

Predicting food challenge outcomes for baked milk: role of specific IgE and skin prick testing

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ABSTRACT

Background: Cow's milk allergy is the most common food allergy in childhood. Many children with IgE-mediated cow's milk allergy may tolerate baked milk products, but few data exist on predictors of outcomes of baked milk challenges.

Objective: To determine the relation of milk protein allergen specific IgE (sIgE) levels and skin prick test (SPT) wheal size with baked milk challenge outcomes.

Methods: A retrospective medical record review was conducted of 35 baked milk challenges. SPT results, sIgE levels, demographic characteristics, and food challenge results were analyzed.

Results: Thirty-five children underwent open challenges to baked milk and 29 (83%) passed. Of those who failed, 3 (50%) passed the initial clinic challenge but developed symptoms to ongoing exposure at home, days to months later. One child who ultimately failed at home required epinephrine. Compared with those who passed, children who failed were younger (median age, 8.9 and 3.7 years, respectively; $P = .02$). Children with a milk SPT wheal less than 12 mm were more than 90% likely to pass a baked milk challenge, and no child with a milk SPT wheal less than 7 mm failed a baked milk challenge. We were also able to establish more than 90% predictive values for passing baked milk challenges with a casein SPT wheal of 9 mm, a milk sIgE level of 1.0 kU/L, and a casein sIgE level of 0.9 kU/L.

Conclusion: Most children allergic to cow's milk tolerated baked milk. Milk protein SPT wheal may be more reliable than sIgE level in predicting outcomes of baked milk challenges. It is important to be aware of the possibility of late reactions to ongoing baked milk exposure.

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Introduction

Cow's milk allergy is the most common childhood food allergy, affecting 2% to 3% of children.^{1,2} Although most children will develop tolerance to cow's milk, recent studies demonstrate that children are outgrowing their cow's milk allergy at a later age than previously reported.^{2,3} With food allergies on the rise⁴ and children outgrowing cow's milk allergies later in life,^{2,3} there is an increasing population of patients avoiding cow's milk. Recent evidence suggests that 70% to 80% of children with IgE-

mediated cow's milk allergy will tolerate baked milk products.⁵ Furthermore, children with milk allergy who tolerate baked milk products may outgrow their milk allergies faster than those who cannot tolerate baked milk,¹ possibly via the induction of milk-specific regulatory T cells.⁶

Cow's milk proteins can be divided into 2 broad groups based on their solubility at pH 4.6: whey and casein.⁷ Whey proteins, which account for 20% of the total milk protein, include α -lactalbumin and β -lactoglobulin. Caseins, which consist of α_{s1} -, α_{s2} -, β -, and κ -caseins, comprise the remaining 80% of milk proteins. Caseins and α -lactalbumin are more heat stable than β -lactoglobulin.⁸ Exposing milk proteins to high temperatures through baking reduces allergenicity by destroying the conformational epitopes.^{9,10} Analysis of serum samples of children with persistent cow's milk allergy demonstrates a significantly higher ratio of specific IgE (sIgE) to linear vs conformational epitopes compared with children who have achieved tolerance.¹¹ In addition to heating, milk protein allergenicity may also be decreased through interaction with other

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food proteins in a complex food, such as in a wheat matrix in a baked muffin or cupcake.¹²

Strict avoidance of cow's milk results in significant stress for patients and families.¹³ Identifying milk allergic children who might tolerate baked milk products would not only increase dietary choices and potential for maintaining adequate nutrition and decrease stress but may also hasten resolution of milk allergy.¹ Few studies have evaluated predictive factors for passing food challenges to baked milk. In this study, we sought to determine whether skin prick test (SPT) wheal size and/or serum sIgE levels to milk proteins could be used to predict successfully passing a baked milk challenge.

Methods

Study design

A retrospective medical record review was performed of all patients who underwent oral challenges to baked milk at Boston Children's Hospital from September 2009 through September 2011. All patients had a history of prior allergic reactions to milk (either baked or unheated) documented in the medical record by an allergist and detectable milk protein sIgE, as determined by a positive SPT result or elevated serum sIgE level. The study was approved by the institutional review board of Boston Children's Hospital.

Allergy evaluation

SPTs were performed according to previously published methods¹⁴ using the Multi-Test II device (Alk-Abello, Round Rock, Texas) and commercially prepared extracts (Greer Laboratories, Lenoir, North Carolina). Control tests for SPTs were performed with histamine (positive control) and normal saline (negative control). Wheal diameters were measured 15 minutes after the skin test was placed, in a standard fashion.¹⁴ A SPT wheal diameter at least 3 mm larger than the negative control was considered a positive result.¹⁵

All children underwent SPTs to milk and 34 (97.1%) underwent SPTs to casein documented in the medical record before oral food challenge (median time before challenge, 3.0 months; range, 0.82–32.0 months). All children underwent serum sIgE testing to milk and 33 (94.3%) also underwent sIgE to casein, α -lactalbumin, and/or β -lactoglobulin before oral food challenge (median time before challenge, 3.1 months; range, 1.15–34.6 months). All patients underwent either SPTs or sIgE measurement within 1 year of the food challenge, and 29 (83%) underwent both within 1 year of the food challenge. Serum samples were analyzed for sIgE using an ImmunoCAP fluorescence enzyme immunoassay (Phadia AB, Portage, Michigan). The lowest limit of detection of the assay was 0.35 kU/L. For analysis, we evaluated the most recent SPT and sIgE test documented before the food challenge.

Oral challenge

Baked milk challenges were performed as open challenges under physician supervision at Boston Children's Hospital, in a standard manner based on previously published methods.⁸ Parents were instructed to prepare muffins or cupcakes at home according to a specific protocol that our clinic provided. Each muffin or cupcake contained 1.3 g of milk protein from nonfat dry milk powder. The muffins or cupcakes were baked at 350°F in an oven for 30 minutes. A standard graded open food challenge consisted of increasing increments every 15 minutes of one-fourth (325 mg), half (650 mg), and 1 1/4 (1,625 mg) muffin or cupcake, totaling 2.6 g of milk protein. Patients were monitored throughout and for 30 to 60 minutes after the completion of the challenge. Challenges were discontinued at the first objective sign of reaction,¹⁵ and treatment was initiated at the discretion of the supervising physician.

Table 1
Characteristics of the study population^a

Characteristic	Passed baked milk challenge (n = 29)	Failed baked milk challenge (n = 6)	P value
Age, median (range), y	8.9 (3.9–18.1)	3.7 (3.1–11.0)	.02
Male, No. (%)	20 (69.0)	5 (83.3)	.65
Other atopic conditions, No. (%)			
Asthma	16 (55.2)	2 (33.3)	.40
Atopic dermatitis	15 (51.7)	6 (100)	.06
ARC	15 (51.7)	4 (66.7)	.68
Other food allergies	24 (82.8)	6 (100)	.56

Abbreviations: ARC, allergic rhinoconjunctivitis.

^aStatistical significance for age was calculated using the Wilcoxon rank sum test. Statistical significance for the remaining parameters was calculated using the Fisher exact test. $P < .05$ was considered statistically significant.

Statistical analysis

Median values were calculated for patient ages at the time of oral challenge and for milk protein sIgE and SPT wheal sizes. Prevalence rates of baseline characteristics were calculated, and differences were compared between those who passed or failed baked milk challenges. The Wilcoxon rank sum test was used to compare baseline characteristics between individuals who passed or failed baked milk challenges, including age, serum sIgE, and SPT wheal sizes. Dichotomous variables were analyzed using the Fisher exact test. $P < .05$ was considered statistically significant.

Oral food challenge outcome was used as the criterion standard by which performance characteristics (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) were calculated. The PPV refers to a level above which it is a given percentage likely that a patient will react and therefore have a failed food challenge outcome. The NPV refers to a level below which it is a given percentage likely that a patient will not react and therefore have a passed food challenge outcome.¹⁶ Receiver operator characteristic (ROC) curve analysis was used to determine a threshold that would differentiate patients who were allergic or tolerant to baked milk. The relationship between SPT or sIgE and food challenge outcome was analyzed using logistic regression. Fitted predicted probability curves were plotted using results from the logistic regression.

Results

Study population

Thirty-five children (median age, 8.1 years; range, 3.1–18.1 years) underwent food challenges to baked milk (Table 1). Twenty-nine children (83%) passed a baked milk challenge and 6 children (17%) failed. This pass rate is consistent with what is reported in the literature.^{5,8} Compared with those who passed, children who failed the baked milk challenges were younger (median age, 8.9 and 3.7 years, respectively; $P = .02$). Sex was not a factor in passing a baked milk challenge. Atopic conditions, including diagnoses of asthma, allergic rhinoconjunctivitis, and other food allergies, were not associated with higher risk of failing a baked milk challenge. Children with atopic dermatitis were more likely to fail a baked milk challenge, although this finding did not reach statistical significance ($P = .06$). Symptoms at first reported milk reaction did not differ among the groups (Table 2).

Oral food challenge failures

Children failed baked milk challenges for a variety of reasons (Table 3). No child who failed the challenge in our clinic developed anaphylaxis or required epinephrine. Patients 1 through 3 reacted to 2.6 g, 325 mg, and 1.7 g of milk protein, respectively. Interestingly, 3 children (50%) who reacted to baked milk (patients 4–6) passed the initial

Table 2
Symptoms at first reported milk reaction^a

Symptom	No. (%) of patients	
	Passed baked milk	Failed baked milk
Atopic dermatitis	7 (24.1)	2 (33.3)
Cutaneous	17 (58.6)	4 (66.7)
Gastrointestinal	10 (34.5)	1 (16.7)
Upper airway	3 (10.3)	1 (16.7)
Lower airway	3 (10.3)	1 (16.7)
Cardiovascular	1 (3.4)	0
Anaphylaxis	9 (31.0)	2 (33.3)

^aAtopic dermatitis: either in relation to milk ingestion or milk allergy evaluated and milk exclusion recommended in the setting of atopic dermatitis. Cutaneous: hives, angioedema, rash, or pruritus. Gastrointestinal: abdominal pain, vomiting, or diarrhea. Upper airway: rhinoconjunctivitis, oral pruritus, tongue swelling, or stridor. Lower airway: wheezing or coughing. Cardiovascular: hypotension or lethargy. Anaphylaxis: defined by the clinical criteria from the Second Symposium on Anaphylaxis.¹⁷

physician-supervised challenge but reacted to ongoing baked milk ingestion at home. One child who reacted at home required epinephrine (patient 4). None of the children who failed at home returned to our clinic to undergo repeat baked milk challenges. The clinical courses of the patients with delayed reactions are subsequently described. Because these reactions occurred at home, details regarding the amount of milk protein ingested, degree of baking, and frequency of baked milk consumption are limited.

Ten months after passing the initial clinic challenge, patient 4 reacted to cake containing yogurt baked at 350°F for 30 to 35 minutes. Patient 4 immediately developed anaphylaxis with hives, lip swelling, and vomiting and was treated with epinephrine.

Patient 5 experienced oral pruritus during the clinic challenge with a baked milk cupcake, but the symptoms resolved and the patient ultimately passed the challenge. Several days later, patient 5 refused to eat a baked milk cupcake made from the same recipe. However, while consuming a brownie that contained baked milk, the patient again experienced oral pruritus.

Patient 6 passed the initial clinic challenge to a baked milk muffin and continued consuming baked milk daily at home. However, 3 weeks later, patient 6 was given a muffin made from the same recipe, but instead of plain muffin mix it was made with the same brand's blueberry muffin mix. The patient did not have a documented blueberry allergy and had consumed blueberry multiple times previously without reactions. While eating the muffin, this patient immediately developed hives.

Predictive value of allergy testing for the outcome of baked milk challenge

No significant difference was found in milk protein skin or blood test results among the groups who passed or failed baked milk challenges (Table 4). All patients had either SPT or sIgE measurement performed within 1 year of the food challenge and most within 3 months of the food challenge.

Casein SPT had 66.7% sensitivity, 85.7% specificity, and a 92.3% NPV using a cutoff of 9 mm. In other words, if a patient has a casein SPT wheal less than 9 mm, it is 92.3% likely that the patient will not react and will pass a baked milk challenge. No child with a casein wheal greater than 15 mm passed the food challenge (Table 4 and Supplemental Fig 1A and B). Milk SPT had 66.7% sensitivity, 72.4% specificity, and a 91.3% NPV using a cutoff of 13 mm (Fig 1). A greater than 90% NPV was also achieved for milk SPT using a cutoff of 17 mm; however, at that level sensitivity decreased and PPV increased to 75%. Notably, all patients with a milk SPT wheal less than 7 mm passed the challenge. No child with a casein SPT wheal greater than 15 mm, a casein sIgE level greater than 10.3 kU/L, or a milk sIgE level greater than 20.6 kU/L passed the challenge. The ROC curve analysis for casein and milk SPT revealed an area under the curve of 0.64 and 0.70, respectively.

Sensitivity, specificity, and PPV for α -lactalbumin and β -lactoglobulin were poor. A greater than 90% NPV was established for α -lactalbumin using a cutoff of 0.35 kU/L. A β -lactoglobulin sIgE level of 0.35 kU/L had an 84.2% NPV, and a greater than 90% NPV could not be established. Casein sIgE had poor sensitivity and specificity in predicting oral food challenge outcomes, and a greater than 90% NPV was established using a cutoff of 0.9 kU/L (Supplemental Fig 1C and D). Milk sIgE also had poor sensitivity and specificity, and a greater than 90% NPV was established using a cutoff of 1.0 kU/L (Supplemental Fig 1E and F). The ROC curve analysis for casein and milk sIgE revealed an area under the curve of 0.56 and 0.60, respectively.

Discussion

Cow's milk allergy is the most common food allergy among children.¹ However, 70% to 80% of children with cow's milk allergy may tolerate milk in well-baked goods, and these children may outgrow their milk allergies faster than those who exclude milk completely.⁵ For these reasons, it is critically important to identify milk allergic children who may be able to consume baked milk products. To date, reliable markers for selecting participants for

Table 3
Failed baked milk challenge data

Patient No.	SPT wheal, mm		Serum antigen specific IgE, kU/L				Prior reaction	Reason for failure	Treatment
	Casein	Milk	α -Lactalbumin	β -Lactoglobulin	Casein	Milk			
1	22	20	0.36	<0.35	1.56	2.63	Hives, periorbital swelling	Rhinorrhea, hives	Diphenhydramine
2	0	7	<0.35	<0.35	<0.35	<0.35	Atopic dermatitis	Hives	Diphenhydramine
3	9	7	2.15	<0.35	1.11	2.15	Atopic dermatitis	Tongue itching, cough	None
4	9	13	0.40	0.91	0.91	1.16	Hives	Passed clinic challenge; 10 months later, hives, lip swelling, and vomiting with cake baked with yogurt	Epinephrine, diphenhydramine, steroids
5	20	20	4.67	2.07	31.5	31.0	Hives, vomiting	Oral pruritus but symptoms resolved and passed clinic challenge; several days later, oral pruritus with baked milk brownie	None
6	0	17	0.82	1.66	1.03	2.80	Cough, tongue swelling, hives	Passed clinic challenge; 3 weeks later, hives with baked milk muffin	Diphenhydramine

Abbreviation: SPT, skin prick test.

Table 4
Immunologic responses to milk proteins^a

Response	Passed baked milk challenge	Failed baked milk challenge	P value
Casein SPT wheal, median (range), mm	5 (0–15)	9 (0–22)	.26
Milk SPT wheal, median (range), mm	10 (0–20)	15 (7–20)	.12
α -Lactalbumin sIgE, median (range), kU/L	0.73 (<0.35–8.63)	0.61 (<0.35–4.67)	.50
β -Lactoglobulin sIgE, median (range), kU/L	<0.35 (<0.35–15.4)	0.63 (<0.35–2.07)	.62
Casein sIgE, median (range), kU/L	1.05 (<0.35–10.3)	1.07 (<0.35–31.5)	.69
Milk sIgE, median (range), kU/L	1.93 (<0.35–20.6)	2.39 (<0.35–31.0)	.50

Abbreviations: sIgE, specific IgE; SPT, skin prick test.

^aStatistical significance was calculated using the Wilcoxon rank sum test. $P < .05$ was considered statistically significant.

baked milk challenge have not been clearly established. Therefore, oral food challenge to baked milk remains the criterion standard. The aim of this study was to determine whether milk protein sIgE levels or SPT wheal sizes were predictors of passing a baked milk challenge. We addressed this question by examining these parameters in a retrospective review of patients who underwent baked milk challenges in our allergy clinics. Our study is one of the largest evaluating baked milk challenges. Furthermore, this study reports on the largest number of milk proteins and is the first study evaluating the relationship of casein SPT and α -lactalbumin sIgE, the heat stable milk proteins,⁸ with baked milk challenge outcome.

We found that 83% of milk allergic children in our study population passed baked milk food challenges, which is consistent with the rate reported in the literature.⁸ Younger children were more likely to fail a baked milk challenge, which may reflect the natural history of baked milk allergy.²

We found that α -lactalbumin and β -lactoglobulin sIgE levels were poor predictors of baked milk challenge outcome. Casein and milk sIgE performed slightly better, with greater than 90% NPVs estab-

lished at decision points of 0.9 and 1.0 kU/L, respectively. However, the milk sIgE decision point is unlikely to be of additional clinical utility because it is the same decision point reported by Sampson and Ho¹⁶ as the greater than 90% NPV in differentiating unheated milk allergic from tolerant individuals. One patient who reacted to baked milk in our study had a milk sIgE level less than 0.35 kU/L. This is in contrast to the results of Nowak-Węgrzyn et al,⁸ who reported that no patient with a milk sIgE level less than 0.35 kU/L reacted to baked milk. Casein SPT had a greater than 90% NPV at a decision point of 9 mm. However, 2 patients failed baked milk challenges with negative casein SPT results, suggesting that milk proteins other than casein are important in determining tolerance to baked milk. No patient with a milk SPT wheal less than 7 mm failed the baked milk challenge in our study. This finding is similar to the results of the study by Nowak-Węgrzyn et al,⁸ who found that no patients with a milk SPT wheal less than 5 mm reacted to baked milk.

Three of the patients who reacted to baked milk in our study passed the initial physician-supervised clinic challenges but reacted to ongoing exposure at home. Reactions to ongoing exposure at home have been reported by other groups. In one recent study,⁸

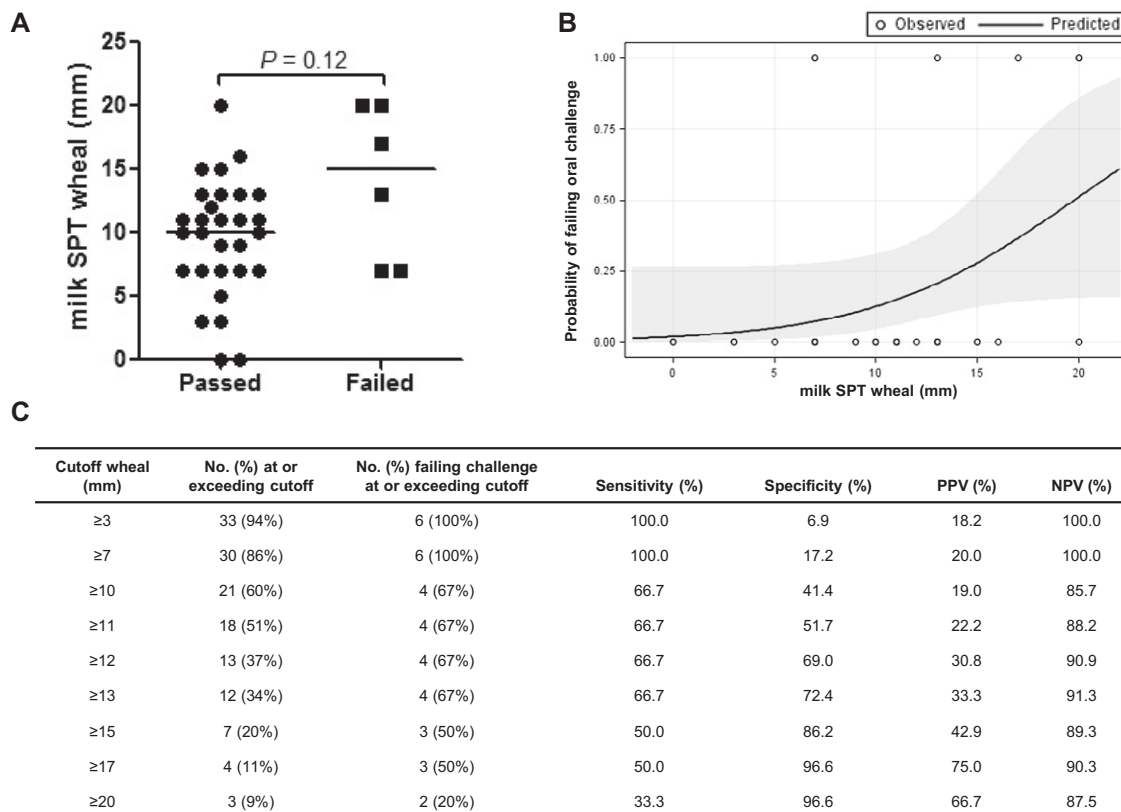


Figure 1. Performance characteristics of milk skin prick test (SPT). **A**, Milk SPT wheal sizes grouped by baked milk challenge outcome. Each data point represents an individual patient and medians are indicated by horizontal lines ($N = 35$). P value determined using the Wilcoxon rank sum test. **B**, Estimated probability curve for failing oral baked milk challenge at a given milk SPT wheal size derived from logistic regression. Open circles represent individual patients. Shaded regions indicate 95% confidence intervals. **C**, Performance characteristics of milk SPT wheal size at various cutoff values.

1 of the 68 patients who passed the supervised baked milk challenge developed oral pruritus with homemade bread and Belgian waffles at home. The patient tolerated a repeat supervised baked milk challenge without symptoms. It was thus determined that the homemade products were not sufficiently baked. In another study,¹ 1 of the 65 patients who passed the supervised baked milk challenge reacted to a waffle that the authors deemed “unintentionally undercooked.” However, the family declined to undergo a repeat baked milk challenge to confirm nonreactivity. Interestingly, this phenomenon of late reactions has not been reported in patients who underwent challenges to baked egg.^{18,19}

Among the patients in our study who reacted to ongoing baked milk exposure at home, it is possible that they also reacted to unintentionally undercooked products. A key intervention is to stress to families the importance of adequately cooking the baked milk products so that no residual unheated protein remains, which could potentially trigger an allergic reaction. It is also possible that the milk protein quantity used during the food challenge was not large enough to confirm tolerance and larger amounts of protein may have been ingested in the products prepared at home. The source of milk protein used in baking may also be important. In the case of patient 4, it is possible that using yogurt as a milk source, rather than nonfat dry milk powder, may have caused the severe reaction that required epinephrine. Different forms of milk (eg, nonfat dry milk powder, liquid milk, yogurt) may contain different conformational epitopes, some of which may be more resistant to heat denaturation and therefore more likely to trigger an allergic reaction. These possibilities will be important to address in future studies.

Several limitations exist in our study. Children were identified as milk allergic based on history and suggestive laboratory studies and not confirmed by failed unheated milk food challenges. Therefore, it is possible that some of the patients who passed the baked milk challenges may be tolerant to unheated milk as well. Although most patients had both sIgE measurement and SPTs performed within 1 year of the baked milk challenge, and most within 3 months, it is possible that obtaining sIgE and SPT results closer to the time of challenge could improve reliability. Among patients who failed baked milk challenges at home, it would have been beneficial to repeat a physician-supervised baked milk challenge to determine whether they reacted because the milk was inadequately baked or because they were indeed intolerant to baked milk. Given the retrospective nature of this study, we did not have longitudinal data on patients after passing the baked milk challenge, subsequent intake of baked milk at home, or progression to tolerance of unheated milk products. A future, longitudinal, prospective study may further our understanding of such patients. Furthermore, we acknowledge that the predictability of outcomes for sIgE and SPT may be enhanced by a larger sample size, and future larger studies are needed to confirm these findings.

Despite these limitations, our study is important because it is one of the few studies evaluating predictors of baked milk challenge outcomes and reports on the largest number of milk proteins in association with baked milk challenges to date. Our study provides practical, real-world implications and evidence that patients who pass these challenges may tolerate products with baked milk that are ubiquitous in various foods, even if they may not tolerate unheated milk. Our food challenge protocol was conducted in a manner that other practicing allergists may adapt into their practices. Our study also raises the important point that passing a baked milk challenge may not guarantee prolonged tolerance to baked milk products, possibly due to inconsistencies in baking of milk products at home, which is a key consideration when this type of protocol is to be continued in a less highly controlled home setting.

In summary, we defined novel decision points based on serum sIgE and SPT results that may be useful in predicting outcomes to baked milk challenges. We found that milk SPT wheal size was a better marker for food challenge outcome to baked milk compared with

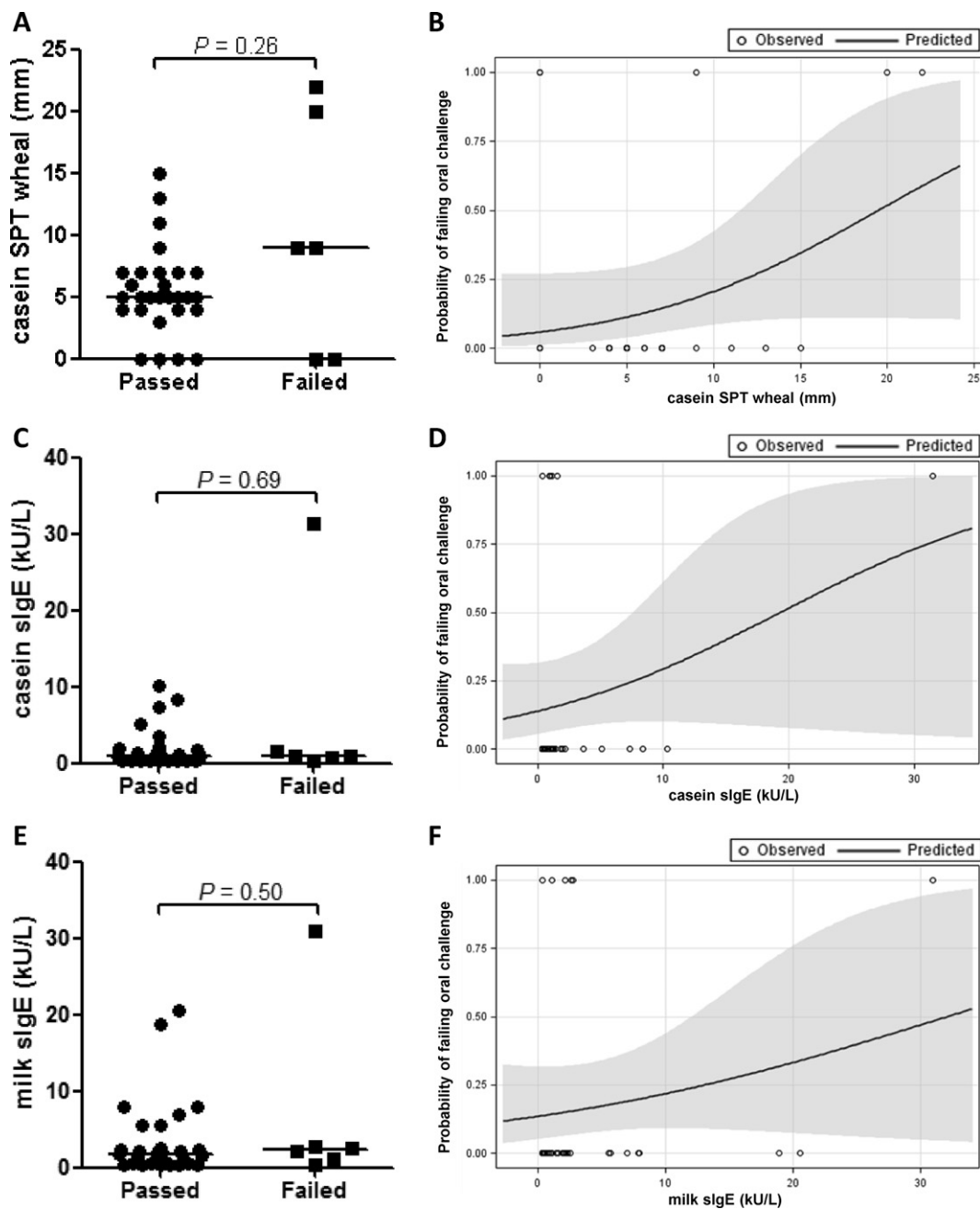
casein SPT and milk protein sIgE levels. We identified more than 90% NPVs for baked milk challenge outcomes for milk and casein SPT wheal sizes and sIgE levels. Furthermore, a milk SPT wheal size less than 7 mm was predictive of passing the baked milk challenge in 100% of cases. On the basis of our findings, we propose using a milk SPT wheal of 12 mm or less to identify those who would pass a baked milk challenge (approximately 90% rate of passing baked milk challenge). Furthermore, a milk SPT wheal less than 7 mm could identify patients who may be candidates for home introduction of baked milk (100% rate of passing baked milk challenge). We identified SPT and sIgE levels above which no patient passed a challenge, but we recognize that our sample size is small, and because most milk allergic children tolerate baked milk, perhaps higher cutoffs will be identified once more patients are challenged. Future studies will be needed to validate our findings in a larger patient population. Our study also demonstrates the need to caution families and health care workers of the possibility of late reactions to ongoing baked milk exposure and to monitor patients over time to verify that clinical tolerance to baked milk is maintained, with hopeful progression toward tolerance of all forms of cow's milk.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at [doi.10.1016/j.anai.2012.07.026](https://doi.org/10.1016/j.anai.2012.07.026).

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eFigure 1. Performance characteristics of milk protein skin prick test (SPT) and specific IgE (sIgE) results. A, C, and E, Milk protein SPT and sIgE levels grouped by baked milk challenge outcome. Each data point represents an individual patient, and medians are indicated by horizontal lines. P value determined using the Wilcoxon rank sum test. $N = 34$ for casein SPT, $n = 33$ for casein sIgE, and $n = 35$ for milk sIgE. B, D, and F, Estimated probability curves for failing baked milk challenges at a given milk SPT or sIgE level, derived from logistic regression. Open circles represent individual patients. Shaded regions indicate 95% confidence intervals.