

A TRIAL OF AN ANAMNESIS-BASED SCORE APPLIED AS A DIAGNOSTIC TOOL FOR COW'S MILK PROTEIN ALLERGY IN CHILDREN

Ana Muñoz-Urribarri M.D. Pediatric Gastroenterologist at Hospital Nacional Edgardo Rebagliati Martins. Tutor of Pediatric Gastroenterology Programme at Hospital Nacional Edgardo Rebagliati Martins. Lima-Perú

Aderbal Sabrá M.D. PhD. Professor of Food Allergy and Autism, Universidade do Grande Rio, School of Medicine. Member of the Brazilian Academy of Medicine. Rio de Janeiro-Brazil

Selmá Sabrá M.D. Associated Professor of Pediatrics, Universidade do Grande Rio, School of Medicine. Chief of Pediatric Endoscopy at Hospital Antonio Pedro, Universidade Federal Fluminense. Rio de Janeiro-Brazil

Yovani M. Condorhuamán M.D. PhD. Associated Professor, Faculty of Pharmacy and Biochemistry, Universidad Nacional Mayor de San Marcos. Lima-Perú

Correspondence:

Ana Muñoz-Urribarri M.D. Address: Jr. José del Llano Zapata N° 316 Apt. 603, Miraflores, Lima, Perú. Email ana.munoz.u@upch.pe. Phone +51-1-999397238

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Autorship

Ana Muñoz-Urribarri M.D.

- Substantial contributions to the conception and design of the work; acquisition, analysis, and interpretation of data for the work; AND
- Drafting the work for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Aderbal Sabrá M.D. PhD.

- Substantial contributions to the conception of the work; AND
- Revising the work critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Selmá Sabrá M.D.

- Substantial contributions to conception of the work; AND
- Revising the work critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Yovani M. Condorhuamán

- Substantial contributions to the analysis, and interpretation of data for the work; AND
- Revising the work critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Abstract:

This study presents an anamnesis-based questionnaire as a diagnostic tool for cow's milk protein allergy (CMPA) in children. We applied 24 dichotomous yes/no questions to 51 cases diagnosed by oral challenge and 31 controls. All patients were recruited at the pediatric gastroenterologist outpatient practice. CMPA patients presented with a family history of atopy/autoimmunity, Caesarean delivery, use and/or change of formulas, use of antacids/antibiotics in the first 6 months of life, an overly clean caregiver, multisystem clinical presentation, and the absence of seasonal symptoms. The CMPA group had an average score of 10.4 versus 3.2 for the control group. We identified a cut-off score of 7, which had 94.4% sensitivity and 96.9% specificity to distinguish CMPA from the control population. Cases were younger and showed different symptoms than controls. This study shows the usefulness of an anamnesis-based clinical score to guide the diagnosis of CMPA in children.

Keywords: food allergy, diagnostic score, CMPA in children

What is known:

- The double-blind, placebo-controlled oral challenge is the current gold standard for diagnosing cow's milk protein allergy (CMPA), but it is difficult to implement and potentially life-threatening if an emergency department access is unavailable.
- ☐ Diagnosis is fragmented into subspecialties, which can hinder a consensus.

What is new:

- ☐ An anamnesis-based questionnaire in children with gastrointestinal symptoms is a useful tool for the diagnosis of CMPA.
- ☐ Prospective validation, with stratification by age, is needed to establish the optimal cut-off level in the presumptive diagnosis.

Introduction

Cow's milk protein allergy (CMPA) is increasing in prevalence worldwide, but its diagnosis remains difficult and requires clinical acumen and experience (1,2). Currently, the double-blind oral challenge is the gold standard diagnostic test for CMPA (3).

CMPA mainly affects children and has systemic and pleomorphic manifestations that can lead to an incorrect diagnosis. Thus, it is important to develop a more practical clinical diagnostic tests like those that have been developed for other pleomorphic conditions, such as systemic lupus erythematosus. We investigated application of a questionnaire based on a comprehensive anamnesis comprising genetic-environmental risk factors and clinical presentation typical of CMPA. Our diagnostic instrument is an improvement over previous questionnaires because it integrates risk factors and clinical clues and could be used across the pediatric age range (4, 5).

Methods

This is an observational, cross-sectional study of children aged 0 to 14 years who came to our pediatric gastroenterology outpatient department between 2014 and 2015 at the Golf Clinic, Lima-Perú. We offered the study to all eligible children in that period, and 82 children with digestive symptoms were enrolled.

Fifty one subjects were diagnosed with CMPA based on clinical suspicion and confirmatory open oral challenge (CMPA+), and 31 did not have CMPA (no history of symptoms upon ingestion of milk protein or negative open oral challenge (CMPA-)). The sampling was non-probabilistic. All cases and controls that unequivocally met the definition of CMPA+ and CMPA- were included. Exclusion criteria were known digestive malformations and neurodegenerative disorders.

The protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Universidad Nacional Mayor de San Marcos's Ethics Committee. A letter explaining the rationale of the present study was provided to all enrolled children's parents, and written informed consent was obtained in all cases. All patient-identifiable data were deleted or modified prior to analysis. No additional procedures for the study were performed on the patients.

The symptom-based questionnaire applied in this study was an adaptation of the CMPA score previously developed by one of the authors (Aderbal Sabrá, 2012) and has 26 questions. Three experts validated the relevance, suitability, and clarity of the questionnaire; 24 out of 26 questions were kept and applied to both the 51 CMPA+ and 31 CMPA- patients. All 26 questions were dichotomous (Yes/No). IBM SPSS™ software was used for statistical analysis; $p < 0.05$ was considered to show statistical significance. A Fischer's test was used to study categorical variables, and Anova or Mann-Whitney U test was applied for continuous variables. Results are expressed as mean or median $\pm$ SD. Analysis of receiver operating characteristic (ROC) curves was performed to identify the best cut-off point to distinguish CMPA + from CMPA- cases.

Results

CMPA+ subjects were on average 21.9 ± 30.9 months old. CMPA- cases were significantly older, 96.4 ± 47.1 months of age ($p < 0.05$). No gender difference between CMPA+ and CMPA- cases was found. We grouped subjects into two subgroups: those under 5 years of age (46 CMPA+ cases) and those over 5 years of age (5 CMPA+ cases). Almost all CMPA- cases were in the over-5 group (Table 1, <http://links.lww.com/MPG/C146>).

Responses to 16 of 24 questions differed significantly between CMPA+ and CMPA- subjects. The group with CMPA+ had an average total score of 10.4 ± 2.4 versus 3.2 ± 1.5 for CMPA-,

of a total of 24 possible points ($p < 0.05$). These differences were not consistently maintained when analyzed by subgroups (Table 1, <http://links.lww.com/MPG/C146>).

The ROC curve showed an area under the curve of 0.997 (Figure 1). The cut-off score to distinguish between CMPA+ and CMPA- cases was 7.0 (94.4% sensitivity and 96.9% specificity). The cut-off score to distinguish between CMPA+ and CMPA- was 7.0 in the under-5 group and 6.0 in the over-5 group. Comparing our survey with the gold standard, the under-5 group showed a similar result, with a sensitivity of 93.5 (CI 0.86 -1.01) and a specificity of the 83.3% (CI 0.54-1.13) (positive predictive value 0.98 [CI 0.93-1.02] and negative predictive value 0.83 [CI 0.29-0.96])

The chief complaint differed significantly between CMPA+ and CMPA- subjects. Fifteen of 31 (48.4%) CMPA- patients had functional constipation, based on Rome IV criteria (6). Others presented with vomiting or abdominal pain secondary to acute viral hepatitis (6 patients, 19.3%), non-alcoholic fatty liver disease (4 overweight patients, 12.9%), or rectal bleeding secondary to polyps (6 patients, 19.4%). One-third of CMPA+ patients presented to the pediatric gastroenterologist because of vomiting and/or colic and had been diagnosed with gastroesophageal reflux disease (20 patients, 39.2%). Another third had feces with mucus and/or blood secondary to allergic proctocolitis (16 patients, 31.4%), and a smaller number had poor weight gain due to small bowel enteropathy (9 patients, 17.6%) or Rome IV criteria for functional constipation (6 patients, 11.8%).

Analysis by age subgroups showed even more noticeable differences, specifically between cases and controls under the age of 5. All CMPA+ cases with gastroesophageal reflux disease; enteropathy or allergic proctocolitis were diagnosed in this age group, and only a few cases had constipation. We only had 6 patients in the control group, and their main complaints were constipation and gastroesophageal reflux ($p = 0.03$) (Table 1, <http://links.lww.com/MPG/C146>).

Specific immunoglobulin E for cow's milk protein was found in 7.8% of CMPA+ cases. The diagnosis of CMPA was delayed by 15 ± 27.4 months; in 50% of cases, the diagnosis of CMPA was delayed by ≥ 4 months. One third of the CMPA+ cases had other food allergies, detected by food challenge, to soybean, beans, egg, or vegetables. None of the controls had diagnosed food allergies.

Discussion

These results demonstrate that our anamnesis-based questionnaire is both sensitive and specific to diagnose CMPA in children with gastrointestinal symptoms compared with the gold standard (4,5). Our questionnaire was designed based on extensive personal experience and the scientific literature (7,8,9). Variation in the responses to individual questions of CMPA subjects was found; each child had a unique fingerprint of genetic/environmental backgrounds and clinical clues. Using the aggregate score, we could accurately distinguished CMPA+ from CMPA- subjects accurately, especially in the under-5 age group.

Another advantage is that although the questionnaire was administered by clinicians who have experience diagnosing CMPA, the performance of the instrument is independent of clinical expertise. This is because the questionnaire focuses on the search for historical variables relevant to the development of CMPA and not on symptoms, like current symptom-based questionnaires (10). The symptom-based questionnaires are not sensitive enough as CMPA is pleomorphic, and given the absence of pathognomonic symptoms, they are also not specific enough. Therefore, an empiric trial with an elemental formula is sometimes necessary to diagnose CMPA+ cases.

The questions included in the questionnaire can be used to search for food allergies in general

and not just CMPA. However, several reasons exist to focus on CMPA mainly in children under 5 years of age: 1) CMPA is the most common food allergy in children around the world, 2) insufficient identification of allergen profiles in most countries (11); 3) the possibility of greater nutritional deterioration if approached as food allergy at the outset (12); 4) it is a cost-effective strategy that is useful even in settings that have sufficient resources to do an extensive first approach (13).

Considering CMPA in the initial assessment of a child with gastrointestinal symptoms, with an objective criterion based on a validated clinical score, would bring the enormous benefit of allowing proper and early management of the condition. Application of this tool may reduce the duration of CMPA, the development of concomitant food allergies (if CMPA is treated in a timely fashion), the risk of anaphylaxis to milk protein, and prevent progression of the “atopic march” as prospective cohorts have shown (14). It is also possible that appropriate treatment of CMPA may prevent the future development of inflammatory bowel disease (15) and irritable bowel syndrome (16).

We believe that the most important differential for gastrointestinal symptoms is CMPA versus functional causes, especially in younger children (4,9,17), since the latter do not require intervention but rather plenty of empathy and excellent communication with parents. This is because parents can unfortunately become dissatisfied and make poor decisions for their babies (8), such as stopping breastfeeding or trying many types of formulas or prescriptions including antibiotics for allergic colitis or antacids for physiologic gastroesophageal reflux. CMPA does require intervention, but only the removal of CMP; the earlier it is achieved, the more effective it is (18,19).

We collected a few cases over 5 years of age to test the usefulness of the instrument in this population. Considerations such as cost-effectiveness (13) and avoiding invasive studies should be considered in deciding whether it is appropriate to include this age group in subsequent validations of the questionnaire.

We recognize that our study does have some design limitations. Not stratifying by age and including patients from a pediatric gastroenterology outpatient practice meant that CMPA+ cases were significantly younger than their CMPA- controls. As a result, the under-5 age group did not have enough CMPA controls with physiological gastroesophageal reflux or functional constipation for example. This resulted in unequal distribution of patients by age subgroups. The sample in our study was not randomized, and all the patients were evaluated by a single researcher. This means that there could have been selection bias, but the researcher followed her regular protocol of clinical suspicion followed by oral challenge to make the diagnosis of CMPA. The oral challenge was not double-blinded. However, digestive symptoms resolved with the elimination of CMP in the diet, and reoccurrence after the reintroduction of CMP confirmed the diagnosis.

While the questionnaire performed very well, it is possible that with additional data and further validation in patients with gastrointestinal symptoms and stratifying by age, we will be able to increase its precision and accuracy.

In conclusion, our anamnesis-based questionnaire accurately identified gastrointestinal CMPA in children. We propose that if gastrointestinal discomfort is a perfect model of what is known in science as the “black box” and symptoms cannot guide us properly, a radical global change of perspective is therefore required: if the child has family and/or personal conditions and/or clinical clues for CMPA, CMPA must be considered as a possible cause (20).

Bibliography

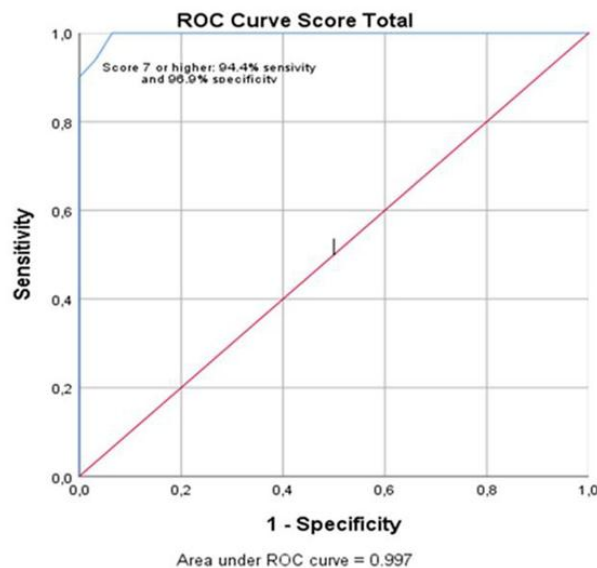
1. Bach JF. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med*. 2002;347(12):911-20.
2. Joyce I. Food allergies in developing and emerging economies: need for comprehensive data on prevalence rates. *Clin Transl Allergy* 2012;2(1): 25.
3. Fiocchi A, Brozek J, Schünemann H, et al. World Allergy Organization (WAO) Special Committee on Food Allergy. World Allergy Organization (WAO) Diagnosis and Rationale for Action Against Cow's Milk Allergy (DRACMA) Guidelines. *Pediatr Allergy Immunol*. 2010;21(Suppl 21):1–125.
4. Gibbons T, Patil S, Frem J. et al. Non-IgE mediated cow milk allergy is linked to early childhood clusters of commonly seen illnesses: A pilot study. *Clin Pediatr (Phila)*. 2012;51(4): 337-344.
5. Vandenplas Y, Althera Study Group, Steenhout P, Grathwohl D. A pilot study on the application of a symptom-based score for the diagnosis of cow's milk protein allergy. *SAGE Open Medicine* 2014;2: 2050312114523423.
6. Benninga M, Nurko S, Faure C et al. Childhood Functional Gastrointestinal Disorders: Neonate/Toddler. *Gastroenterology* 2016; 150:1443–1455
7. Sabrá, Aderbal. Manual de Alergia Alimentar. Rio de Janeiro: Editora Rubio Ltda; 2015.
8. Forsyth BW, McCarthy PL, Leventhal JM. Problems of early infancy, formula changes, and mothers' beliefs about their infants. *J Pediatr* 1985; 106(6):1012-7.
9. Hirsch AG, Pollak J, Glass TA et al. Early-life antibiotic use and subsequent diagnosis of food allergy and allergic diseases. *Clin Exp Allergy*. 2017;47(2): 236-244.
10. Vandenplas Y, Dupont C, Mukherjee R et al. Protocol for the validation of sensitivity and specificity of the Cow's Milk related Symptom Score (CoMiSS) against open food challenge in a single blinded, prospective, multicentre trial in infants. *BMJ Open* 2018;8(5):e019968.
11. Hossny E, Ebisawa M, El-Gamal Y et al. Challenges of managing food allergy in the developing world. *World Allergy Organ J*. 2019;12(11): 100089.
12. Skypala IJ, Venter C, Meyer R et al. 2015. The development of a standardized diet history tool to support the diagnosis of food allergy. *Clin Transl Allergy*. 2015;5:7.
13. Teoh T, Mill C, Chan E, et al. Liberalized versus strict cow's milk elimination for the treatment of children with eosinophilic esophagitis. *Can Assoc Gastroenterol*. 2019;2(2): 81-85
14. Høst A, Halken S, Jacobsen HP et al. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. *Pediatr Allergy Immunol* 2002: 13

(Suppl. 15): 23–28.

15. Virta L, Kautiainen H, Kolho K. Symptoms suggestive of cow milk allergy in infancy and pediatric inflammatory bowel disease. *Pediatr Allergy Immunol.* 2016;27(4):361-7
16. Koloski N, Jones M, Walker M et al. Population based study: atopy and autoimmune diseases are associated with functional dyspepsia and irritable bowel syndrome, independent of psychological distress. *Aliment Pharmacol Ther.* 2019;49(5): 546-555
17. Iacono G, Carroccio A, Cavataio F, Montalto G et al. Chronic constipation as a symptom of cow milk allergy. *J Pediatr.* 1995;126(1): 34-9.
18. Urashima M, Mezawa H, Okuyama M et al. Primary prevention of cow's milk sensitization and food allergy by avoiding supplementation with cow's milk formula at birth: A Randomized Clinical Trial. *JAMA Pediatr.* 2019;173(12):1137-1145.
19. Diaferio L, Caimmi D, Verga M et al. May failure to thrive in infants be a clinical marker for the early diagnosis of cow's milk allergy? *Nutrients.* 2020;12:12.
20. Saps M and Langhaw A. An ounce of prevention may be worth many pounds of cure. *J Pediatr* 2018; 195:13-15.

Figure

Figure 1. Sensitivity and specificity of observed total scores according to gold standard



Table

Table 1. Main Symptom Frequency and Results of Questionnaire in 51 CMPA(+) children compared with 31 CMPA(-) controls

Variable		Below 5 (Age I) n= 52				Above 5 (Age II) n = 30				Total
Description of Variable		Control n=6	Case n=46	p** n=52	Se/Sa*	Control n=25	Case n=5	p** n=30	Se/Sa*	p** n=82
Age	Age in months	25(32.5)	28.5(39)	0.076		112.5(79.2)	100.8(39)	0.516		0
Gender	Frequency of male	5(83.3)	25(54.3)	0.226		15(60)	2(40)	0.628		0.361
Main symptom										
Constipation		4(66.7)	3(6.5)			11(44)	3(60)			0.354
Vomiting		2(33.3)	15(32.6)			3(12)	1(20)			0.028
Abdominal pain/colic		0	3(6.5)	0.003		1(4)	1(20)	0.4		0.4
Faeces with mucus and/or blood		0	16(34.8)			6(24)	0			0
Overweight		0	0			4(16)	0			
Failure to thrive		0	9(19.6)			0	0			
Questionnaire items (Q01-Q24)										
Q01	All three systems affected (gastrointestinal, respiratory, and dermic)	0	30(65.2)	0.004	65.2/100	1(4)	5(100)	0	100/96	0
Q02	Two of the three systems affected (gastrointestinal, respiratory, and/or dermic)	1(16.7)	16(34.8)	0.65	34.8/83.3	5(20)	0	0.556	0/80	0.307
Q03	One of the three systems affected (gastrointestinal, respiratory, or dermic)	4(66.7)	0	0	0/33.3	15(60)	0	0.042	0/40	0
Q04	Gastrointestinal, respiratory, or dermic discomfort all year round, regardless of the season	1(16.7)	46(100)	0	100/83.3	0	5(100)	0	100/100	0
Q05	Persistent facial pallor	1(16.7)	39(84.8)	0.002	84.8/83.3	0	4(80)	1	80/100	0
Q06	Digestive symptoms (severe cramping, reflux, nursing strike, early constipation, blood in stools) with exclusive breastfeeding	1(16.7)	23(50)	0.199	50/83.3	3(12)	2(40)	0.183	40/96	0
Q07	Born by caesarean delivery	3(50)	37(80.4)	0.127	80.4/50	8(32)	4(80)	0.128	80/68	0
Q08	Absence of breastfeeding	0	4(8.7)	1	8.7/100	0	0		0/100	0.292
Q09	Mixed breastfeeding from birth (and stopped breast milk before the first month of life)	0	9(19.6)	0.574	19.6/100	0	0		0/100	0.012
Q10	Cow's milk formula in the first few days of life prior to exclusive breastfeeding	2(33.3)	18(39.1)	1	39.1/67.7	4(16)	2(40)	0.254	40/84	0.087
Q11	Exclusive breastfeeding for 4 months	0	6(13)	1	13/100	4(16)	0	1	0/84	1
Q12	Exclusive breastfeeding for 6 months	3(50)	4(8.7)	0.026	8.7/50	16(64)	1(20)	0.138	20/96	0
Q13	Exclusive breastfeeding for 8 months	1(16.7)	3(6.5)	0.397	6.5/83.3	2(8)	1(20)	0.433	20/92	1
Q14	Mixed breastfeeding until 8 months of age	2(33.3)	12(26.1)	0.655	26.1/66.7	4(16)	2(40)	0.254	40/84	0.442
Q15	Use of antacids or antibiotics in the first six months of life	1(16.7)	20(43.5)	0.382	43.5/83.3	2(8)	1(20)	0.433	20/92	0.003
Q16	Diarrhoea, colds, otitis, or dermal infection in the first six months of life	0	21(45.7)	0.07	45.7/100	0	1(20)	0.167	20/100	0
Q17	Caregiver reports that an excessive standard of cleanliness is maintained for the child and its environment	0	35(76.1)	0.001	76.1/100	2(8)	3(60)	0.022	60/92	0
Q18	Several episodes of colic, vomiting, bloating, dermatitis, rhinitis, or hoarseness while exclusively breastfeeding	1(16.7)	24(52.2)	0.194	52.2/83.3	1(4)	2(40)	0.064	40/96	0
Q19	Parents try different formulas for gastrointestinal "intolerance"	0	40(86.9)	0	87/100	0	2(40)	0.023	40/100	0
Q20	Both parents have an allergy of some kind other than a food allergy (asthma, rhinitis, sinusitis, dermatitis, drug allergy)	0	5(10.9)	1	10.9/100	0	1(20)	0.167	80/100	0.078
Q21	Both parents have a food allergy or had one as a child	0	22(47.8)	0.033	47.8/100	0	1(20)	0.167	80/100	0
Q22	Sibling(s) or only one parent has a food allergy or had one as a child	0	26(56.5)	0.023	56.5/100	0	1(20)	0.167	80/100	0
Q23	Sibling(s) or only one parent has an allergy of some kind other than a food allergy (asthma, rhinitis, sinusitis, dermatitis, drug allergy)	4(66.7)	21(45.7)	0.411	45.7/33.3	5(20)	4(80)	0.019	80/80	0.106
Q24	Any family member, including parents or siblings, diagnosed with an autoimmune disease	0	24(52.2)	0.025	52.2/100	1(4)	2(40)	0.064	40/96	0

Scores reported are n or n(%), otherwise mean or median (standard deviation)

ty; Sp = Specificity

exact test used, otherwise Anova/U-Mann Whitney

*Se=Sensitivity

** Fischer's