ORIGINAL ARTICLE | Eur Ann Allergy Clin Immunol | Vol 52, N.4, 160-164, 2020

A. Castro Neves[1], A. M. Romeira[1], J. G. Marques[1,2,3], V. Matos[4], P. Leiria-Pinto[1,3]

Blood or skin: what is best in predicting cow's milk allergy diagnosis?

[1]Immunoallergology Service, Centro Hospitalar Universitário de Lisboa Central EPE, Lisbon, Portugal
[2]Immunoallergology Unit, Centro Hospitalar de Lisboa Ocidental EPE, Lisbon, Portugal
[3]NOVA Medical School, Comprehensive Health Research Center (CHRC), Lisbon, Portugal
[4]Clinical Pathology Service, Centro Hospitalar Universitário de Lisboa Central EPE, Lisbon, Portugal

Key words
IgE; milk allergy; sensitivity; specificity; skin tests.

Corresponding author
Ana Castro Neves
Hospital Dona Estefania
Jacinto Marto Street 8A
Lisbon, Portugal
E-mail: ananeves6@hotmail.com

Doi: 10.23822/EurAnnACI.1764-1489.123

Summary
Cut-off values for both skin prick tests (SPT) and specific IgE (sIgE) levels for predicting cow´s milk allergy (CMA) diagnosis are not universally defined. This study is a retrospective analysis of consecutive children (0-18 years-old) with suspected CMA tested with SPT and sIgE for cow's milk (CM) and its fractions between 2016-2017. CMA diagnosis was defined by a positive oral food challenge or a highly suggestive clinical history of CMA and SPT and/or sIgE positive to CM and/or its fractions. One hundred and five patients were included, 58% males with a median age of 2.5 (P25-P75:1-6) years and the diagnosis was confirmed in 83 patients (79%). The variables associated with CMA diagnosis were SPT with CM (p<0,05) and casein (p<0,05) and all sIgE to CM and its fractions (α-Lactalbumin, β-Lactoglobulin and casein; p<0,05 for all). Optimal cut-off points (Youden's index) for CMA diagnosis were 4.5mm for the mean wheal diameter to Cow's milk and 3mm to casein. For sIgE levels the optimal cut-off points were: were: CM 4.36 KUA/L , alfa-lactalmunin 1.6 KUA/L, b-lactoglobulin 1.7 KUA/L and casein 2.6KUA/L. The role of SPT and sIgE levels to cow's milk and its fractions is unequivocal in CMA follow-up. Moreover, sIgE levels seem to be more discriminatory than SPT.

Introduction

Cow's milk allergy (CMA) affects 1 to 3% of children and is one of the most common food allergies in pediatric age (1,2). CMA is classified according to the immunological reaction to milk proteins as IgE mediated or non-IgE-mediated, although a combination of both reactions may occur (1,3). The most common reactions are IgE-mediated, which are immediate, appearing within minutes to up to two hours after ingestion of cow's milk, and may affect one or more organs including systemic reactions as anaphylaxis (1,3). The delayed reactions are typically non-IgE mediated, occurring several hours after cow's milk ingestion and affect mainly the gastrointestinal system (3-5). Cow's milk contains several potentially sensitizing proteins, which are found in the whey and casein fractions, including α-lactoalbumin, β-lactoglobulin and casein allergens (5). When a patient is diagnosed with CMA, dairy exclusion with replacement with dietary alternatives is indicated, for maintaining adequate nutrition, growth and development. Strict avoidance of cow's milk has a negative influence on the quality of life of these patients and families (6-8). The diagnosis of IgE-mediated CMA is based on a detailed clinical history, sIgE levels and/or SPT to whole milk and the main sensitizing proteins, α-lactalbumin, β-lactoglobulin and casein, followed by oral food challenge (OFC), which is the diagnostic gold standard. In the literature there are several studies that try to estimate the accuracy of SPT and sIgE levels for predicting the result of the OFC, in order to decrease the risk of a positive OFC (9,10). At this moment, there are no universally defined cut-off values, due to a lack of reproducibility in different populations (9,11-15).
The aim of this study was to estimate the accuracy of sIgE levels and SPT to cow's milk and its fractions in CMA diagnosis.

Materials and methods

Study population and design

Retrospective analysis of the clinical records of children (0-18 years-old) with suspected CMA, followed at the Immunoallergy Department, who were tested to cow's milk proteins (whole milk protein, α-lactoalbumin, β-lactoglobulin and casein) between 2016-2017. In this analysis, we included consecutive patients with SPT and sIgEs performed within 6 months previous to the OFC. Patients that did not perform OFC but had a highly suggestive clinical history of CMA (more than one allergic episode upon cow's milk protein ingestion in the previous 6 months with at least one positive SPT and/or positive sIgE) were also included. Patients with non-IgE mediated CMA were excluded. Demographic data, clinical manifestations and diagnostic procedures were evaluated. The diagnosis of CMA was considered when OFC was positive or there was a suggestive clinical history with the criteria defined above.

Skin prick tests and specific IgE assessment

Skin prick tests were performed in all patients with whole cow's milk extract (5 mg/mL), α-lactoalbumin (5 mg/mL,) β-lactoglobulin (1 mg/mL), and casein (10 mg/mL), from Bial Aristegui®. Histamine (10 mg/mL, ALK-Abelló®) was used as a positive control and glycerosaline was used as a negative control. SPT were evaluated 15 minutes after testing. The appearance of a wheal with a mean diameter ≥ 3 mm was considered positive (16). The levels of specific IgE antibodies to whole cow's milk, α-lactoalbumin, β-lactoglobulin and casein were determined using the ImmunoCAP® method (Thermo Fisher Scientific®, Uppsala, Sweden). A result of ≥ 0.35 kUA/L was considered positive (16).

Oral food challenges

Oral food challenges (OFC) were performed in the Allergy Unit of the Hospital with increasing doses of cow's milk, given at regular intervals according to the standard protocol of the Unit (236 mL of milk divided in 7 incremental doses every 15-30 minutes). All the OFC were performed with an open protocol just as routine clinical practice. Informed consent was previously obtained from the parents. All patients remained for, at least, 2 hours under observation after the last milk dose intake, before being discharged. If a clinical reaction appeared, the challenge was discontinued and treatment was provided and the test was considered positive.

Statistical analysis

Statistical analyses were conducted using IBM SPSS for Mac version 20.0 (SPSS, Chicago, IL, USA) and MedCalc 14.10.2 (MedCalc Software bvba, Ostend, Belgium). Continuous variables were expressed as mean ± standard deviation, median (25th to 75th percentiles) and categorical variables were expressed as counts (percentages). The relationship between sensitivity and specificity and the optimal decision points for sIgE and SPTs were determined by analysis with the receiver-operating characteristic (ROC) curve. Correlation between the SPT wheal diameters and sIgE levels was evaluated with Pearson's correlation coefficient. The Yates and Fisher chi-squared test was used for comparison between groups. The Mann-Whitney non-parametric test was used to compare continuous variables of the two groups. The relationship between SPT or sIgE and food challenge outcome was analysed using logistic regression. Youden's index (17) was used to calculate the optimal cut-offs for the considered variables associated with CMA diagnosis. An alternative cut-off was also calculated for maximization of the specificity (specificity=100%). The level of significance $\alpha = 0.05$ was considered.

Results

In this analysis, we included 105 patients, 61 (58%) males. The median age of the children evaluated was 2.5 years (P25-P75: 1-6 years). The group with confirmed allergy consisted of 83 patients (79%) and the control group (allergy excluded) of 22 (21%). In the group of patients with confirmed allergy, 37 (45%) were included for having had positive oral food challenge and the remaining (46, 55%) due to a strongly suggestive clinical history of CMA. Among the patients with confirmed allergy, 57% had a previous history of anaphylaxis, 87% had cutaneous manifestations (hyperemia, urticaria and angioedema), 54% gastrointestinal (vomiting, diarrhea) and 13% respiratory symptoms (rhinorrhea, sneezing, laryngeal stridor, hoarseness, coughing, dyspnea).

The main characteristics of the patients with confirmed allergy and the patients with excluded allergy regarding age, gender, SPT and sIgE are shown in **table I**.

The associations between the evaluated variables and the CMA diagnosis are shown in **table II**.

The associations between the SPTs and the sIgE to milk and milk fractions were: cow's milk rho=0.234 (p=0.023); α-lactalbumin rho=0.372 (p<0.0001); β-lactoglobulin rho=0.349 (p=0.001); Casein rho=0.489 (p<0.0001).

The ROC curves constructed from the ratio between sensitivity and specificity of SPTs and sIgE levels are shown in **figure 1**.

Using Youden's index, only taking into consideration the variables with a statistically significant association to CMA diagnosis, the optimal cut-off points, description of the sensitivity (S), specificity (Sp), positive predictive value (PPV) and negative predictive values (NPV) for mean wheal diameters in SPTs and sIgE levels are presented in **table III**.

Table I *- Main characteristics of the patients with excluded and confirmed cow's milk allergy.*

	Excluded allergy (n=22)	**Confirmed allergy (n=83)**	**p-value**
Male gender (%)	54.50	59.00	0.809
Age (Median; P25-P75)	2.00 (0.90-3.00)	3.00 (0.92-3.00)	0.126
Skin prick tests (mean wheal diameter in millimetres); median (P25:P75)			
Cow's milk extract	4.00 (0.00-6.00)	7.00 (5.00-10.00)	0.013
α-Lactalbumin	9.50 (6.00-11.00)	8.80 (6.50-12.10)	0.537
β-Lactoglobulin	6.00 (0.00-7.50)	7.00 (4.50-10.00)	0.136
Casein	3.00 (0.00-6.00)	7.30 (5.00-10.30)	0.001
Cow's milk extract	0.66 (0.28-3.24)	11.40 (2.90-25.60)	<0.0001
α-Lactalbumin	0.56 (0.16-1.31)	1.86 (0.69-11.80)	0.002
β-Lactoglobulin	0.23 (0.06-1.06)	1.89 (0.43-9.02)	<0.0001
Casein	0.30 (0.03-1.34)	6.01 (0.99-14.00)	<0.0001

Specific IgE levels (kUA/L); median (P25:P75).

Table II *- Associations between the evaluated variables and cow's milk allergy diagnosis.*

	Odds-ratio (CI 95%)	**p-value**
Male gender	1.201 (0.466-3.094)	0.704
Age	1.127 (0.974-1.304)	0.114
Skin prick tests		
Cow's milk	1.242 (1.041-1.481)	0.017
α-Lactalbumin	1.034 (0.935-1.144)	0.513
β-Lactoglobulin	1.133 (0.979-1.311)	0.090
Casein	1.280 (1.085-1.510)	0.003
Specific IgE levels		
Cow's milk	1.393 (1.137-1.707)	<0.001
α-Lactalbumin	1.264 (0.995-1.606)	0.049
β-Lactoglobulin	2.142 (1.176-3.898)	0.012
Casein	1.712 (1.166-2.514)	0.006

CI 95%: Confidence-interval.

Table III *- Sensitivity (S), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) for mean wheal diameters in SPT and sIgE levels.*

SPT cut-offs by Youden's index	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Cow's milk extract (>4.50 mm)	77%	60%	93%	38%
Casein (>3.00mm)	92%	60%	31%	13%
sIgE cut-offs by Youden's index				
Cow's milk extract (>4.36 kUA/L)	71%	95%	100%	30%
α-Lactalbumin (>1.60 kUA/L)	55%	86%	100%	52%
β-Lactoglobulin (>1.70 kUA/L)	58%	95%	100%	44%
Casein (>2.60 kUA/L)	64%	95%	100%	37%

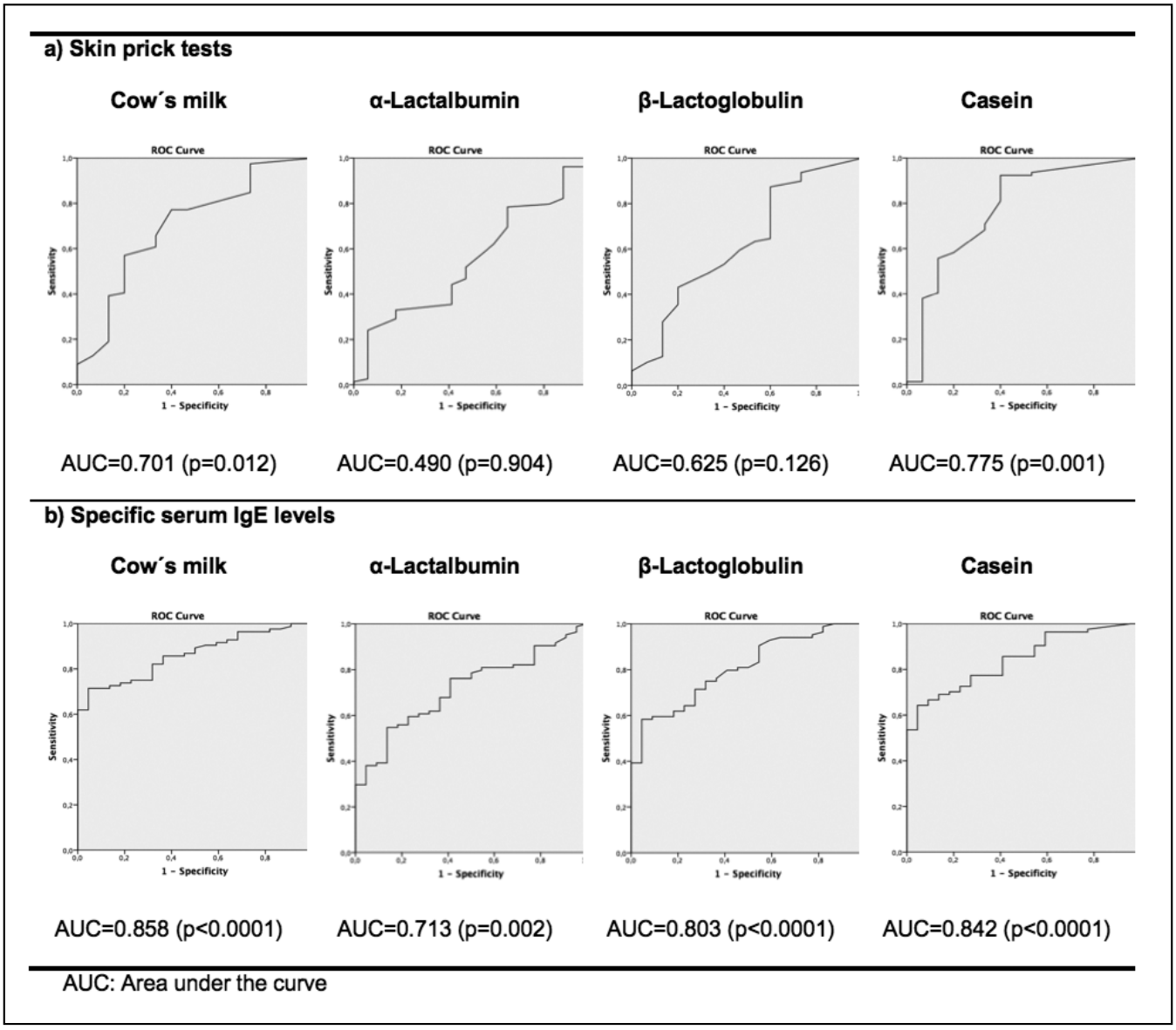
sIgE specific IgE; SPT: skin prick tests.

Using alternative cut-offs for maximization of the specificity, only taking into consideration the variables with a statistically significant association to CMA diagnosis, the optimal cut-off points for the mean wheal diameter to cow's milk was 12.5 mm and casein 20mm. For sIgE, also using maximum specificity criteria, the optimal cut-off points for cow's milk extract was of 8.2 kUA/L, α-Lactalbumin of 8.6 kUA/L, β-Lactoglobulin of 3.1 kUA/L and casein of 4.2 kUA/L.

Discussion

We investigated the accuracy of sIgE levels and SPT mean wheal diameters in the management of children with CMA diagnosis. In our sample, children's age distribution was similar to the published data about CMA, suggesting that most of patients achieve tolerance within 3 to 4 years (5). Age was not associated with CMA diagnosis and this may be influenced by the fact that

Figure 1 *- ROC curves to obtain optimum levels of mean wheal diameter of SPT (a) and sIgE levels (b).*



patients followed in our Department are high-risk patients with decreased chances of spontaneous CMA outgrow.

From the evaluated variables analysed, the ones that had a statistically significant association with CMA diagnosis were the SPT with cow's milk and casein and all the sIgE to cow's milk and its fractions. In the evaluated patients, and consistently with available literature, allergic patients had higher levels of sIgE to cow's milk and its fractions although the differences where less notorious on SPT (10).

Comparing the optimal cut-offs for sIgE found in our study with available literature they were similar when using Youden's index criteria (Franco et al. (10); milk: 5.17 kUA/L; α-Lactalbumin: 0.95 kUA/L; β-Lactoglobulin: 0.82 kUA/L; casein: 0.72 kUA/L) but inferior when considering the maximum specificity (Franco et al. (10); milk: 77.7 kUA/L; α-Lactalbumin: 20.7 kUA/L; β-Lactoglobulin: 50.8 kUA/L; casein: 15.9 kUA/L). Recent systematic reviews have reported the heterogeneity in these cut-offs (9) and our study adds more data to

clarify his question. A previous study conducted in our Hospital (18) also found different cut-offs for sIgE levels to cow's milk extract using Youden's index (sIgE milk cut-off: 2.15 kUA/L) and maximum specificity criteria (sIgE milk cut-off: 25 kUA/L) but a different methodology was used in patient inclusion as only were included patients that performed OFC (18).

The optimal cut-offs for SPT found in our study compared with available literature were similar when using Youden's index criteria (Franco et al. (10); milk: 3.5 mm; casein: 3.0 mm) but superior when considering the maximum specificity (Franco et al. (10); milk: 5.0 mm; casein: 10.0 mm).

Our study has some limitations, as not all patients performed OFC to confirm CMA. Nevertheless, with the methodology used, this analysis reflects routine clinical practice, with OFC protocols and postponement of OFC when clinical history is highly suggestive of active CMA. Another potentially limitation is that the accuracy found for SPT and sIgE levels may only apply to patients in a tertiary allergy unit and with a higher risk of having severe manifestations. We may speculate that these results may not be applicable to other clinical settings. Nevertheless, it was used a large sample of consecutive patients with CMA suspicion with different ages what strengthens our conclusions.

From a clinical practice perspective, we must highlight that the definition of optimal cut-offs for sIgE and SPT to cow's milk and correspondent fractions is extremely important. This may avoid stressful and hard to implement cow's milk eviction and, on the other hand, may avoid unnecessary and potentially dangerous oral food challenges (3).

Conclusions

The role of SPT and sIgE to cow's milk and its fractions is unequivocal in CMA follow-up. Moreover, sIgE levels seem to be more discriminatory than wheal diameters of SPT in CMA confirmation. Optimal cut-offs for confirmed CMA are still not universally defined and our study adds data to clarify this question.

Conflict of interests

The authors declare that they have no conflict of interests.

References

1. Luyt D, Ball H, Makwana N, Green MR, Bravin K, Nasser SM, et al. BSACI guideline for the diagnosis and management of cow's milk allergy. Clin Exp Allergy 2014;44(5):642-72.
2. Sicherer SH. Epidemiology of food allergy. J Allergy Clin Immunol 2011;127(3):594-602.
3. Koletzko S, Niggemann B, Arato A, Dias JA, Heuschkel R, Husby S, et al. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI Committee practical guidelines. J Pediatr Gastroenterol Nutr 2012;55(2):221-9.
4. Hochwallner H, Schulmeister U, Swoboda I, Spitzauer S, Valenta R. Cow's milk allergy: from allergens to new forms of diagnosis, therapy and prevention. Methods 2014;66(1):22-33.
5. Fiocchi A, Schunemann HJ, Brozek J, Restani P, Beyer K, Troncone R, et al. Diagnosis and Rationale for Action Against Cow's Milk Allergy (DRACMA): a summary report. J Allergy Clin Immunol 2010;126(6):1119-28 e12.
6. Lebovidge JS, Strauch H, Kalish LA, Schneider LC. Assessment of psychological distress among children and adolescents with food allergy. J Allergy Clin Immunol 2009;124(6):1282-8.
7. Teufel M, Biedermann T, Rapps N, Hausteiner C, Henningsen P, Enck P, et al. Psychological burden of food allergy. World J Gastroenterol 2007;13(25):3456-65.
8. Antolin-Amerigo D, Manso L, Caminati M, de la Hoz Caballer B, Cerecedo I, Muriel A, et al. Quality of life in patients with food allergy. Clin Mol Allergy 2016;14:4.
9. Cuomo B, Indirli GC, Bianchi A, Arasi S, Caimmi D, Dondi A, et al. Specific IgE and skin prick tests to diagnose allergy to fresh and baked cow's milk according to age: a systematic review. Ital J Pediatr 2017;43(1):93.
10. Franco JM, Pinheiro A, Vieira SCF, Barreto IDC, Gurgel RQ, Cocco RR, et al. Accuracy of serum IgE concentrations and papule diameter in the diagnosis of cow's milk allergy. J Pediatr (Rio J) 2018;94(3):279-85.
11. Verstege A, Mehl A, Rolinck-Werninghaus C, Staden U, Nocon M, Beyer K, et al. The predictive value of the skin prick test weal size for the outcome of oral food challenges. Clin Exp Allergy 2005;35(9):1220-6.
12. Calvani M, Alessandri C, Frediani T, Lucarelli S, Miceli Sopo S, Panetta V, et al. Correlation between skin prick test using commercial extract of cow's milk protein and fresh milk and food challenges. Pediatr Allergy Immunol 2007;18(7):583-8.
13. Mehl A, Niggemann B, Keil T, Wahn U, Beyer K. Skin prick test and specific serum IgE in the diagnostic evaluation of suspected cow's milk and hen's egg allergy in children: does one replace the other? Clin Exp Allergy 2012;42(8):1266-72.
14. Celik-Bilgili S, Mehl A, Verstege A, Staden U, Nocon M, Beyer K, et al. The predictive value of specific immunoglobulin E levels in serum for the outcome of oral food challenges. Clin Exp Allergy 2005;35(3):268-73.
15. Rolinck-Werninghaus C, Niggemann B, Grabenhenrich L, Wahn U, Beyer K. Outcome of oral food challenges in children in relation to symptom-eliciting allergen dose and allergen-specific IgE. Allergy 2012;67(7):951-7.
16. Martorell-Aragones A, Echeverria-Zudaire L, Alonso-Lebrero E, Bone-Calvo J, Martin-Munoz MF, Nevot-Falco S, et al. Position document: IgE-mediated cow's milk allergy. Allergol Immunopathol (Madr) 2015;43(5):507-26.
17. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3(1):32-5.
18. Moscoso T, Gaspar-Marques J, Loureiro V, Martins P, Leiria-Pinto P. Determination of specific IgE in children with allergy to cow's milk proteins. Rev Port Imunoalergologia 2013;21(4):283-4.