

# Cut-Off Values of Specific IgE and Skin Prick Test to Predict Oral Food Challenge Positivity in Children with Cow's Milk Allergy

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## What is already known on this topic?

- The cut-off values for the cow's milk-skin prick test (CM-SPT) diameters and CM-specific IgE measurements are used to predict the result of the oral food challenge (OFC) test in clinical practice for the diagnosis of cow's milk allergy (CMA).

## What this study adds on this topic?

- The diagnostic power of SPT was determined to be higher when compared to CM-sIgE in the diagnosis of CMA in patients  $\leq 2$  years of age, whereas 2 tests had similar diagnostic power in patients  $> 2$  years of age. Additionally, the significant cut-off levels for the patients younger than 1-year-old were very low compared to other age groups.

## ABSTRACT

**Objective:** The cut-off values for the skin prick test diameters and cow's milk-specific IgE measurements are used to predict the result of the oral food challenge test for the diagnosis of cow's milk allergy. This study aimed to determine the diagnostic values of skin prick test and cow's milk-specific IgE according to age groups and compare the diagnostic powers of these 2 methods.

**Materials and Methods:** In total, 153 children who had a preliminary diagnosis of cow's milk allergy were evaluated. Group A (n = 90) consisted of cow's milk allergy patients whose diagnosis was confirmed by a positive oral food challenge or a history of anaphylaxis. Group B (n = 63) was composed of patients with a negative oral food challenge. The demographic, clinical, and laboratory findings of 2 groups were compared.

**Results:** The cut-off points for cow's milk-specific IgE and cow's milk-skin prick test were determined as  $>2.12$  kUA/L and  $>5$  mm, respectively. The area under the curve was 0.844 for cow's milk-skin prick test (sensitivity 73%, specificity 84%) and 0.745 for cow's milk-specific IgE (sensitivity 67%, specificity 86%). The diagnostic power of skin prick test was determined to be higher when compared to cow's milk-specific IgE ( $P=.02$ ). According to the predicted probability curves, decision points for cow's milk-specific IgE and cow's milk-skin prick test with 95% probability were determined as follows, respectively: for  $\leq 24$  months: 22 kUA/L, 11.3 mm; for  $>24$  months: 44.1 kUA/L, 15.1 mm. The lowest cut-off value with a positive predictive value of 95% and a specificity of 96% was found in patients  $<1$ -year-old ( $>3.3$  kUA/L)

**Conclusion:** The use of high probability diagnostic values of communities for specific IgE and skin prick test along with a significant clinical history may provide accurate and rapid diagnosis of cow's milk allergy and facilitate patient follow-up.

**Keywords:** Children, cow's milk allergy, decision point, skin prick test, specific IgE

## INTRODUCTION

Cow's milk allergy (CMA) is the most common (2%-3%) food allergy in children. It may develop by IgE-associated and/or non-associated immune-mediated mechanisms.<sup>1</sup> Skin prick test (SPT), CM-specific IgE (sIgE) measurement, and the oral food challenge (OFC) test are used for the diagnosis of IgE-associated CMA.<sup>2,3</sup> In CMA, the basis of treatment is the elimination of milk and milk products from the diet until tolerance development is the basis of treatment, and symptomatic treatment is adjusted based on clinical findings.<sup>3-5</sup> It is classically known that the percentage of patients developing tolerance to CM increases by age; 45%-50% at the age of 1, 60%-75% at the age of 2, and 85% at the age of 3.<sup>6</sup> Some

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newer studies reported lower ratios of tolerance reaching at most to 70% (57%–68%) at the age of 16.<sup>7-9</sup> The elimination diet has negative effects on the quality of life of both the patient and the family.<sup>10</sup> Long-term elimination diets may cause nutritional disorders in these children.<sup>11,12</sup> Thus, it is important to make the correct diagnosis before starting the elimination diet to prevent unnecessary dietary practices.

The OFC, which is the gold standard in the definitive diagnosis of CMA, is a troublesome test with the risk of anaphylaxis and should be performed in experienced centers.<sup>13-15</sup> The increasing frequency of food allergies in recent years has made it even more important to evaluate patients quickly and avoid unnecessary OFC testing in appropriate cases. Cow's milk-specific IgE and SPT are safer and frequently used in clinical practice, but the positive results sometimes show only allergic sensitization and may not correlate with the clinical findings.<sup>16</sup>

Detection of cut-off values that predict the result of OFC test accurately for both tests would facilitate the diagnosis and follow-up of patients with CMA, especially when the conditions are not suitable for performing an OFC test. Children living in different countries have different dietary habits and food sensitivities. In our country, the most common food allergy is CMA, but there are few studies to determine cut-off values with high diagnostic power for SPT and CM-sIgE in children with CMA.

In this study, it was aimed to determine cut-off values for CM-SPT and CM-sIgE in different age groups and compare the diagnostic power of these tests in order to reduce the need for OFC in the diagnosis of CMA.

## MATERIALS AND METHODS

### Study Population and Selection Criteria

This is a retrospective methodological study that evaluated the diagnostic power of SPT and sIgE to predict OFC test results in other words presence of CMA diagnosis. The patients who had a sudden allergic reaction associated with intake of CM or some non-specific allergic complaints of unknown origin were investigated for IgE-associated CMA with SPT and CM-sIgE measurement. A total of 153 patients in whom cow's milk sensitivity was determined by a positive result in 1 or both of these tests were included in the study. The patients underwent an OFC test unless there was a clear-cut history of anaphylaxis after milk ingestion. This study was carried out in accordance with the principles of the Helsinki Declaration. Ethics committee approval was received from the Ethics Committee of Ege University (number and date: 15-4/15, 2015) and written informed consent was provided by the parents or legal guardians of the patients.

### Study Protocol

Hospital files of the patients were evaluated and demographic, clinical characteristics, laboratory findings (total IgE, SPTs, CM-specific IgE), and OFC test results were recorded.

The included patients were divided into 2 groups according to the presence of CMA.

**Group A** (n = 90) comprised IgE-mediated CMA patients who had a positive OFC result or a clear-cut history of

anaphylaxis after milk ingestion diagnosed in accordance with guidelines.<sup>17,18</sup>

**Group B** (n = 63) comprised patients who were found to be non-reactive to milk with a negative OFC test result.

## Assessment Methods

### Skin Prick Test

Skin prick tests were performed with fresh milk (1 drop of each fresh milk containing 3.5% fat). Single-peak lancets (1 mm diameter) (Stallerpoint, Stallergenes SA laboratories) were used to prick the skin. Histamine (10 mg/mL) was used as positive control and NaCl (0.9%) was used as negative control. A wheal size  $\geq 3$  mm larger than the negative control was accepted as positive.

### Cow's Milk-Specific Immunoglobulin E Measurement

The total serum IgE and CM-sIgE levels were measured using the CAP system-Fluorescent enzyme immunoassay (FEIA) (Pharmacia Upjohn, NJ, USA). Cow's milk-sIgE titers  $\geq 0.35$  kUA/L were defined as positive.

### Oral Food Challenge Test Protocol

Oral food challenge tests were started using 0.1 mL diluted pasteurized CM with 3.3% protein content (1 : 10, milk : water) and were continued with increasing amounts of undiluted cow's milk every 15-30 minutes until a reaction was noted. If no reaction occurred with total amount of 200 mL (6540 mg milk protein) of CM, the child continued to receive at least 200 mL of CM or CM-based formula for the next week, and the parents were instructed to observe the child for late reactions. Oral food challenge results were considered positive when objective symptoms such as urticaria, angioedema, airway obstruction signs, vomiting, and anaphylaxis were developed.<sup>14,19</sup>

### Statistical Analysis

Statistical analyses were performed using the Statistical Package for Social Sciences version 23.0 software (IBM Corp.; Armonk, NY, USA) and MedCalc Statistical Software trial version (MedCalcSoftware, Ostend, Belgium, 2016). Continuous variables were presented as mean  $\pm$  standard deviation (SD) or median (minimum:max) according to the distribution pattern of variables. The assumption of normality was tested using Kolmogorov-Smirnov or Shapiro-Wilk tests. Whether the distribution of each variable in the data set fits the normal distribution was tested and variables that were not normally distributed were evaluated by non-parametric tests. Mann-Whitney *U* test was used in binary-independent group comparison.

Categorical variables were compared by chi-square test and Fisher's exact test. In order to estimate the sensitivity and specificity of CM-sIgE and CM-SPT measurements for predicting CMA, receiver operator characteristic (ROC) curve analysis was performed. Area under the ROC curve values with 95% CIs were reported. Different cut-off values for sIgE with  $>95\%$  PPV,  $>90\%$  specificity, and highest sensitivity and specificity were determined and predicted probability curves were created. A *P*-value less than .05 was considered statistically significant.

In the present study, post hoc power analysis was performed considering the CM-SPT measurements for study groups. The

effect size value was calculated as  $d = 1.28$ . With this effect size calculated, the power value obtained from the study at the level of  $\alpha = 0.05$  was determined as  $>95\%$ .

**RESULTS**

In the study, 153 children (61% male) with a median age of 12 (2 : 84) months were evaluated. The symptoms at presentation were urticaria (68.6%,  $n = 105$ ), urticaria and angioedema (17%,  $n = 26$ ), and respiratory distress (11.1%,  $n = 17$ ). Five patients who had a history of anaphylaxis after exposure to CM were included in group A without performing an OFC test. The median reaction dose was 6.0 (0-98) mL (0.19 g milk protein) in patients with a positive OFC test. Uneasiness was an accompanying symptom in 18 (11.7%) of the patients. Concomitant diseases were atopic dermatitis (37.2%,  $n = 57$ ), asthma (10.4%,  $n = 16$ ), allergic rhinitis (6.5%,  $n = 10$ ), and colitis (4%,  $n = 6$ ).

Five patients who had a history of anaphylaxis after exposure to CM were included in group A without performing an OFC test. The median reaction dose was 6.0 (0-98) mL (0.19 g milk protein) in patients with a positive OFC test.

Two groups of patients with and without CMA (groups A and B) were similar in terms of age ( $P=.58$ ) and gender ( $P=.56$ ). Urticaria-angioedema symptom was more frequently seen ( $P<.01$ ) in group A, while frequencies of other symptoms at presentation ( $P>.05$ ) and concomitant diseases ( $P>.05$ ) were similar in 2 groups. There was no difference in median total IgE levels ( $P=.06$ ) and wheal diameters of histamine ( $P=.76$ ) between 2 groups. The median CM-SPT wheal diameter ( $P<.001$ ) and CM-slgE level ( $P<.001$ ) were higher in group A (Table 1).

Group A patients were grouped according to age as  $\leq 24$  and  $>24$  months old. Two groups had similar presenting symptoms except uneasiness which was more frequent in patients  $\leq 24$  months old (16% vs. 0%,  $P<.001$ ). Concomitant asthma was more frequent in patients  $>24$  months old (24.4% vs. 5.4%,  $P<.001$ ), while atopic dermatitis was more frequent in patients  $\leq 24$  months old (43% vs. 22%,  $P=.01$ ). Histamine and CM wheal diameters in SPT were similar between 2 groups. Total IgE and CM-slgE levels were higher in patients  $>24$  months old than in patients  $\leq 24$  months old (respectively, total IgE:190 (4.36:2142) kU/L, 67.50 (0.01:1584) kU/L,  $P<.001$ ; CM-slgE: 2.10 (0.03:100) kUA/L, 1.70 (0.01:79.10) kUA/L,  $P=.04$ ) (Table 2).

**Receiver Operator Characteristic Curve Analysis**

Receiver operator characteristic (ROC) curve analysis was performed in the whole group and 2 groups according to age ( $\leq 24$  and  $>24$  months old). In the whole group, ROC curve analysis was performed to estimate the sensitivity and specificity of CM-SPT and CM-slgE for predicting the presence of CMA. The cut-off points for CM-slgE and CM-SPT were determined as  $>2.12$  kUA/L and  $>5$  mm, respectively.

The area under the curve (AUC) for CM-SPT was 0.844 (sensitivity 73%, specificity 84%), showing that CM-SPT  $> 5$  mm was significantly related to an increased risk of the presence of CMA. The AUC for CM-slgE was 0.745 (sensitivity 67%, specificity 86%).

**Table 1.** Demographic, Clinical, and Laboratory Features of the Patients According to the Groups

	Group A (n = 90)	Group B (n = 63)	P
Median age (months)	12 (2 : 74)	12 (4 : 84)	.580
Sex distribution (male)	59% (n = 53)	63.5% (n = 40)	.566
Uneasiness	9 (10%)	9 (14.30%)	.418 <sup>a</sup>
Urticaria	54 (60%)	51 (80%)	.509 <sup>a</sup>
Urticaria and angioedema	22 (24.40%)	4 (6.30%)	.003 <sup>a</sup>
Anaphylaxis	5 (5.6%)	0	.078 <sup>b</sup>
Respiratory distress	9 (10%)	8 (12.6%)	.875 <sup>a</sup>
Colitis	4 (4.4%)	2 (3.2%)	$>.99$ <sup>b</sup>
Allergic rhinitis	3 (3.3%)	7 (11.1%)	.093 <sup>b</sup>
Asthma	9 (10%)	7 (11.1%)	.825 <sup>a</sup>
Atopic dermatitis	34 (37.8%)	23 (36.5%)	.873 <sup>a</sup>
Median total IgE (kU/L)	114.50 (3.29 : 2142)	64.70 (0.01 : 1706)	.061
Median histamine-SPT (mm)	6 (3 : 13)	6 (3 : 12)	.766
Median CM-SPT (mm)	9 (0 : 35)	0 (0 : 12)	$<.001$
Median CM-slgE (kUA/L)	3.87 (0.01 : 100)	1.04 (0.01 : 40.30)	$<.001$

<sup>a</sup>Chi-square test;  
<sup>b</sup>Fisher's exact test.  
 SPT, skin prick test; CM, cow's milk; slgE, specific immunoglobulin E.

When the OFC was taken as a basis, comparison of the AUCs showed that the diagnostic power of CM-SPT was significantly higher than CM-slgE ( $P=.02$ ) (Figure 1, Table 3).

Among the patients  $\leq 24$  months old, the AUC for CM-SPT was 0.838 (sensitivity 71%, specificity 84%), and AUC for CM-slgE was 0.736 (sensitivity 62%, specificity 86%). The difference between the diagnostic power of 2 tests was significant ( $P=.04$ ) (Figure 1, Table 3).

In the patients  $>24$  months old, CM-slgE and CM-SPT were not found to be superior to each other ( $P=.41$ ) in the diagnosis of CMA (Figure 1, Table 3).

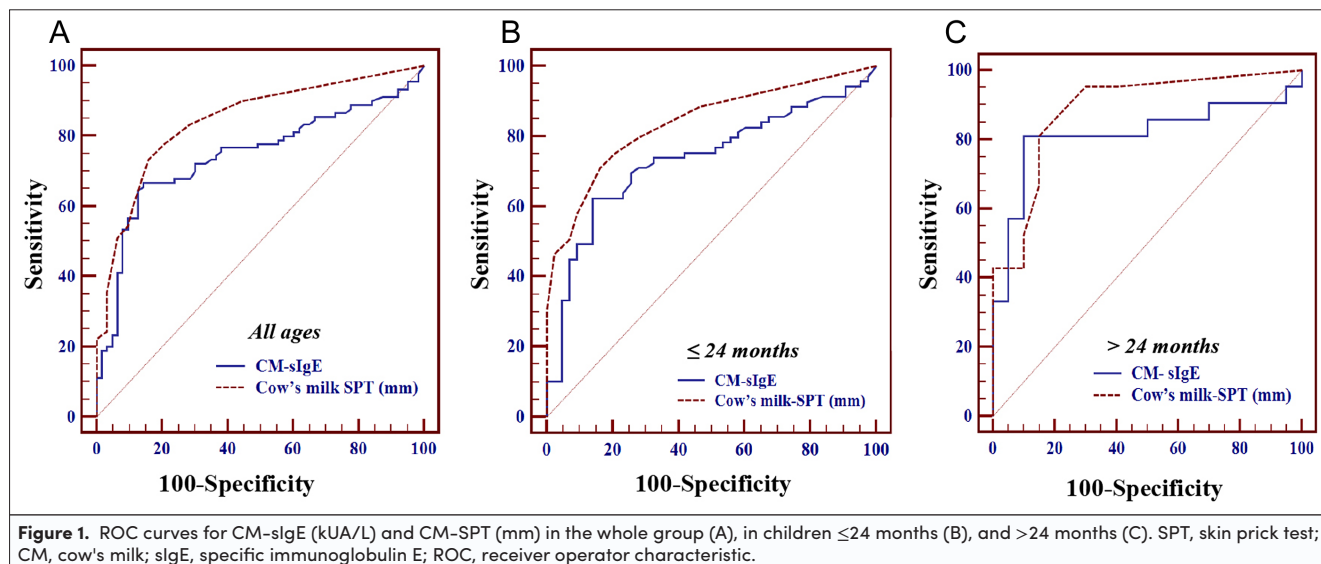
**Predicted Probability Curves Showing Positive Cow's Milk Challenge Test Results**

We calculated the predicted probabilities for a positive clinical reactivity at a given CM-slgE level using the logistic regression model proposed by Sampson.<sup>20</sup> According to the predicted

**Table 2.** Laboratory Features of the Patients According to the Age of the Patients

	Age of Patients		P
	$\leq 24$ months (n = 112)	$>24$ months (n = 41)	
Median total IgE	67.50 (0.01 : 1584)	190 (4.36 : 2 142)	$<.001$ <sup>c</sup>
Median histamine-SPT (mm)	6 (3 : 12)	6 (4 : 13)	.260 <sup>c</sup>
Median CM-SPT (mm)	5 (0 : 35)	5 (0 : 20)	.542 <sup>c</sup>
Median CM-slgE (kUA/L)	1.70 (0.01 : 79.10)	2.10 (0.03 : 100)	<b>.042<sup>c</sup></b>

<sup>c</sup>Mann-Whitney U test.  
 SPT, skin prick test; CM, cow's milk; slgE, specific immunoglobulin E.



probability curves, decision points for CM-sIgE and CM-SPT with 95% probability were determined as follows, respectively: whole group 32.6 kUA/L, 12.4 mm;  $\leq 24$  months 22 kUA/L, 11.3 mm;  $> 24$  months 44.1 kUA/L, 15.1 mm (Figure 2, 3).

**Immunoglobulin E Cut-off Levels for the Prediction of Clinical Reactivity**

The CM-sIgE cut-off values were examined separately for ages from 1 to 5 years. Three separate CM-sIgE levels that correspond to decision points with  $> 95\%$  PPV,  $> 90\%$  specificity, and highest sensitivity and specificity were found for each group. The lowest cut-off value with a PPV of 95% and a specificity of 96% was in patients  $< 1$ -year-old ( $> 3.3$  kUA/L) (Table 4).

**DISCUSSION**

In the previous studies carried out, quite different CM-sIgE levels and CM-SPT induration diameters were reported as cut-off values to predict CMA.<sup>20-25</sup> In this study, significant cut-off values for CM-sIgE and SPT, with 95% decision points predicting CMA, and the diagnostic power of these tests were investigated. Sampson et al<sup>20</sup> reported that CM-sIgE levels diagnostic for CMA with 95% PPV were 5 kUA/L for patients  $< 2$  years old and 15 kUA/L for patients  $> 2$  years old. In this study, higher cut-off levels ( $> 7.6$  kUA/L) with 91% PPV were

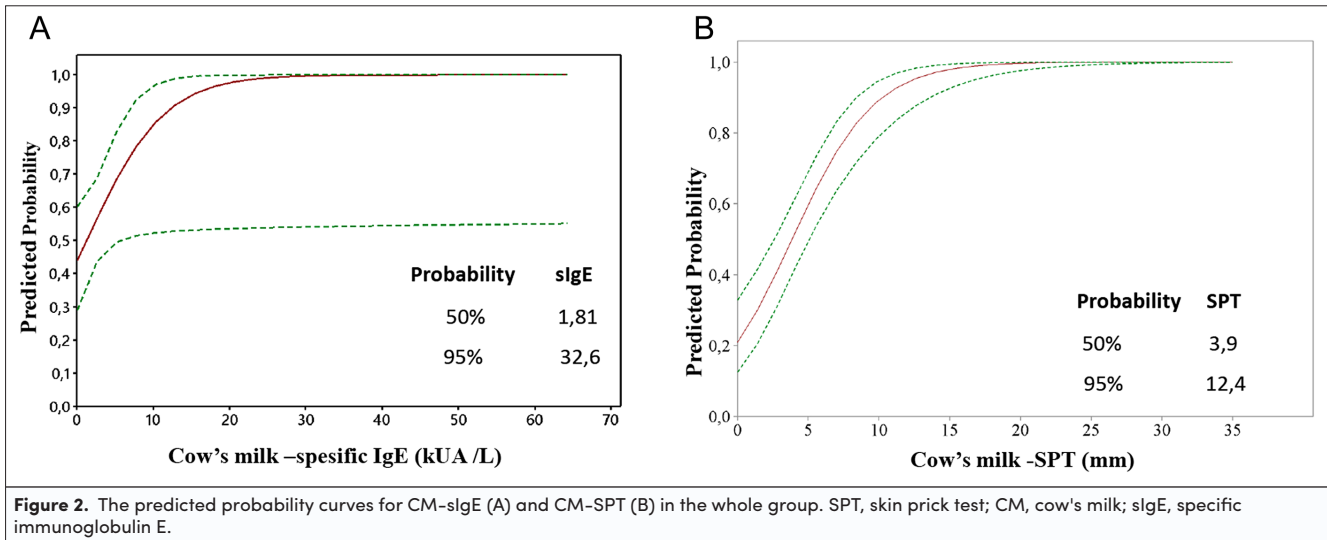
determined in patients  $\leq 2$  years old. PPV and specificity were found to be lower under this level. But in patients  $< 1$ -year-old, a quite lower cut-off ( $> 3.3$  kUA/L) had a PPV of 95% and specificity of 96%. This result suggests that patients younger than 1-year-old should be evaluated with separate cut-off values. The cut-off level of  $> 14.2$  kUA/L was significant for CMA diagnosis with 93% PPV and 95% specificity in patients  $> 2$  years old similar to Sampson's study. In patients over 5 years of age, the PPV and specificity for this cut-off even reached 100%.

In different studies, CM-sIgE levels corresponding to 90%-95% at predicted probability curves were found to be in good correlation with clinical reactivity and helpful in making a diagnosis. The CM-sIgE levels diagnostic for CMA with 90% predicted probability were determined to be higher in children younger than 2 years (31.4 kUA/L) than in children older than 2 years (10.1 kUA/L) by Kim et al.<sup>24</sup> Çelik-Bilgili et al<sup>21</sup> reported higher cut-off levels with 90% predictive values in children younger than 1 year (25.8 kUA/L) and at all ages (88.8 kUA/L). This broad range of cut-off values for CM-sIgE found in different studies may be due to the differences in the study groups in terms of diagnosis, age, and disease severity distributions, as well as differences in the community characteristics such as the frequency of allergy, onset time, and consumption amount of that supplementary food.

**Table 3.** Performance Characteristics of Cow's Milk-sIgE Levels and Cow's Milk-SPT Diameters in the Determination of CMA (Whole Group and 2 Age-Based Groups)

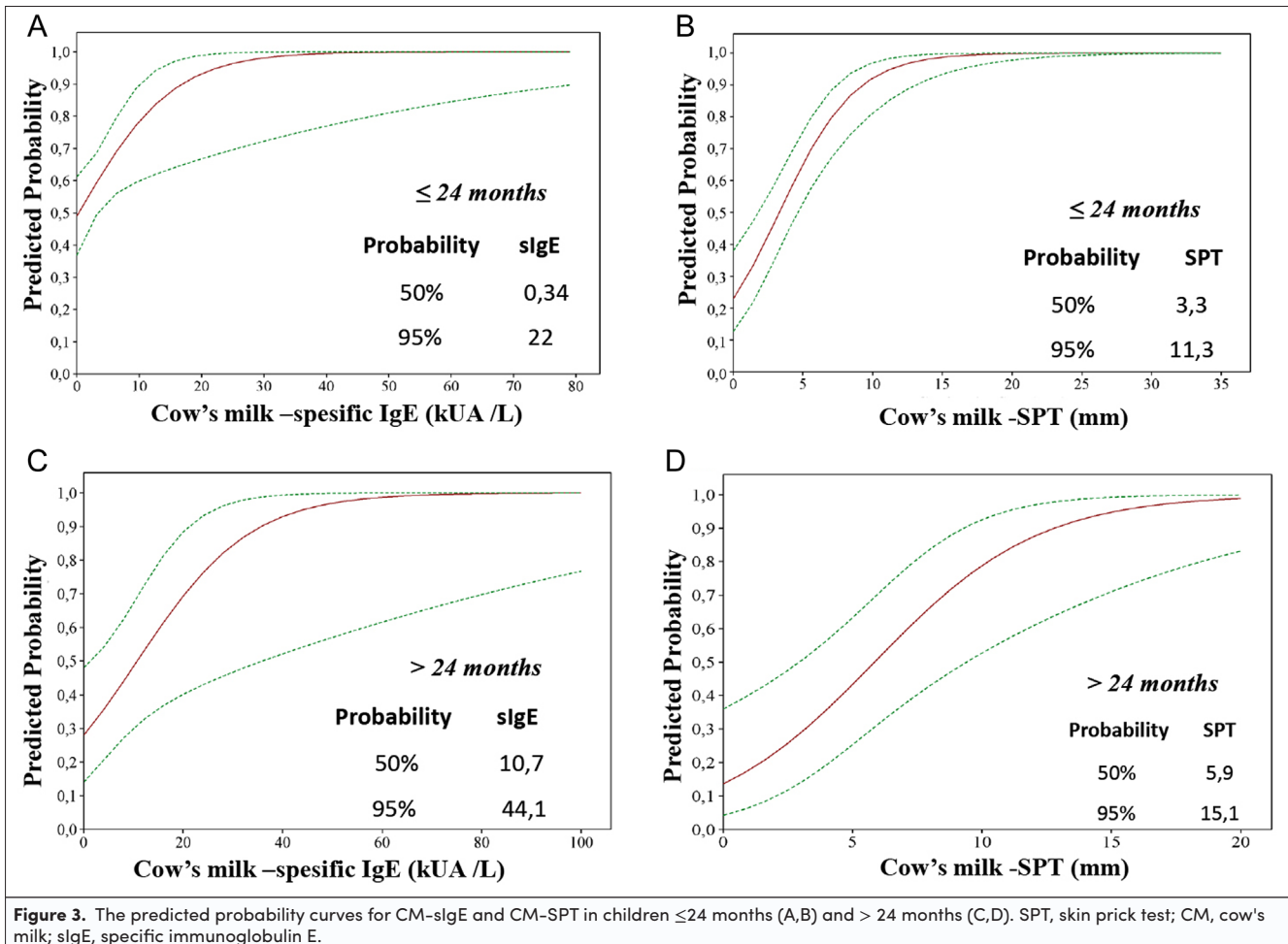
Performance Characteristics	Whole Group		$\leq 24$ months		$> 24$ months	
	CM- sIgE (kUA/L)	CM-SPT (mm)	CM- sIgE (kUA/L)	CM-SPT (mm)	CM- sIgE (kUA/L)	CM-SPT (mm)
Cut-off value	$> 2.12$	$> 5$	$> 2.12$	$> 5$	$> 2.12$	$> 5$
Sensitivity (%)	67	73	62	71	62	81
Specificity (%)	86	84	86	84	86	85
PPV (%)	87	87	88	88	88	85
NPV (%)	64	69	59	64	59	81
AUC	0.745	0.844	0.736	0.838	0.735	0.886
P	<b>.02</b>		<b>.04</b>		<b>.41</b>	

SPT, skin prick test; CM, cow's milk; sIgE, specific immunoglobulin E; PPV, positive predictive; NPV, negative predictive value; AUC, area under the curve.



In this study, CM-sIgE cut-off levels with 100% PPV and >90% specificity were displayed for different age groups (Table 4, values in the second line for each age group). We propose that these values can be used for CMA diagnosis without performing OFC test.

Yavuz et al<sup>22</sup> also reported CM-sIgE levels with 95% diagnostic value for CMA in Turkish children, but cut-off levels were lower than our study. They found that in children <2 years old, the cut-off level significant with 95% PPV and 94% specificity (9.3 kUA/L) was also supported by predictive probability curves





**Table 4.** Cow's Milk-sIgE Cut-off Values for Prediction of Clinical Reactivity

Age, Years	n	Performance Characteristics	AUC	Cut-off	Sens	Spec	PPV	NPV	P
<1	68	>95% PPV		>3.33	45	96	95	55	<.001
		>90% Spec.	0.743	>20.30	10	100	100	44	
		Highest Sens. & Spec.		>2.10	63	89	89	63	
≥1	85	>95% PPV		>25.90	26	97	93	48	<.001
		>90% Spec.	0.748	>40.30	18	100	100	46	
		Highest Sens. & Spec.		>2.40	68	86	87	65	
<2	108	>95% PPV		>7.60	31	95	91	48	<.001
		>90% Spec.	0.721	>25.90	9	100	100	42	
		Highest Sens. & Spec.		>2.12	60	86	87	59	
≥2	45	>95% PPV		>14.20	52	95	93	61	<.001
		>90% Spec.	0.830	>40.30	28	100	100	53	
		Highest Sens. & Spec.		>2.40	84	90	91	82	
<3	120	>95% PPV		>7.60	34	95	93	46	<.001
		>90% Spec.	0.737	>25.90	13	100	100	40	
		Highest Sens. & Spec.		>2.12	63	86	89	58	
≥3	33	>95% PPV		>14.20	64	95	90	78	<.001
		>90% Spec.	0.872	>40.30	36	100	100	68	
		Highest Sens. & Spec.		>2.40	86	89	86	90	
<5	141	>95% PPV		>25.90	18	98	94	44	<.001
		>90% Spec.	0.737	>40.30	11	100	100	42	
		Highest Sens. & Spec.		>2.12	66	86	88	62	
≥5	12	>95% PPV		>1.53	80	86	80	86	<.001
		>90% Spec.	0.886	>14.20	40	100	100	70	
		Highest Sens. & Spec.		>1.21	100	71	71	100	
All	153	>95% PPV		>25.90	19	98	94	46	<.001
		>90% Spec.	0.745	>40.30	11	100	100	44	
		Highest Sens. & Spec.		>2.12	67	86	87	64	

Sens, sensitivity; Spec, specificity; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value.

with 95% decision points (11.1 kUA/L), whereas, in our study, the value significant with 95% predicted probability (22 kUA/L) was closer to the diagnostic value (>25.9 kUA/L;100% PPV and 100% specificity) instead of 95% PPV. Also in children >2 years old, they found lower significant and diagnostic levels (>4 kUA/L, with 93% PPV, 91% specificity) compared to our study (>14.2 kUA/L, with 93% PPV, 95% specificity).<sup>22</sup> But according to the predicted probability curves, decision points for CM-sIgE with 95% probability were higher in our study for >24 months (44.1 kUA/L).

As in CM-sIgE, induration diameters in CM-SPT significant for CMA diagnosis with ≥95% PPV were detected in a wide range (5-15 mm) in different studies.<sup>25-28</sup> Sampson<sup>20</sup> reported that 10 mm induration diameter is considered significant. In our study, induration diameters diagnostic for CMA with 95% predicted probability were found as 11.3 mm for patients ≤2 years old and 15.1 mm for patients >2 years old. The diagnostic value found for the whole group (12.4 mm) was closer to the value of patients ≤2 years old, likely due to the early diagnosis in majority of the study population.

This study is also important because it compared the diagnostic strengths of CM-sIgE and CM-SPT in CMA. When ROC curves for CM-sIgE and CM-SPT were compared, SPT was found to be superior in the diagnosis of CMA in patients ≤24 months of age. Therefore, SPT can be suggested to be the first choice in this group. In those >24 months of age, diagnostic power of 2 tests was similar. This result is important for health centers

that cannot do CM-sIgE testing and that would use the high predictive probability values in SPT for the diagnosis of CMA. It also shows how sensitive the SPT is in young children.

This is a study that evaluates CM-sIgE and SPT together and compares them in predicting the presence of CMA. Results that would show the presence of clinical signs at high probability were calculated. We determined the CM-sIgE level of 32.6 kUA/L and CM-SPT induration diameter of 12.4 mm as threshold values for CMA diagnosis (with 95% predicted probability) when the age is not taken into account.

In this study, different and heterogenous CM-sIgE cut-off levels at high specificity and PPV were found for different ages. But prominently, lower cut-off level that was found for the patients younger than 1 year suggested that this group should be evaluated as a separate and special group.

The present study has some limitations. We did not perform double-blind, placebo-controlled OFC, which is the gold standard. Instead, the open OFC was applied and only the objective findings were recorded. Yet, we had no doubt about the diagnosis of IgE-mediated CMA because all patients in group A had a reaction history after the intake of milk and/or milk products.

Another limitation of the study is that there are differences between age groups in terms of number of patients and

diagnosis. In addition, the fact that the patients included in the study were high-risk patients who applied to the pediatric allergy outpatient clinic and had an increased probability of diagnosis may have caused higher cut-off values compared to the general population.

## CONCLUSION

In this study, we determined the CM-SPT and CM-sIgE cut-off values in different age groups that can be used to predict the result of OFC test results. The cut-off points for CM-sIgE and CM-SPT were determined as  $>2.12$  kUA/L and  $>5$  mm, respectively. According to the predicted probability curves, decision points for CM-sIgE and CM-SPT with 95% probability were determined as follows, respectively: for  $\leq 24$  months 22 kUA/L, 11.3 mm; for  $>24$  months 44.1 kUA/L, 15.1 mm. Additionally, we showed that the lower cut-off values for CM-sIgE were significant under the age of 1 ( $>3.3$  kUA/L). The use of high probability diagnostic values for CM-sIgE and SPT, along with a significant clinical history, strengthens the diagnosis of CMA and directs the clinician by predicting the OFC results. Considering that different decision points have been determined in different societies, it would be appropriate to use the diagnostic decision values obtained in cases with CMA pre-diagnosis.

**Ethics Committee Approval:** This study was approved by Ethics Committee of Ege University, (Approval No: 15-4/15, 2015).

**Informed Consent:** Written informed consent was provided by the parents or legal guardians of the patients.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – N.C.G., E.D.; Design – N.C.G., S.E.A., E.D.; Supervision – F.G., R.T.; Materials – N.C.G., S.E.A., F.G., C.M.B.; Data collection &/or processing – N.C.G., M.Aç, C.M.B.; Analysis and/or interpretation – N.C.G., E.D.

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