Letter to the Editor

Utility of casein-specific IgE levels in predicting reactivity to baked milk

To the Editor:

Cow's milk (CM) allergy is the most common food allergy, affecting 2% to 3% of infants,[1] half of which is estimated to be IgE mediated and responsible for up to 13% of fatal food-induced anaphylaxis.[2] The overall prognosis is favorable, although recent studies have shown a later acquisition of tolerance to CM in a subset of patients.[3,4] It was recently reported that the majority (75%) of children with CM allergy tolerate baked milk products (eg, muffins, waffles, cakes, and breads).[5] Moreover, the addition of baked milk to the diets of children tolerating such foods appears to accelerate the development of regular milk tolerance compared with strict dietary avoidance.[6] In contrast, children reactive to baked milk have a more severe phenotype of CM allergy, with a higher risk of severe anaphylaxis and a more protracted course. Identification of patients tolerating baked milk is therefore essential but challenging.

Although the clinician-supervised oral food challenge (OFC) is considered the gold standard, it is resource consuming, has limited availability, and is associated with a risk for anaphylaxis. The diagnostic value of skin prick tests (SPTs) and serum specific IgE measurements to CM in distinguishing baked milk–reactive from baked milk–tolerant children is not well defined. Recently, major interest has focused on component-resolved diagnostics in the diagnosis of CM allergy, particularly measurement of specific IgE to casein.[7] On the basis of 2 large cohorts of patients, the aim of this study was to evaluate the clinical utility and added diagnostic value of measurements of specific IgE and IgG_4 antibodies to CM, casein, and β-lactoglobulin.

We analyzed baseline data from 225 patients evaluated for tolerance to baked milk. Ninety-seven patients were prospectively recruited at the Mount Sinai allergy clinics between 2004 and 2007 from an original study on tolerance to baked milk.[5] A second cohort of 128 patients with milk allergy was recruited prospectively between 2008 and 2010 at the Mount Sinai allergy clinics and was used for comparison with results obtained from the initial cohort. Characteristics of patients from both cohorts are shown in Table E1 in this article's Online Repository at www.jacionline.org. More detailed clinical characteristics, as well as the inclusion and exclusion criteria for both studies, are described elsewhere[5,8] and in Appendix E1 in this article's Online Repository at www.jacionline.org. The studies were approved by the Mount Sinai Institutional Review Board, and informed consent was obtained before enrollment.

Tolerance to baked milk was determined by means of OFC, and subjects were categorized as baked milk reactive (group A), tolerant to baked milk only (group B), and tolerant to both baked and regular milk (ie, no longer milk allergic; group C). At baseline, serum samples were collected from subjects to measure CM-, casein-, and β-lactoglobulin–specific IgE, as well as casein- and β-lactoglobulin–specific IgG_4 antibody concentrations with the UniCAP system (ThermoFisher Scientific, Phadia US, Portage, Mich).

Statistical analyses and comparisons of data between the baked milk–reactive (group A) and baked milk–tolerant (groups B and C) patients were performed with the Mann-Whitney *U* test. A probability value of less than 5% was considered significant. *P* values were adjusted for multiple testing. Receiver operating characteristic curves were generated for specific IgE to CM, casein, and β-lactoglobulin. The areas under the curves (AUCs) were estimated and compared by using PROC LOGISTIC in SAS (SAS Institute, Cary, NC).

In both cohorts of patients, the levels of specific IgE to CM, casein, and β-lactoglobulin were significantly higher in baked milk–reactive patients (group A) compared with those seen in baked milk–tolerant subjects (groups B and C, $P < .05$, see Table E1). As previously reported for the first cohort,[5] casein- and β-lactoglobulin–specific IgG_4 levels did not differ significantly between subjects reactive and tolerant to baked milk. In the second cohort only casein-specific IgG_4 levels were greater in reactive subjects, with borderline significance ($P = .045$). Casein- and β-lactoglobulin–specific IgE/IgG_4 ratios were significantly higher in baked milk–reactive subjects in comparison with those seen in baked milk–tolerant subjects in both cohorts ($P < .05$). However, the IgE/IgG_4 ratio seems to be driven mostly by levels of specific IgE itself based on analysis of covariance using ranks.

Regarding the performance of tests evaluated in the combined cohort of patients (n = 225), casein-specific IgE measurement had a significantly greater accuracy for predicting baked milk reactivity compared with measurement of specific IgE to CM ($P = .01$) and β-lactoglobulin ($P = .02$; Fig 1, *A*). All patients with undetectable levels of specific IgE to casein (n = 25 [11.1%]) tolerated baked milk. Various cutoff levels of specific IgE to CM, casein, and β-lactoglobulin have been analyzed (Table I). We used specific IgE levels representing the 95% specificity of the tests as the positive decision points and levels representing the 95% sensitivity as the negative decision points.[9] We chose these values because they are not influenced by the prevalence of the disease. The positive decision point for reactivity to baked milk was 20.2 kU_A/L for casein-specific IgE (UniCAP) based on the combined cohort. In practice, this means that patients with casein-specific IgE antibody levels of greater than this value are unlikely to pass a baked milk challenge, and baked milk products should be avoided. In contrast, a concentration of less than approximately 0.94 kU_A/L (negative decision point) indicates a very low risk of reacting to baked milk, even though the patient might very well react to regular milk. When giving equal weight to sensitivity and specificity, the optimal cutoff point was 4.95 kU_A/L.

In addition, we analyzed both cohorts separately (see Table E2 in this article's Online Repository at www.jacionline.org). Again, the AUCs for specific IgE levels to casein were greater than the AUCs for specific IgE levels to CM, although the difference was not statistically significant in the first cohort of patients (Fig 1, *B*). Moreover, we found very similar values for the positive and negative decision points (ie, 21.4 and 1.0 kU_A/L in the first cohort and 20.2 and 0.7 kU_A/L in the second cohort, respectively; see Table E2). On the basis of cross-validation analysis, similar results for AUCs were obtained in both cohorts of patients, which suggests adequate reliability of these cutoffs. The distribution of casein-specific IgE is shown in Fig E1 in this article's Online Repository at www.jacionline.org.

On the basis of the largest cohort of children with baked milk allergy to date, we conclude that quantitative measurements of casein-specific IgE antibodies using UniCAP are useful in the

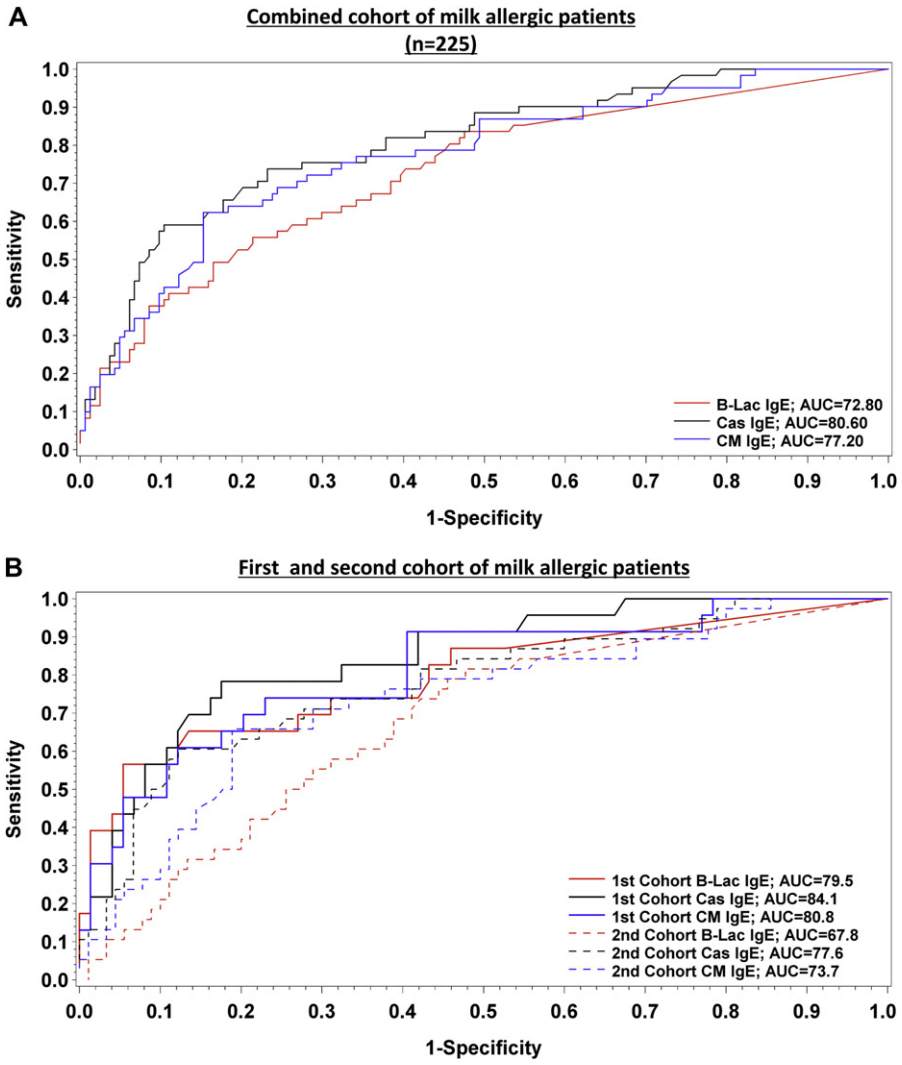

FIG 1. Full receiver operating characteristic curves for the 3 predictors tested (CM-, casein *[Cas]*-, and β-lactoglobulin *[B-Lac]*–specific IgE levels) in the combined cohort of patients with milk allergy (n = 225; **A**), as well as in each cohort **(B)**.

management of CM allergy. Casein is a major allergen in CM and has been previously identified as the best performing diagnostic component in the diagnosis of CM allergy.[7] This is likely due to the fact that casein retains its allergenicity after extensive heating, as opposed to whey proteins, which have low heat stability.[10] Combined with clinical history and the expertise of the physician, the use of cutoff decision points for specific IgE levels to casein could identify the optimal candidates for baked milk OFCs and improve the management of children with suspected CM allergy (Table I). The choice of a cutoff that a physician wishes to use will be based on individual risk assessment, which can vary according to circumstances under which the challenges are offered. For example, a casein-specific IgE cutoff of 0.94 kU_A/L (negative decision point) had a negative predictive value of 96% but a specificity of 32%. Of note, this testing should not replace OFCs because some patients might still react to baked milk. On the other hand, using a cutoff of 20.2 kU_A/L (positive decision point) would decrease the negative predictive value to approximately 78%, a number that might still be acceptable to some clinicians; however, the specificity would increase to 95%, thus capturing most children tolerant to baked milk. In addition to enhancing quality of life by removing unnecessary dietary restrictions, consumption of baked milk could change the natural evolution of milk allergy by promoting the development of tolerance to regular CM.[6] Although promising, our results should be validated in other populations and age groups before applying them in clinical practice.

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TABLE I. Specificity, sensitivity, and predictive values for various serum casein- and CM-specific IgE levels in the combined cohort of patients with milk allergy (n = 225)

Casein-specific IgE (kU_A/L)	Sensitivity	Specificity	Negative predictive value	Positive predictive value
0.94*	95%	32%	96%	34%
4.95†	74%	77%	89%	54%
20.2‡	30%	95%	78%	69%

CM-specific IgE (kU_A/L)	Sensitivity	Specificity	Negative predictive value	Positive predictive value
1.21*	95%	27%	94%	33%
9.97†	62%	85%	86%	60%
24.5‡	30%	95%	78%	69%

*The negative decision point is defined as the cutoff level producing a sensitivity of 95%.
†Optimal cutoff point, whereby equal weight is given to sensitivity and specificity.
‡The positive decision point is defined as the cutoff level producing a specificity of 95%.

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APPENDIX E1

The eligibility criteria included patients between the ages 0.5 and 21 years, a positive SPT response or detectable serum milk-specific IgE level, and a history of an allergic reaction to milk within 6 months before study entry or milk-specific IgE levels or SPT responses that were highly predictive of clinical reactivity (if 2 years old, >5 kU_A/L; if >2 years old, >15 kU_A/L; SPT wheal diameter, 8 mm). Exclusion criteria included a negative SPT response and an undetectable milk-specific IgE level; unstable asthma, allergic rhinitis, or atopic dermatitis; milk-induced eosinophilic gastroenteropathy; a recent reaction to a heated milk product; or pregnancy.

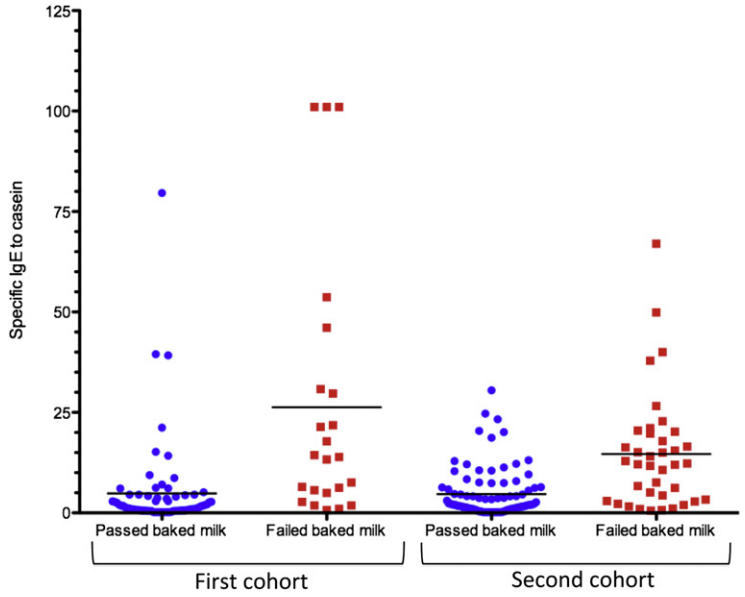

FIG E1. Full distribution of specific IgE levels to casein in both cohorts of patients with milk allergy.

TABLE E1. Study participants' characteristics

Outcome of the oral challenge	First cohort				Second cohort			
	Baked milk reactive (group A)	Baked milk tolerant* (group B)	Outgrown milk† (group C)	*P* value‡	Baked milk reactive (group A)	Baked milk tolerant* (group B)	Outgrown milk† (group C)	*P* value‡
No. of patients (%)	23 (23.7)	66 (68.0)	8 (8.3)		38 (29.7)	83 (64.8)	7 (5.5)	
Age (y)								
Median	7.3	6.5	5.8	.55	8.0	7.5	5.9	.1
Range	3.4-14.9	2.1-17.3	2.6-15.0		4.4-10.9	4.0-11.0	4.1-10.9	
Sex, no. (%)								
Male	14 (60.9)	43 (65.2)	4 (50)	.83	27 (71.1)	59 (71.1)	4 (57.1)	.74
Female	9 (39.1)	23 (34.8)	4 (50)		11 (28.9)	24 (28.9)	3 (42.9)	
Specific IgE (kU_A/L)								
CM								
Median	11.6	2.5	0.9	.00	11.9	4.0	0.7	.00
Range	0.7-101	0.2-79.1	0.2-6.1		0.8-50.5	0.2-42.3	0.2-4.8	
Casein								
Median	13.9	1.4	1.5	.00	12.2	2.3	0.4	.00
Range	0.7-101	0.2-79.6	0.5-3.7		0.5-67.0	0.2-30.5	0.2-2.0	
β-lactoglobulin								
Median	4.6	0.5	0.2	.00	2.0	0.6	0.2	.00
Range	0.2-101	0.2-13.8	0.2-2.3		0.2-15.4	0.2-19.8	0.2-7.8	
Specific IgG_4 (mg_A/L)								
Casein								
Median	1.5	0.6	1.4	.11	1.2	0.8	0.3	.05
Range	0.0-6.7	0.0-23.8	0.2-31		0.1-10.3	0.0-13.1	0.1-1.9	
β-lactoglobulin								
Median	0.6	0.4	1.3	.15	0.4	0.4	0.1	.72
Range	0.0-8.4	0.0-31	0.1-31		0.0-2.3	0.0-3.9	0.0-2.4	
IgE/IgG_4 ratio								
Casein								
Median	10.6	2.5	1.34	.00	6.0	3.0	1.6	.00
Range	1.9-69.3	0-868	0.1-3.7		0.2-109.8	0.1-63.7	0.1-5.0	
β-lactoglobulin								
Median	6.3	2.4	0.1	.02	3.9	1.9	3.5	.01
Range	0.11-54.7	0-238	0.1-2.4		0.2-81.1	0.1-80.5	0.1-13.7	

*Includes patients tolerating baked milk but reacting to regular milk or not challenged to regular milk.

†Includes children with a diagnosis of milk allergy who were asymptomatic during baked milk and regular milk challenges.

‡Comparison between patients reacting to baked milk and patients tolerating baked milk (group A vs groups B and C).

TABLE E2. Characteristics of diagnostic tests for determination of clinical reactivity to baked milk

	First cohort (n = 97)			Second cohort (n = 128)		
Diagnostic test	**Specific IgE to CM**	**Specific IgE to casein**	**Specific IgE to β-lactoglobulin**	**Specific IgE to CM**	**Specific IgE to casein**	**Specific IgE to β-lactoglobulin**
AUCs	80.8%	84.1%	79.5%	73.7%	77.6%	67.8%
Optimal cutoff point*	4.7	4.9	2.4	10.1	10.7	0.5
Sensitivity, specificity	73.9%, 77.0%	78.3%, 82.4%	65.2%, 86.5%	65.8%, 81.1%	60.5%, 87.8%	81.6%, 52.2%
PPV, NPV	50.0%, 90.5%	58.1%, 92.4%	60.0%, 88.9%	59.5%, 84.9%	67.7%, 84.0%	41.9%, 87.0%
Positive decision point†	28.4	21.4	6.1	25.3	20.2	7.9
Sensitivity, specificity	34.8%, 96.0%	39.1%, 96.0%	43.5%, 96.0%	21.1%, 95.6%	23.7%, 95.6%	10.5%, 95.6%
PPV, NPV	72.7%, 82.6%	75.0%, 83.5%	76.9%, 84.5%	66.7%, 74.1%	69.2%, 74.8%	50.0%, 71.7%
Negative decision point‡	0.8	1.0	ND	1.21	0.7	ND
Sensitivity, specificity	95.7%, 23.0%	95.7%, 44.6%		97.4%, 20.0%	97.4%, 21.1%	
PPV, NPV	27.9%, 94.4%	34.9%, 97.1%		33.9%, 94.7%	34.3%, 95.0%	

ND, Not determined because the highest sensitivity is less than 95%; *NPV*, negative predictive value; *PPV*, positive predictive value.

*Defined as the shortest distance to the receiver operating characteristic.

†Defined as the cutoff level producing a specificity of 95%.

‡Defined as the cutoff level producing a sensitivity of 95%.