

Usefulness of analytic tests for the diagnosis of cow's milk protein allergy

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ABSTRACT

Introduction. Cow's milk protein allergy is the most common food allergy among children. It can be diagnosed based on a guided history taking and using an oral food challenge (OFC), serum specific immunoglobulin E levels (sIgE), and skin prick tests (SPT). However, it is difficult to establish their diagnostic performance in the local population. Our objective was to assess the usefulness of tests used to diagnose cow's milk protein (CMP) allergy in the studied population.

Population and methods. Retrospective analysis of data from patients seen at the Unit of Allergy of a tertiary care pediatric hospital between 2015 and 2018. SPT and sIgE tests were done for milk, alpha-lactalbumin, beta-lactoglobulin, and casein, followed by an OFC, and the diagnostic usefulness of each test, as well as their combination, was established.

Results. The tests of 239 patients were assessed. OFC was performed at the hospital in 54.8% of cases, via a rechallenge test at home in 35.5%, and through CMP intake by the mother in 9.6%. The highest specificity was observed with the casein SPT (96.7%; 95% confidence interval [CI]: 90.8-99.3) and the highest sensitivity, with the 4-allergen SPT and sIgE combination (55.3%; 95% CI: 45.7-64.6).

Conclusions. The study established the diagnostic usefulness of SPT and sIgE in the studied population.

Key words: milk hypersensitivity, diagnostic tests, predictive value of tests.

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INTRODUCTION

The worldwide prevalence of food allergy has increased,¹ both in developed and developing countries.² This is specially problematic among children and affects their quality of life.³ Worldwide, between 220 and 250 million people may have food allergy.⁴

Cow's milk protein allergy (CMPA) is the most common food allergy during childhood,⁵ with a prevalence between 0.5 and 3% in developed countries.^{3,5} A study conducted in Argentina reported an 0.8% prevalence.⁶

CMPA is defined as an hypersensitivity response to cow's milk protein, resulting from exposure to these allergens intake.³ Typically, such reaction is mediated by immunoglobulin E (IgE); however, its assessment is complex due to other mechanisms, including non-IgE-mediated or mixed reactions and non-immune mechanisms.^{3,7}

CMPA diagnosis is based on careful assessment, including laboratory tests, and a definite determination using a double-blind, placebo-controlled food challenge (DBPCFC), considered to be the gold standard test.³ In practice, an unblinded oral food challenge (OFC)⁷ may be considered an appropriate option to DBPCFC. However, they are both costly in terms of time and resources and pose a risk for anaphylaxis.^{3,8,9}

Skin prick tests (SPT) and serum specific IgE (sIgE) may be useful to diagnose CMPA, together with symptomatology.

However, their usefulness has not been clearly determined and varies depending on the different publications¹⁰⁻¹⁷ and, to the authors'

knowledge, there are no articles about the diagnostic usefulness of these tests in Argentina.⁵

OBJECTIVE

To assess the usefulness of tests used to diagnose to CMPA in the studied population.

POPULATION AND METHODS

Data collection

The medical records of the hospital's Unit of Allergy were digitized in Excel® and assessed for suspected CMPA in the 2015-2018 period, for a total of 623 records. This was a cross-sectional, retrospective study submitted to and approved by the hospital's Ethics Committee.

The variables included the following data: date of birth and first consultation; symptoms at onset; development of symptoms; test conduct, date, and results; feeding received throughout the different stages of life, and patient follow-up.

Patients in whom an OFC -considered the standard for diagnosis- was recorded were selected for analysis.

Urticaria, angioedema, anaphylaxis, rhinitis, recurrent obstructive bronchitis, asthma, and vomiting were considered a group of IgE-mediated symptoms.

Unit

This is a public children's hospital located in the south of the Autonomous City of Buenos Aires, Argentina.

During the study period, a total of 353 766 first-time consultations were made, with an annual average of 88 442 consultations. In the same period, the Unit of Allergy received 5024 first-time consultations, with an annual average of 1256 consultations.

Diagnostic algorithm

The hospital's Unit of Allergy assesses suspected cases of CMPA in accordance with the DRACMA guidelines,^{7,18,19} as per the algorithm shown in *Figure 1*. It includes an *ad hoc* medical record designed to collect systematic and comprehensive information to reflect the guidelines (*Figure 2*).

Diagnostic tests

SPT: the following commercial allergens (Q allergy) were used: whole milk, alpha-lactalbumin (alpha), beta-lactoglobulin (beta), casein, with negative (saline solution) and positive controls (histamine). Disposable, single

use, sterile lancets (Morrow-Brown model) were used for the procedure. Results were reviewed at 15 minutes. A wheal diameter ≥ 3 mm was considered positive.

sIgE: the Allergen® method is based on a capture enzyme-linked immunosorbent assay (ELISA) that uses a solid phase (strips of wells coated with human anti-IgE antibodies) for all tests with the different allergens (milk, alpha, beta, casein) and calibration curves. Results > 0.35 kU/L were considered detectable.

OFC: a previous 4-week period of strict elimination diet without CMP was required. Patients were assessed for CMPA, regardless of symptoms.

In the case of children who were exclusively breastfed, their mother's diet carefully excluded any CMP product and a calcium supplement was added.

In patients with exclusive formula-feeding or mixed-feeding, the use of a free amino acid formula was indicated to ensure absolute elimination of CMP. A weekly clinical control was conducted.

The diagnosis of CMPA was disregarded in patients who did not show improvement.

OFC was done in patients who showed a significant symptom improvement after the 4-week period. It consisted in an oral challenge with CMP while the patient was hospitalized at the day hospital in a room where oxygen, a laryngoscope, and anaphylaxis medicine (epinephrine injection, antihistamines, corticosteroids, bronchodilators, saline solution) were available and where a venous line was ready to administer drugs.

Oxygen saturation, heart rate, respiratory rate, and symptoms at initiation and after each dose up-titration were monitored.

The initial dose was 20 mL, which was doubled every 20 minutes until reaching a dose adequate for age, weight, and nutritional status.

The test was stopped once symptoms developed; the necessary treatment actions were taken and diagnosis was confirmed.

If no symptoms appeared in the 2 hours after the last administration, the patient was discharged with indications for feeding with CMP, warning signs, and outpatient follow-up at 24 and 48 hours and weekly for a month. If symptoms did not occur, CMPA was ruled out; otherwise, when symptoms occurred again, CMP was eliminated from the diet and diagnosis was confirmed (*Figure 1*).

Statistical analysis

Variables for the 4-allergen SPT combination (combined SPT) and for the 4-allergen sIgE combination (combined sIgE) were developed; the result was considered positive if any assessment was positive, otherwise it was considered negative. The same was done for the combination of the 8 tests (combined SPT + sIgE).

For each test or combination, the area under the curve (AUC), sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) were estimated, considering the cut-off points

described in the diagnostic test section.

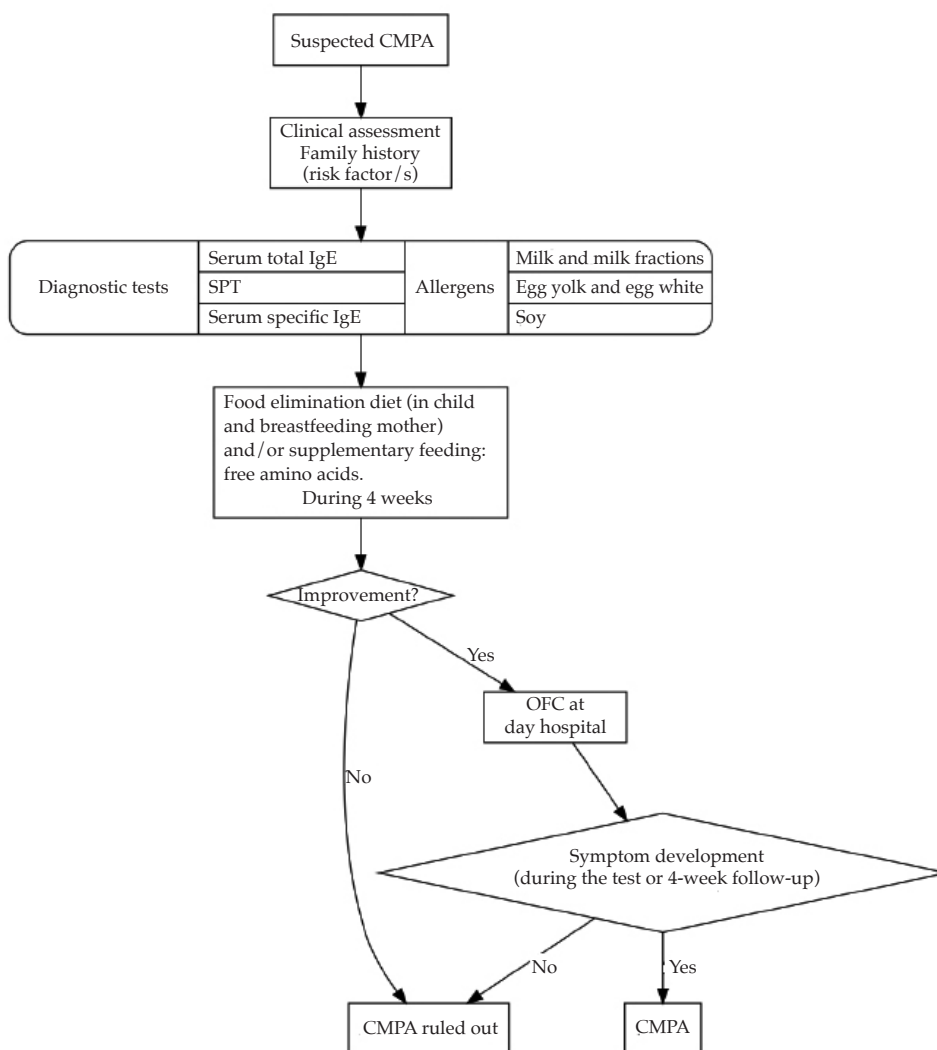
Based on the prevalence of CMPA in the studied population as a pre-test probability, the post-test probabilities were estimated using the maximum LR+ and LR- obtained. Based on these values, Fagan's nomograms were also plotted.²⁰

The Optimal Cutpoints and Uncertain Interval packages, part of the R software®, version 3.6.1, were used.

RESULTS

Out of 623 medical records collected, 239 were selected because the performance of an OFC was verified. Among these, 126 corresponded

FIGURE 1. Diagnostic algorithm for the assessment of cow's milk protein allergy used in the Unit of Allergy of Hospital Elizalde according to the DRACMA guidelines



IgE: immunoglobulin E; SPT: skin prick test; OFC: oral food challenge; CMPA: cow's milk protein allergy.

FIGURE 2. Ad hoc sheet designed and used by the hospital's Unit of Allergy to assess patients suspected of CMPA

History of cow's milk protein allergy (CMPA)														
Date of birth:/...../..... ID no.: Age: Weight: Height:														
Referred by:														
Last names:							First names:							
Address:										Telephone:				
Reason for consultation	Digestive symptoms		Diarrhea		Vomiting		Intestinal bleeding		Reflux		Other			
	Skin symptoms		Urticaria		Angioedema		Atopic dermatitis		Other					
	Respiratory symptoms		ROB/asthma		Rhinitis		Other							
	Growth disorder		Other											
Date of symptom onset:														
Family history of allergy	Eczema		Asthma		Rhinitis		Atopic dermatitis		CMPA		Food allergy			
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No		
Personal history	Gestational age			Weight at birth:										
	Age at initiation		Breastfeeding with maternal diet		Breastfeeding without maternal diet		Breastfeeding and formula-feeding		Formula-feeding					
Previous type of feeding	Term newborn infant													
	Preterm newborn infant													
	0-6 months													
	6-9 months													
	9-12 months													
	> 1 year													
	>5 years													
Skin prick test / /	Histamine		Saline solution		Milk		Alpha		Beta		Casein			
	Egg		Egg yolk		Egg white		Soy							
Specific IgE / /	IgE		Milk		Alpha		Beta		Casein					
	Egg yolk		Egg white		Soy									
Food challenge / /	Previous control	Week 1												
		Week 2												
		Week 3												
		Week 4												
	Symptoms at the time of food challenge													
	Control of course	Week 1												
		Week 2												
		Week 3												
		Week 4												
	Result		Positive						Negative					

to patients with a definite diagnosis of CMPA, resulting in a prevalence of 52.7% (95% confidence interval [CI]: 46.4-59.0%) in the studied population.

The mean age at symptom onset in these patients was 9.1 months and their median age was 5 months (1st quartile = 1.6; 3rd quartile = 10). *Figure 3* shows the distribution of symptoms.

The mean age at the time of the first consultation was 18.8 months and the median age was 10.4 months (1st quartile = 5.4; 3rd quartile = 21).

Before the consultation, patients were receiving different types of feeding. Exclusive breastfeeding was the most common type (41% of cases). The most common sequence before the consultation was breast milk followed by bottle-feeding (53% of cases).

The mean follow-up of patients was 10.36 months, with a median follow-up of 6.1 months.

OFC was done at the hospital in 54.8% of patients. In 35.5%, the occurrence of symptoms at home before rechallenge with CMP was considered a diagnosis. In 9.6%, diagnosis was obtained by reintroducing CMP in the mother's diet, if the patient was exclusively breastfed.

Among positive tests, mediate reactions occurred in 10.5% of patients with IgE-mediated symptoms (n = 163) and in 14% of those with non-IgE-mediated symptoms (n = 76).

Diagnostic usefulness was estimated in the 3 groups (total: 239 patients) and in each subgroup of IgE-mediated and non-IgE-mediated

symptoms.

Results can be observed in *Table 1*. It is worth noting that the highest specificity was obtained in the casein SPT (96.7% specificity) and the highest sensitivity, in the 4-allergen SPT and sIgE combination (55.3% sensitivity).

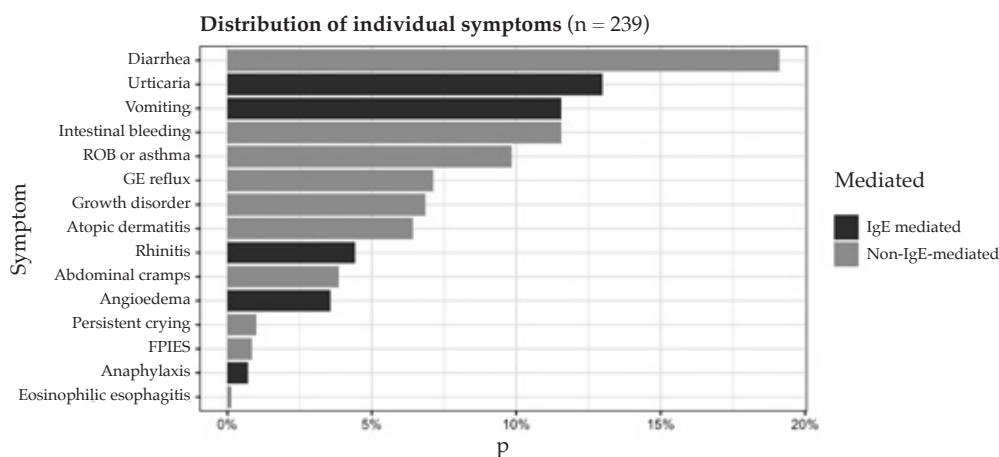
When results are discriminated by IgE-mediated symptoms, the highest specificity value was obtained in the casein SPT (specificity = 93.9%) and the highest sensitivity, in the 4-allergen SPT and sIgE combination (sensitivity = 57.9%). Among patients with non-IgE-mediated symptoms, the highest specificity value was obtained in the beta sIgE (specificity = 97.9%) and the highest sensitivity, in the 4-allergen SPT and sIgE combination (sensitivity = 42.1%). *Figure 4* shows a plot comparing the different diagnostic performances, by type of presentation.

The LR+ and LR- values can be used to plot Fagan's nomograms, which allow to estimate the post-test probability value in a theoretical patient with a pre-test probability equal to the prevalence observed in the studied population. To develop the plot, the test results that obtained the highest LR+ and the lowest LR- were selected (*Figure 5*).

In the case of the casein SPT, the probability of a patient being finally diagnosed with CMPA changes to 83.0%, whereas the probability of ruling out CMPA changes to 49.6%.

However, for the 4-allergen SPT and sIgE combination, these values were 63.5% and 43.5%, respectively.

FIGURE 3. Bar chart showing the proportion of individual occurrence of each symptom in our patients. The dark gray bars indicate probably IgE-mediated symptoms



GE: gastroesophageal; ROB: recurrent obstructive bronchitis; FPIES: food protein induced enterocolitis syndrome; IgE: immunoglobulin E.

DISCUSSION

In general, results showed high specificity and low sensitivity values. This indicates that the tests may be more appropriate to confirm a diagnosis than to screen for CMPA.²¹

The highest specificity values were associated with the use of casein as an allergen, both sIgE and SPT (94.1% and 96.7%, respectively).

The highest sensitivity values were obtained with the combination of at least 1 positive result among the 8 tests (55.3% sensitivity) and, when considered individually, those that used milk as an allergen (SPT = 34.7%, sIgE = 24.6%).

In general, when tests are analyzed by type of symptoms (IgE-mediated and non-IgE-mediated), the highest sensitivity values were observed in the IgE-mediated group, with a higher PPV. The latter is related to a higher prevalence of CMPA in this group.

The high prevalence observed here is explained by the fact that the study site is a referral hospital that receives a large number of patients referred by other units and facilities. This is in line with what has been reported in similar studies.²²⁻²⁷

Other authors have assessed the usefulness of CMPA diagnostic tests, but many times, a direct comparison is not possible because both antigens used and cut-off points for decision-making vary

greatly.¹¹ The cut-off points used here are based on the DRACMA guidelines.¹⁸

In comparison, the cut-off points proposed by other authors are much higher than those established in the guidelines.¹¹

Our results are different from those obtained by other authors that used the same cut-off points, both with the sIgE^{22,26-31} and the SPT.^{10,22-27,29-32} However, in the studies that used the 4 antigens, the highest sensitivity was obtained with milk –as in our study–, whereas the remaining tests showed a higher specificity than sensitivity, both with the SPT^{10,29,32} and the sIgE.²⁹ Furthermore, the greater specificity for SPT resulted in the same sense for casein.^{10,29,32}

The differences mentioned above cannot be explained by the differences in cut-off points because they were the same in those studies.

The commercial reagents and equipment used in different studies vary greatly and, at least to the authors' knowledge, there is no standard that would allow to compare methodologies directly. This is a potential source of variability.

Another potential difference lies in the studied population because it included patients assessed with an OFC, regardless of their symptoms. However, other authors^{25,29} included only patients with symptoms suggestive of an IgE-mediated mechanism.

TABLE 1. Diagnostic performance values obtained with the skin prick test and specific immunoglobulin E, individually and with the different allergens combined

Test	Cutoff point	AUC	Se (%; 95% CI)	Sp	PPV	NPV	LR+	LR-
Milk SPT	3	0.61	34.7 (26.3-44.0)	86.4 (78.2-92.4)	75.0 (63.0-81.5)	53.0 (43.0-68.2)	2.55	0.76
Alpha SPT	3	0.56	21.6 (14.5-30.1)	91.3 (83.6-96.1)	75.8 (60.2-83.1)	48.0 (36.2-68.8)	2.48	0.86
Beta SPT	3	0.56	20.7 (13.7-29.2)	91.6 (84.1-96.3)	75.0 (59.3-82.6)	48.6 (36.6-69.3)	2.46	0.87
Casein SPT	3	0.56	14.5 (8.7-22.2)	96.7 (90.8-99.3)	85.0 (65.2-90.5)	47.1 (33.3-81.5)	4.46	0.88
Casein sIgE	0.35	0.58	21.7 (14.6-30.4)	94.1 (87.6-97.8)	80.7 (64.9-86.8)	51.6 (39.6-74.9)	3.70	0.83
Beta sIgE	0.35	0.57	19.3 (12.5-27.7)	94.1 (87.5-97.8)	78.6 (61.9-85.5)	50.8 (38.2-74.3)	3.25	0.86
Milk sIgE	0.35	0.56	24.6 (17.1-33.4)	87.3 (79.2-93.0)	69.1 (55.4-77.4)	50.0 (38.8-66.1)	1.93	0.86
Alpha sIgE	0.35	0.54	15.5 (9.5-23.4)	92.2 (85.1-96.6)	69.2 (55.3-79.0)	49.0 (35.3-69.6)	1.98	0.92
Combined								
SPT + sIgE	1	0.60	55.3 (45.7-64.6)	64.6 (53.3-74.9)	68.5 (57.6-76.2)	51.0 (41.4-62.9)	1.56	0.69
Combined SPT	3	0.61	39.7 (30.7-49.2)	83.1 (73.7-90.2)	75.4 (63.6-81.9)	51.4 (41.6-66.5)	2.35	0.73
Combined sIgE	0.35	0.58	34.5 (25.8-44.0)	80.8 (71.7-88.0)	67.2 (55.2-75.4)	52.0 (41.7-65.4)	1.80	0.81

CMPA: cow's milk protein allergy, SPT: skin prick tests, sIgE: serum specific immunoglobulin E, Se: sensitivity, Sp: specificity, NPV: negative predictive value, PPV: positive predictive value, LR: likelihood ratio, AUC: area under the curve, Alpha: alpha-lactalbumin, Beta: beta-lactoglobulin.

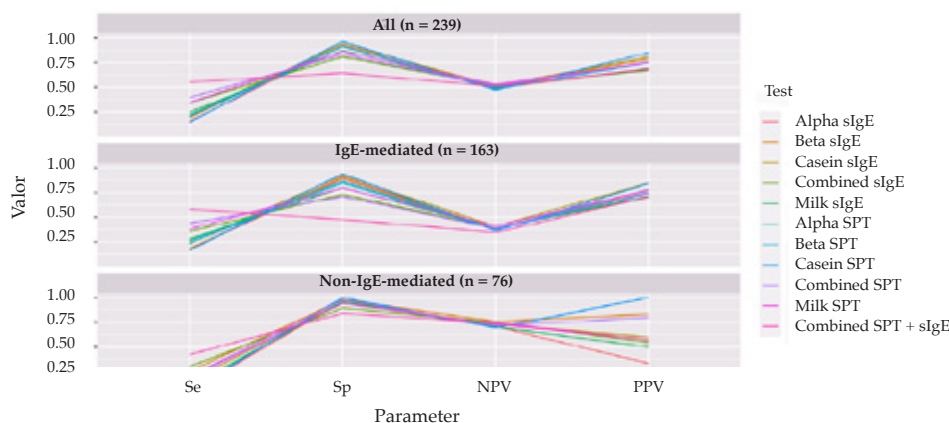
An interesting point that is worth noting is the exclusive use of formula based on amino acids during the elimination diet before the OFC. Such decision is based on the potential development of allergy to hydrolyzed formulas, which may occur in approximately 10% of individuals with CMPA.³³ According to the preceding, and in accordance with the authors' experience, the use of formulas based on amino acids warrants a faster diagnosis and minimizes the impact on the

quality of life of both the family and the patients who continue with allergy symptoms in spite of being fed with extensively hydrolyzed formulas.³⁴

Lastly, it is important to note the population's genetic composition. As mentioned above, no other local study was found to allow the comparison with similar populations.

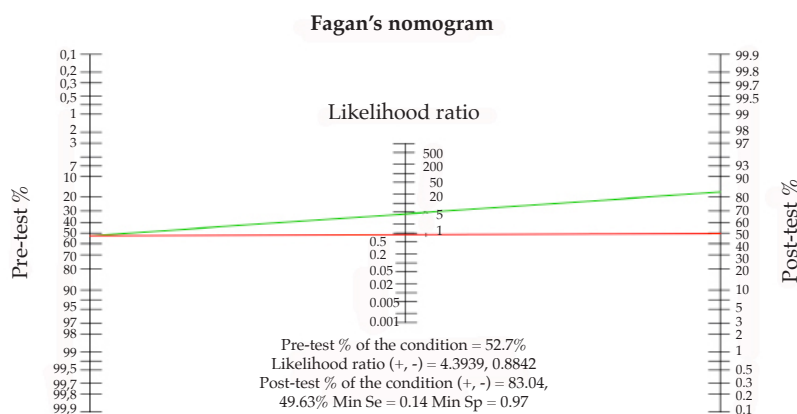
In addition, we should also note that the variability observed here may be due to the limitations implied by a retrospective study.

FIGURE 4. Proportion of each test's usefulness in terms of sensitivity, specificity, negative predictive value, and positive predictive value, by patients with or without probably IgE-mediated symptoms



Se: sensitivity; Sp: specificity; NPV: negative predictive value; PPV: positive predictive value; sIgE: specific immunoglobulin E; Alpha: alpha-lactalbumin; Beta: beta-lactoglobulin; SPT: skin prick tests.

FIGURE 5. Fagan's nomogram showing the change in the probability of diagnosing cow's milk protein allergy, before and after the skin prick test using the antigen casein, regarding the prevalence of allergy at the time of the first assessment at the Unit of Allergy of Hospital Elizalde as the initial probability



The green line accounts for the change in the probability of cow's milk protein allergy (CMPA) after the skin prick test, based on the use of a positive likelihood ratio, applied to a pre-test probability of 52.7%, which corresponds to the baseline prevalence.

Consistent with the higher specificity values observed, PPV were higher than NPV. The study by Calvani et al.,¹⁰ assessed SPT with a cut-off point of 3 mm, and found a better NPV for milk and a better PPV for casein. They also analyzed the combined SPT and found the best NPV. However, predictive values are correlated to the population prevalence³⁵ and are not as useful for comparisons. These authors,¹⁰ based on considerations made by Sampson et al.,²⁷ used individual and combined SPT as predictors of OFC positivity, and found that SPT may be useful to avoid performing an OFC if predicted probability exceeded 95%.

The observed likelihood ratios showed an intermediate or low discrimination power. LR+ values are higher, which is consistent with the better possibility of confirming a diagnosis.³⁶ However, it is interesting to wonder how these tests may be used to make decisions in patients with varying pre-test probabilities, based on the symptoms at onset.

The authors consider that, once SPT and sIgE usefulness values are established in the studied population, these tests may help, together with symptoms that indicate a different probability, to make decisions, especially in relation to patients with IgE-like symptoms, and may render OFC unnecessary if the post-test probability exceeded the 95% threshold.

CONCLUSIONS

This study allowed to establish that, although SPT and sIgE showed a low sensitivity and NPV, they may be useful to make decisions in the studied population.



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