁷ a follow-up of these patients should be carried out to test the moment allergic reactions to food are outgrown. Consequently, longitudinal studies that provide specific IgE cutoff levels at different times are required to predict tolerance.

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