

# Baked Tolerance in Cow's Milk Allergy: Quite Frequent, Hard to Predict!

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## Keywords

Food allergy · Milk allergy · Skin prick test · Oral food challenge · Casein

## Abstract

**Introduction:** Cow's milk protein allergy (CMA) is the most common type of food allergy in childhood and exclusion diet is a challenge for patients. **Objective:** The study aim was to investigate the frequency of tolerance to baked foods containing milk and evaluate immediate skin prick test (SPT) and specific IgEs for different cow's milk (CM) protein types as predictors of tolerance to baked foods containing milk for CMA patients. **Methods:** A cross-sectional study was performed. Fifty-four CMA patients were enrolled and oral food challenge (OFC) was performed with baked product, 6 different milk SPTs and specific IgEs to CM, casein,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin. **Results:** Thirty-nine (72.2%) patients tolerated OFC with baked milk cupcake. CM-specific IgE and casein SPT showed statistical difference between positive and negative OFC groups. Probability curves for baked milk tolerance were created for specific CM IgE ( $Z = 2.542$ ,  $p < 0.0110$ ) and casein SPT ( $Z = 2.290$ ,  $p < 0.0220$ ) using logistic regression. **Conclusions:** The high percentage of patients able to tolerate baked goods enables an improvement in in-

take possibilities and quality of life of CMA patients and families. Specific CM IgE and casein SPT demonstrated to be useful predictors in relation to baked milk tolerance.

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## Introduction

Cow's milk protein allergy (CMA) is the most common type of food allergy in childhood presenting a variable prevalence worldwide. Currently, the traditional therapeutic approach in food allergy is the total food diet exclusion, regardless of its presentation [1, 2]. The extensive use of cow's milk (CM) and its derivatives in the regular diet, especially in the Western countries, makes this approach a great challenge [3–5].

Infancy CM protein exclusion is commonly associated with inadequate food intake due to the costs of special formulas, leading a delay in the child growth and development. As CM is present in a great number of culinary recipes, its total exclusion makes the full socialization process for preschoolers and scholars difficult and is associated with a reduction in the quality of life of patients and their families [6, 7].

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CM has diverse proteins with different molecular structures and thermal and digestive properties, resulting in different impacts in the allergenic potential according to the preparations of recipes using CM [4, 6]. The CM protein exposed to high temperatures, especially in culinary recipes associated with a matrix (wheat, vegetable oil, and sugar), demonstrated the changes in its structure and reduction of the allergenic potential [5, 8–10]. In this context, baked foods containing CM protein could be an opportunity to increase options for CMA children; however, some patients remain reactive and could present anaphylactic reactions [8, 10, 11]. Therefore, introduction of baked recipes containing CM for allergic patients should be done under medical supervision by oral food challenge (OFC), a procedure not accessible in most of the medical units, leading to a limited use of baked goods for CMA patients. Studies comparing baked CM goods OFC and allergic tests could help the introduction, once allergy tests are largely accessible and relatively inexpensive, but these studies are scarce in the world literature [1, 10–12].

The study aim was to investigate the frequency of tolerance to baked foods containing milk in our population, as well as to evaluate immediate skin prick test (SPT) and specific IgE for different CM protein types as predictors of tolerance to baked foods containing milk.

## Methods

A cross-sectional study was performed from July 2014 to June 2017 at the Pediatric Allergy and Immunology Outpatient Clinic of Hospital das Clínicas, Universidade Federal de Uberlândia. The study inclusion criteria were children under the age of 18 years followed up in our unit presenting history of immediate allergic reaction after contact with milk protein in the last 6 months, allergic sensitization to milk protein, and under exclusion CM diet. These patients were invited to participate in the study; an interview was conducted, and a questionnaire containing clinical, nutritional, and laboratory data was applied; and subsequently, allergic tests and OFC were performed using baked milk. Of the 225 patients followed in the study period, 114 fulfilled the inclusion criteria and were invited to participate initially by an interview and questionnaires application.

### *SPT and Specific IgEs*

The SPTs were performed according to the guidelines of the European Academy of Allergy and Clinical Immunology (EAACI) using commercial extracts of casein,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin (IPI-ASAC, São Paulo, Brazil) and also with raw fresh CM, boiled CM (boiled for 15 min), and baked CM according to the OFC recipe. Punctures were performed on the inner side of the forearm, on the allergen microdroplets using appropriate lancets. The positive control was performed with histamine (IPI-ASAC Brazil, São Paulo, Brazil), and the negative control per-

formed with saline. The reading was performed with a ruler graduated in millimeters after 15 min, and a papule with an average diameter of  $>3$  mm compared with negative control was considered as a positive test, as previously described; however, all values were used for statistical analysis. Specific IgEs to milk, casein,  $\alpha$ -lactalbumin, and  $\beta$ -lactoglobulin were measured using ImmunoCap method (ImmunoCap; Thermo Fisher Scientific, Waltham, USA) in the hospital laboratory. Patients with specific CM IgE  $< 0.35$  kU/L and CM SPT  $< 3$  mm were excluded.

### *Oral Food Challenge*

Open OFC was performed under medical supervision according to the PRACTALL recommendations and Groetch and Nowak-Wegrzyn [9, 13]. Before the OFC, patients, parents, and/or legal guardian received instructions about the test and explanations about the medicine use including antihistamines, corticosteroids, adrenaline, and hospitalization, if necessary. During the OFC, patients received a small cupcake containing 1.3 g of milk protein and 12.5 g of fat and baked in a wheat matrix for 30 min at 180°C. The recipe was developed and prepared by the nutrition team and called “baked milk” (BM) using 2 cups of wheat flour, 1 cup of sugar, 5 tablespoons of canola oil, 4 tablespoons of powdered whole milk, 1 cup of hot water and 1 tablespoon of yeast for 12 units. The cupcake was offered in quarters every 20 min until the consumption of the total portion. The OFC was considered positive and interrupted if the child presented objective signs of an allergic reaction such as hives, angioedema, pruritus, wheezing, dyspnea, cough, vomiting, diarrhea, hypotension, or fainting, and drug treatment was started immediately [11]. Symptoms, period, and cupcake intake were recorded in case of reaction. Subjective symptoms were not considered as a real reaction and patients continued the intake and the observation was also continued. OFC was considered negative if, 2 h after the final ingestion, patients did not present any objective clinical manifestations. The cupcakes were weighed before OFC, and in case of reaction, the leftovers were weighed to estimate the amount of ingested milk protein. Family members and patients were instructed to introduce the baked good tested into the daily dietary routine.

### *Statistical Analysis*

The Smirnov-Kolmogorov test was used to calculate normality, and nominal variables were described as frequencies. Categorical variables were analyzed using the  $\chi^2$  test, and numerical variables using the Mann-Whitney test. The probability curves for specific IgE for LV and SPT for casein in relation to OFC were created and analyzed using logistic regression. The data were analyzed using the GraphPad Prism 8.3 program. We considered  $p < 0.05$  to be statistically significant.

## Results

One hundred and fourteen patients were initially enrolled in the study after medical record analyses and confirmation of meeting the inclusion criteria. Surprisingly, during the interviews for questionnaire application, 60 parents or legal guardian or patients themselves reported the sporadic or habitual consumption of baked foods containing CM, even though in their medical records, to-

tal CM exclusion diet was recommended. These patients were requested to continue the baked milk food intake daily, with no symptoms complaints recurrence in the subsequent follow-up.

Thus, 54 patients continued the other study stages including the performance of allergic tests and OFC. In this group, the patients' age ranged from 6 months to 15 years with a median of 3.14 years, with no difference between genders. Thirty-nine (72.2%) patients completed the cupcake OFC without reactions. Fifteen (27.8%) had objective reactions during OFC (60% cutaneous, 40% gastrointestinal, and 33% respiratory; 13 patients received desloratadine plus prednisolone and 2 patients received epinephrine plus desloratadine plus prednisolone) and were considered allergic to baked CM. The mean period of reaction occurrence was 46 min (3–120 min). The median age of the group presenting negative OFC (OFC–) was 2.81 years (0.53–10.06 years) and positive OFC (OFC+) was 3.91 years (1.07–15.46 years) with no statistical difference ( $p = 0.281$ ). The mean amount of protein intake during the OFC was 0.81 g (0.27–1.30 g) in the reactive group. Regarding allergy to other foods, there was no difference between the groups, as shown in Table 1.

The specific IgE median results to CM,  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, and casein and SPT median diameters for standardized extracts of  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, and casein and also for raw milk, boiled milk, and BM are shown in Table 2. Significant differences were found between OFC– and OFC+ groups only for specific IgE for CM ( $p = 0.002$ ) and for SPT for casein ( $p: 0.027$ ). These data from specific IgEs and SPTs and OFC results were also used to create, using logistic regression, probability curves for BM tolerance. Only the curves for specific CM

IgE ( $Z = 2.542, p < 0.0110$ ) and casein SPT ( $Z = 2.290, p < 0.0220$ ) showed statistically significant relationship between analyzed test and OFC for baked milk. Regarding milk-specific IgE, the chance of tolerance to BM is greater than 80% in patients with IgE  $< 5$  kU/L (Fig. 1a). This probability drops to 50% in those with levels of 42 kU/L and drops to 20% or less in those with values above 85 kU/L, as shown in Figure 1a. For casein SPT, the probability of tolerance to BM is  $>72\%$  if the median wheal diameter is 3.0 mm or less, 50% for 5.5 mm, and  $<30\%$  if the diameter is 10 mm or more (Fig. 1b). ROC curve analysis showed that the cutoff value of the specific CM IgE for prediction of tolerance to baked milk was 5.13 kU/L, with 54% sensitivity and 80% specificity. It was area under curve (AUC) 0.70 (95% confidence interval 0.53–0.87). Additionally, the cutoff value of various tests in predicting clinical reactivity to baked milk was established. 64%

**Table 1.** Data on cow's milk allergy patients under baked milk OFC

	OFC–	OFC+	<i>p</i> value
N	39	15	
Gender, M/F	17/22	9/6	0.279 <sup>a</sup>
Age, years	2.81	3.91	0.281 <sup>b</sup>
Protein intake, g	–	0.81	–
Other food allergies, %	62.0	38.0	0.666 <sup>a</sup>
OFC symptoms, %			
Cutaneous		60.0	
Gastrointestinal		40.0	
Respiratory		33.3	
Anaphylaxis		13.3	

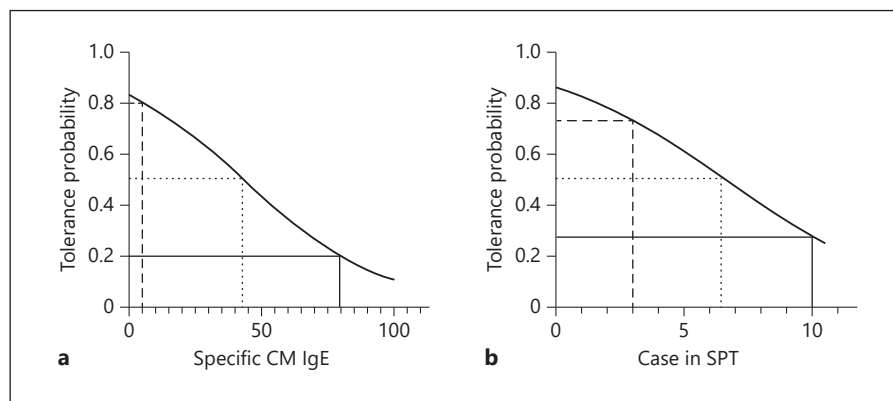
OFC, oral food challenge. <sup>a</sup>  $\chi^2$  test. <sup>b</sup> Mann-Whitney test.

**Table 2.** Allergic tests medians in cow's milk allergy patients under negative and positive OFC with baked milk

Allergic test	OFC– median (CI)	OFC+ median (CI)	<i>p</i> value
IgE CM, kU/L	3.2 (0.10–79.10)	13.2 (0.35–100)	0.002 <sup>a</sup>
IgE $\alpha$ -lactalbumin, kU/L	1.2 (0.10–11.30)	2.8 (0.10–9.63)	0.860
IgE $\beta$ -lactoglobulin, kU/L	2.3 (0.10–6.77)	3.9 (0.20–21.90)	0.604
IgE casein, kU/L	1.9 (0.10–14.30)	3.6 (0.10–56.60)	0.297
SPT CM, mm	5.5 (2.5–12.5)	6.5 (2.5–14.5)	0.539
SPT boiled CM, mm	4.0 (2.5–9.5)	7.0 (6.0–11.0)	0.071
SPT baked CM, mm	3.0 (2.5–7.0)	5.5 (3.0–8.5)	0.075
SPT $\alpha$ -lactalbumin, mm	5.5 (3.5–11.5)	7.5 (3.0–16.0)	0.134
SPT $\beta$ -lactoglobulin, mm	6.0 (2.5–11.5)	7.5 (5.5–14.0)	0.353
SPT casein, mm	2.5 (2.5–7.5)	5.5 (3.5–10.5)	0.027 <sup>a</sup>

OFC, oral food challenge; CM, cow's milk; CI, confidence interval; SPT, skin prick test; IgE, immunoglobulin E. Mann-Whitney test. <sup>a</sup>  $p < 0.05$ .

**Fig. 1.** Tolerance probability curves for allergic tests and baked milk oral food challenge by logistic regression. **a** Specific cow's milk IgE. **b** Skin prick test to casein. Dashed lines used as markers for some points in the curves.



sensitivity and 77% specificity were established for SPT wheal diameters for casein using a cutoff of 3.2 mm, 76% sensitivity and 69% specificity were established for SPT wheal diameters for boiled milk using a cutoff of 5.7 mm, and 78% sensitivity and 46% specificity were established for SPT wheal diameters for baked milk using a cutoff of 3.2 mm.

## Discussion

In the present study, 72.2% of the patients tolerated the BM under OFC and started to consume similar products at home regularly. This percentage is similar to that described in a few studies concerning this OFC type for CMA patients in the literature, varying from 70 to 75% of tolerance [10, 14, 15]. However, considering our initial sample of 114 CMA patients with 60 patients tolerant to BM found during the interview, the total of BM tolerant patients increases to 86.8%, higher proportion than that previously described in the literature.

Previous studies have shown a CMA complete resolution occurring around 5 years of age. Data on the tolerance age for BM data are poor in the literature. Kose et al. [10] found this tolerance to baked goods with an average of 24 months, slightly lower than that observed in our study. This difference may have occurred because we only started BM OFC after the approval of this research project, and probably, some followed patients could be tolerant before the study but they never were tested. Another important data suggesting the median age should be lower is the presence of several patients with negative BM OFC around the age of 1 year.

The CM-specific IgE presented a better profile to predict the OFC results in our study. The median CM IgE value in the OFC+ was 13.2 kU/L, similar to the levels re-

ported in the literature (8.14–12.0 kU/L), while in patients who presented negative OFC, the median was 3.2 kU/L, which is also comparable the previous reports (4.2–4.8 kU/L) [11, 12]. Different from other studies, casein-specific IgE in our cohort showed no difference between reactive and nonreactive individuals [14, 16, 17]. A recent study also demonstrated BM SPT,  $\alpha$ -lactalbumin SPT,  $\alpha$ -lactalbumin IgE, and  $\beta$ -lactoglobulin IgE were statistically different in the groups with positive and negative BM OFC, different from our data, although baked milk and boiled milk SPTs were almost significant and maybe with a huger sample could find statistical difference [17].

The OFC performance requires a multidisciplinary team (physician, nursery, and dietician) and also an infrastructure to manage anaphylactic reactions, turning OFC a real challenge for many physicians around the world. Therefore, once the introduction of baked milk goods for CMA patients requires an OFC, most providers choose to continue the CM diet exclusion until an unintentional intake without symptoms presence. For this reason, the strength of our study is the tolerance probability curves related to baked milk OFC, only possible for specific CM IgE and casein SPT. These curves could be a new tool helping physicians to decide about the ideal moment to perform baked milk OFC, reducing the probability of reactions and the BM OFC number. As far as we know, a previous study generated a similar tolerance probability curve for BM but only for CM SPT performed at the moment of diagnosis, around 6 months before the OFC [10]. In our study, we performed the CM SPT at the same day just before the BM OFC, and they did not show relation ( $Z = 0.5499$ ,  $p = 0.5824$ ).

The limitations of our study are related to the number of participants, which reduced from 114 to 54 after the interviews and the performance of open OFC instead of double-blinded, placebo-controlled OFC. The open OFC option was done because most of our patients were 3 years or



younger, and for older ones, the use of objective symptoms only reduces the chance of false-positive tests. Other weakness is related to the small number of patients who performed a raw milk OFC just after they passed BM OFC without any reaction to make sure they are not fully CM tolerant.

In conclusion, most CMA patients in our cohort were able to tolerate BM. Specific CM IgE and casein SPT demonstrated to be the best allergic test predictors in relation to baked milk tolerance and the tolerance probability curves obtained can be very useful in the decision point to introduce or not baked goods containing milk for patients. The early introduction of dairy products in baked foods improves the calcium intake, reduces the social impacts of a total CM diet exclusion, and creates a new horizon in the treatment of CMA. More studies are necessary to understand the impact of BM intake and the whole CMA resolution.

### Statement of Ethics

The project was conducted in accordance with the Declaration of Helsinki, approved by the local Ethics Committee under the protocol number 32030714.4.0000.5152, and the legal guardian and/or patient signed a consent form for the patient's participation in the study.

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### Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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### Author Contributions

L.K.V.: conceptualization, draft, methodology, supervision, data analysis and interpretation, article writing, and final approval of the version to be submitted; F.A.A.: conceptualization, methodology, statistics analysis, and final approval of the version to be submitted; T.P.S.: investigation, methodology, and final approval of the version to be submitted. T.T.M.: investigation, methodology, and final approval of the version to be submitted; M.F.C.: supervision and final approval of the version to be submitted; G.R.S.S.: conceptualization, methodology, supervision, draft, and final approval of the version to be submitted.