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Oral food challenge test to confirm the diagnosis of cow's milk allergy

Maria das Graças Moura Lins,[1] Márcia Raquel Horowitz,[2] Giselia Alves Pontes da Silva,[3] Maria Eugênia Farias Almeida Motta[4]

Abstract

Objective: To determine the prevalence of cow's milk protein allergy in children with symptoms attributed to cow's milk intake.

Methods: Sixty-five children with symptoms attributed to cow's milk intake were studied. Diagnosis was established after an open oral food challenge test carried out at least 15 days after an elimination diet and absence of symptoms, with a follow-up period of up to 4 weeks after the test. The children who remained asymptomatic after this period were considered negative for cow's milk protein allergy (n = 30), while those whose symptoms reappeared were considered positive (n = 35).

Results: The median age was 5 months (P 25-75% 2-9 months) in the case group and 7 months (P 25-75% 4-11 months) in the comparison group (p = 0.05). The test did not confirm cow's milk protein allergy in 46.8% of the patients with symptoms attributed to cow's milk intake. A delayed reaction occurred in 77.1% (27/35) of the cases testing positive, 18/27 in the first week, 3/27 in the second week, and 6/27 in the third week of follow-up. A statistically significant association was found between cutaneous manifestations and positive test result (p = 0.04). However, there was no association with digestive and respiratory symptoms.

Conclusion: Our results confirm the need of an oral food challenge test to determine which patients really have cow's milk protein allergy and may therefore benefit from a diet free of cow's milk.

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Introduction

Prevalence of cow's milk protein allergy (CMPA) is between 2 and 8%.[1,2] Results are conflicting and difficult to compare because of the different diagnostic criteria and study designs used, with prevalence rates being higher when based solely on clinical manifestations (usually parents' perception) than when using more objective diagnostic tools, such as the oral food challenge test.[3-5]

The natural history of CMPA differs from that observed in other food proteins, which occur later in life, since cow's milk protein is the first foreign protein introduced into the

1. Doutoranda, Programa de Pós-Graduação em Saúde da Criança e do Adolescente, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil.
2. Acadêmica, Medicina, UFPE, Recife, PE. Bolsista, PIBIC/FACEPE/CNPq, Brazil.
3. Doutora, Medicina. Professora associada e coordenadora, Programa de Pós-Graduação em Saúde da Criança e do Adolescente, Departamento Materno Infantil, UFPE, Recife, PE, Brazil.
4. Doutora, Medicina. Professora adjunta, Departamento Materno Infantil, UFPE, Recife, PE, Brazil.

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diet of a body that is still maturing its mechanism of oral tolerance to heterologous proteins, and therefore is more likely to develop food allergies.[6]

Thus, it is important to consider the low specificity of symptoms in the diagnosis of CMPA, as they may also indicate other disorders such as gastroesophageal reflux disease, infectious diarrhea, anatomical changes, organic constipation, etc.[6] Moreover, according to evolutionary medicine, the early use of milk-based formula prevents contact between the digestive tract and the bioactive agents contained in breast milk, stimulating the development of symptoms.[7] These agents contribute to the immune maturation and integrity of intestinal mucosa (hindering the occurrence of CMPA) and assist the maturation of motor function (impairing the appearance of symptoms of functional immaturity).[7,8]

Currently, it is difficulty to establish the diagnosis of CMPA, showing low or high prevalence, and causing the impairment of children's nutrition and family's quality of life, especially in cases of misdiagnosis.[9] Therefore, further studies using more appropriate criteria are needed to establish the diagnosis of CMPA. The objective of this study was to determine the prevalence of CMPA in infants with symptoms attributed to cow's milk intake.

Methods

Sixty-six parents of children referred to the outpatient clinic of Pediatric Gastroenterology, Hospital das Clínicas (HC), Universidade Federal de Pernambuco (UFPE), Recife (PE), Brazil, were invited to participate in the present study. The children had adverse symptoms related to cow's milk and milk derivatives consumption by them or their breastfeeding mothers. The patients had cutaneous (atopic dermatitis and urticaria), respiratory (cough and dyspnea, rhinitis) and digestive (regurgitation, vomiting, rectal bleeding, constipation, diarrhea, and proctitis) symptoms. Constipation was determined when the child had bowel movements two or fewer times a week, hard stool, and painful bowel movement. Rectal bleeding was reported by parents as the presence of any amount of blood before or after bowel movement or mixed with the feces. Proctitis was defined on physical examination by the presence of edema and erythema, with or without cleft in the perianal region while the patients or their mothers were still consuming cow's milk and its derivatives. The same patient could present with one or more gastrointestinal symptoms or an association of these symptoms with skin or respiratory symptoms. The patients with other diseases of the digestive tract, multiple food protein allergy, isolated respiratory symptoms and atopic dermatitis, patients using antihistamines, and very low weight preterm infants were excluded.

After signing the written consent form, the parents answered a structured questionnaire containing data on the patients' individual history. Such information was used to evaluate the symptoms and their relation to cow's milk exposure and to record physical examination data, including assessment of nutritional status and total and specific immunoglobulin E (IgE, anti-casein, anti β-lactoglobulin, and anti α-lactalbumin).

Nutritional status was assessed based on age, weight, and height, as recommended by Gibson.[10] Weight was measured using a digital scale (Filizola™) while the child was undressed. Length or height was measured using a length board for children younger than 2 years and a wall-mounted stadiometer (Tonelli®) while the child was barefoot, keeping his/her spine and lower limbs aligned. The anthropometric indices weight-for-age and height-for-age were calculated using the Epi-Info software, version 3.3.2, based on the reference values of the Centers for Disease Control and Prevention (CDC) 2000.[11] The cutoff point for underweight and stunting was percentile < 5.

Total and specific IgE levels were determined using the 3gAllergy™ Specific IgE Universal Kit and Immulite® 2000 3g, an electrochemiluminescence immunoassay with two-step reaction based on a liquid phase technology and coated beads. Total IgE levels were considered high above the cutoff points according to the age groups (newborn to 1 month: 6.1 IU/mL, 1-2 months: up to 15 IU/mL; 3 months to 5 years: up to 60 IU/mL), and for specific IgE, the cutoff point was equal to or above class 3 (3.5 to 17.5 kU/L).

The open oral food challenge test was carried out to establish the diagnosis of CMPA: children and mothers, while still nursing, were instructed to follow a diet free of cow's milk and its derivatives for at least 2 weeks, using soy protein isolate formula. All patients followed a pre-test elimination diet properly. If there was symptom remission, the children were admitted to the Clinical Pediatrics ward of HC/UFPE for 24 hours with the purpose of undergoing the open oral food challenge test modified from Isolauri & Hill,[12] Sampson,[13] and Chapman,[14] as follows: 1) time 0: about 2 mL of cow's milk was administered to the skin of the left forearm; 2) time 15': about 2 mL of cow's milk was administered to the perioral region; 3) from the time 30' every 15 minutes, cow's milk was gradually offered in portions of 1, 4, 10, 20, 20, 20, and 25% of the total volume calculated (0.5 g of cow's milk protein without lactose/kg) up to the onset of symptoms. The challenge test was discontinued when the patients had the same symptom reported by their family before undergoing the challenge test, including those patients allocated in the CMPA group. If there were no symptoms during hospitalization, the patients continued to consume the usual pre-test amount of cow's milk protein, two to three times a day. A weekly follow-up visit at the outpatient clinic was scheduled to check for the presence of symptoms up to 4 consecutive weeks. Those patients who had the same symptom reported before the challenge test during the follow-up period were

included in the CMPA group after a 4-week follow-up, and the patients who had no symptoms were allocated in the group without CMPA. There were no symptoms different from those present before the test. The patients without CMPA were referred to their original health care facilities for further investigations; while the patients diagnosed with CMPA were followed up at the outpatient clinic of Pediatric Gastroenterology of HC/UFPE.

This study was approved by the Research Ethics Committee of the Center of Health Sciences (CEP/CCS) of UFPE under the no. 197/06.

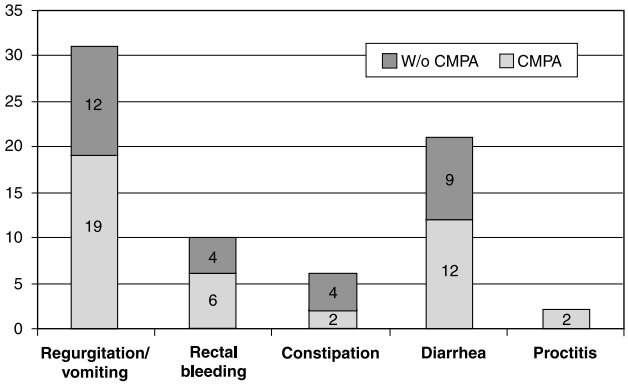
The data of 65 patients collected using forms and additional tests were stored (coding the categorical variables) in a data file prepared using the statistical software Epi-Info, version 3.3.2 for Windows, which was used to carry out the statistical analysis. The ages of the comparison groups were summarized as medians and compared using the Mann-Whitney test. The differences between the categorical variables were determined by the chi-square test. The statistical tests were considered to be significant when $p \leq 0.05$.

Results

Upon inclusion in the study, the patients' age was 5 months (P 25-75% 2-9 months) in the CMPA group and 7 months (P 25-75% 4-11 months) in the group without CMPA ($p = 0.05$). About 50% of the patients with symptoms attributed to cow's milk intake did not confirm CMPA in the oral food challenge test. In most cases the patients reported several symptoms at inclusion in the study; however, after undergoing the oral food challenge test, 24 out of 35 patients presented with one positive symptom, and 11 out of 35 patients presented with two positive symptoms. A delayed reaction occurred in 77.1% (27/35) of the cases testing positive; 18/27 in the first week, 3/27 in the second week, and 6/27 in the third week of follow-up.

As shown in Table 1, we found a statistically significant association between cutaneous manifestations and positive oral food challenge test. The presence of digestive and respiratory symptoms was not statistically associated with the confirmation of the CMPA diagnosis.

Total IgE was high in 17/35 patients (33.8%) in the CMPA group and in 5/30 (16.7%) patients of the group without CMPA, but specific IgE levels were high only in the CMPA group: 3/35 (8.6%) for casein, 3/35 (8.6%) for α-lactalbumin, and 5/35 (14.2%) for β-lactoglobulin. Two of them had vomiting and poor weight gain associated with urticaria, vagal symptoms (pallor and sweating); and three patients only had urticaria. Figure 1 shows the number of patients with digestive signs and symptoms in both groups.



CMPA = cow's milk protein allergy; W/o CMPA = without cow's milk protein allergy.

Figure 1 - Digestive signs and symptoms of patients after oral food challenge test

Table 1 - Demographic and clinical characteristics of children with symptoms suggestive of CMPA based on the confirmation of CMPA

Patients	CMPA		p
	Yes (n = 35), n (%)	No (n = 30), n (%)	
Female	19 (54.2)	18 (60.0)	0.83
Nutritional status			
Weight-for-age (percentile < 5)	7 (20.0)	6 (46.2)	0.75
Height-for-age (percentile < 5)	6 (17.1)	1 (3.3)	*
Symptoms			
Digestive	25 (71.4)	22 (73.3)	0.91
Respiratory	7 (20)	9 (30)	0.52
Cutaneous	24 (68.6)	12 (40.0)	0.04

CMPA = cow's milk protein allergy.
* We did not carry out any statistical tests because the number of patients was not enough to fill out one of the quads.

Discussion

CMPA was not confirmed by the oral food challenge test in almost half (46.8%) of the children with symptoms attributed to cow's milk intake. The patients reported several symptoms at inclusion in the study; however, when the oral food challenge test was carried out, 24/35 patients had one positive symptom, and 11/35 had two positive symptoms, confirming that there are excessive complaints related to cow's milk intake. Such finding shows that even in the presence of a clinical history suggestive of CMPA, further investigations should be performed using the oral food challenge test to confirm the diagnosis.[6,15] CMPA was more prevalent among the youngest patients, and the frequency distribution of gastrointestinal, cutaneous, and respiratory symptoms was similar to that found by Host.[16]

CMPA occurs in a body that has not developed oral tolerance. The factors that contribute to the development of oral tolerance are genetics, intestinal microbiota, and patient's age, as well as the factors associated with antigens.[17] Early in life, exposure to food antigens introduced to the immune system activates regulatory T lymphocytes, causing suppression of the immune response and induction of oral tolerance.[18] This process occurs naturally in most children who are exclusively breastfed, exposing their intestinal mucosa to low doses of food antigens present in breast milk, which induce active suppression of immune reactions by secreting mucous of the transforming growth factor beta (TGF-beta).[18] However, increased intestinal permeability enables the development of CMPA, particularly when heterologous protein is offered to children.[19]

In addition to being in continuous contact with foreign proteins during a phase of vulnerable intestinal permeability, children who are not breastfed do not take advantage of the benefit provided by the bioactive agents contained in breast milk for protection against CMPA.[7] TGF-beta, a cytokine present in breast milk, is involved in the production of immunoglobulin A (IgA) by the intestinal mucosa and in the cell suppression of immune response; reduced levels of IgA promote allergic sensitization and CMPA.[20]

The intestinal microflora acquired in the postnatal period is also required for the development of oral tolerance and regulatory T lymphocytes' expression and function.[21] Exclusive breastfeeding is responsible for the bacterial colonization by the genus bifidobacterium, which actively participates in oral tolerance.[22] These bacteria are reduced in children who consume milk-based formulas, thus promoting the occurrence of CMPA.[22,23]

About 50% of the patients with symptoms attributed to cow's milk intake did not confirm CMPA in the oral food challenge test. This finding draws attention to the fact that the presence of symptoms does not always imply the presence of disease, but it may indicate an evolving functional maturation process. Therefore, physicians are responsible for assessing the parents' interpretation and differentiating between good health and disease.[7,8,24] It is natural for the parents to associate the presence of symptoms in young children with cow's milk intake because, in most cases, it is the sole or main food consumed within short periods of time. The symptoms may depend on the early introduction of milk-based formula, which impairs the maturation of the gastrointestinal motor function due to the absence of stimulation of the bioactive agents of breast milk in the body.[7] Thus, the symptoms in healthy infants are probably not a sign of disease in most situations and they may suggest a problem of immature functional development, coupled with the consumption of milk-based formula. Therefore, regurgitation may result from the increase in time of gastric emptying and shorter intervals between feedings promoted by the use of milk-based formulas; diarrhea may result from solute overloading in the preparation of artificial milk or overeating at short time intervals; difficult bowel movement may indicate lack of coordination between intra-abdominal pressure and relaxation of the pelvic floor in the first months of life; constipation may start with the change from breast milk to milk-based formula or introduction of solids or milk thickeners; and none of these situations mean that the child has a disease, instead they are signs of organic immaturity and/or feeding error.[24-27]

In practice, laboratory tests (skin test, IgE levels) only identify the sensitization by means of IgE positivity and IgE-mediated possible immediate reaction, with negative results in children with gastrointestinal symptoms and cell-mediated possible delayed reaction. The IgE level is associated with persistence of CMPA, particularly in the IgE-mediated mechanism, and follow-up of patient during the atopic march, with prolonged duration of clinical manifestations of CMPA and onset of other future allergic manifestations.[28,29] Absence of symptoms in the short term, even in the initial months, suggests a higher likelihood of developing oral clinical tolerance, which is more often related to cell-mediated mechanisms.[30] Therefore, we cannot consider that symptomatic patients do not have short-term CMPA while immune system maturation and oral tolerance are not reached.[28]

During a diet free of cow's milk, those patients who were not exclusively breastfed used soy protein isolate formula as a substitute for 15 days before undergoing the oral food challenge test. Although the literature does not recommend offering this type of formula to infants younger than 6 months with gastrointestinal clinical manifestations, since there is a risk of the patients also having soy protein allergy (10 to 14% of cases), the clinical situations mentioned above are characterized by a high degree of intestinal permeability and severity of clinical manifestation, severe nutritional impairment (eosinophilic gastrointestinal disease, food protein-induced enterocolitis syndrome, allergic proctocolitis), which was not the case of

our patients, who had isolated symptoms, without presenting any of these specific clinical conditions.[6,31-33] As included in the Brazilian Consensus on Food Allergy, supported by international medical associations, these formulas can be used when managing cell-mediated mild clinical allergies.[34] The follow-up of those participants whose oral food challenge test result was positive showed symptom remission with favorable clinical outcome.

The oral food challenge test is still the best method to demonstrate the causal relation between food antigens and symptoms. Our results corroborate the need of using the oral food challenge test to determine in a more accurate manner which patients actually have CMPA and who will benefit from a diet free of cow's milk.

References

1. Host A. Frequency of cow's milk allergy in childhood. Ann Allergy Immunol. 2002;89:33-7.
2. Sicherer SH, Sampson HA. Food allergy: recent advances in pathophysiology and treatment. Annu Rev Med. 2009;60:261-77.
3. Rona RJ, Keil T, Summers C, Gislason D, Zuidmeer L, Sodergren E, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol. 2007;120:638-46.
4. Aardoom HA, Hirasing RA, Rona RJ, Sanavro FL, van den Heuvel EW, Leeuwenburg J. Food intolerance (food hypersensitivity) and chronic complaints in children: the parents' perception. Eur J Pediatr. 1997;156:110-2.
5. Zuberbier T, Edenharter G, Worm M, Ehlers I, Reimann S, Hantke T, et al. Prevalence of adverse reactions to food in Germany - a population study. Allergy. 2004;59:338-45
6. Boyce JA, Arshad SH, Assa'ad A, Bahna SL, Beck LA, Burks AW, et al. Guidelines for the diagnosis and management of food allergy. 2009. p. 119. http://www3.niaid.nih.gov/topics/foodAllergy/clinical/Who/comments.htm. Access: 30/03/2010.
7. Goldman AS. Modulation of the gastrointestinal tract of infants by human milk. Interfaces and interactions. An evolutionary perspective. J Nutr. 2000;130:S426-31.
8. Udall JN, Colony P, Fritze L, Pang K, Trier JS, Walker WA. Development of gastrointestinal mucosal barrier. II. The effect of natural versus artificial feeding on intestinal permeability to macromolecules. Pediatr Res. 1981;15:245-9.
9. Gupta RS, Kim JS, Barnathan JA, Amsden LB, Tummala LS, Holl JL. Food allergy knowledge, attitudes and beliefs: focus groups of parents, physicians and the general public. BMC Pediatr. 2008;8:36.
10. Gibson RS. Evauation of anthropometric indices. In: Gibson RS. Principles of nutritional assessment. New York: Oxford University; 1990. p. 248-61.
11. Centers for Disease Control and Prevention (CDC-US). 2000 CDC growth charts: United States. http://cdc.gov/growthcharts. Access: 18/01/2002.
12. Isolauri E, Hill DJ. Guide for paediatricians on the diagnosis and treatment of severe cow milk allergy and multiple food protein intolerance in infancy. SHS; 1997.
13. Sampson HA. Update on food allergy. J Allergy Clin Immunol. 2004;113:805-19.
14. Chapman JA, Bernstein L, Lee RE, Oppenheimer J, Nickals RA, Portnoy JM et al. Food allergy: a practice parameter. Ann Allergy Asthma Immunol. 2006;96:S1-68.
15. Nowak-Wegrzyn A, Assa'ad AH, Bahna SL,.Bock SA, Sicherer SH, Teuber SS, et al. Work Group report: oral food challenge testing. J Allergy Clin Immunol. 2009;123:S365-83.
16. Host A. Cow's milk protein allergy and intolerance in infancy. Some clinical, epidemiological and immunological aspects. Pediatr Allergy Immunol. 1994;5:1-36.
17. Chehade M, Mayer L. Oral tolerance and its relation to food hypersensitivities. J Allergy Clin Immunol. 2005;115:3-12.
18. Peron JP, de Oliveira AP, Rizzo LV. It takes guts for tolerance: the phenomenon of oral tolerance and the regulation of autoimmune response. Autoimmun Rev. 2009;9:1-4.
19. Weaver LT, Laker MF, Nelson R. Intestinal permeability in the newborn. Arch Dis Child. 1984;59:236-41.
20. Kalliomäki M, Ouwehand A, Arvilommi H, Kero P, Isolauri E. Transforming growth factor-beta in breast milk: a potential regulator of atopic disease at an early age. J Allergy Clin Immunol. 1999;104:1251-7.
21. Ishikawa H, Tanaka K, Maeda Y, Aiba Y, Hata A, Tsuji NM, et al. Effect of intestinal microbiota on the induction of regulatory CD25+ CD4+ T cells. Clin Exp Immunol. 2008;153:127-35.
22. Tannock GW. What immunologists should know about bacterial communities of the human bowel. Semin Immunol. 2007;19:94-105.
23. Björkstén B, Naaber P, Sepp E, Mikelsaar M. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. Clin Exp Allergy. 1999;29:342-6.
24. Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiau J. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology. 2006;130:1519-26.
25. Issenman RM, Hewson S, Pirhonen D, Taylor W, Tirosh A. Are chronic digestive complaints the result of abnormal dietary patterns? Diet and digestive complaints in children at 22 and 40 months of age. Am J Dis Child. 1987;141:679-82.
26. Douglas PS. Excessive crying and gastro-oesophageal reflux disease in infants: misalignment of biology and culture. Med Hypotheses. 2005;64:887-98.
27. Heacock HJ, Jeffery HE, Baker JL, Page M. Influence of breast versus formula milk on physiological gastroesophageal reflux in healthy, newborn infants. J Pediatr Gastroenterol Nutr. 1992;14:41-6.
28. Shek LP, Soderstrom L, Ahlstedt S, Beyer K, Sampson HA. Determination of food specific IgE levels over time can predict the development of tolerance in cow's milk and hen's egg allergy. J Allergy Clin Immunol. 2004;114:387-91.
29. Jarvinen KM, Beyer K, Vila L, Chatchatee P, Busse PJ, Sampson HA. B-cell epitopes as a screening instrument for persistent cow's milk allergy. J Allergy Clin Immunol. 2002;110:293-7.
30. Sicherer SH, Sampson HA. Cow's milk protein-specific IgE concentrations in two age groups of milk-allergic children and in children achieving clinical tolerance. Clin Exp Allergy. 1999;29:507-12.
31. Bhatia J, Greer F. Use of soy protein-based formulas in infant feeding. Pediatrics. 2008;121:1062-8.
32. ESPGHAN Committee on Nutrition, Agostoni C, Axelsson I, Goulet O, Koletzko B, Michaelsen KG, et al. Soy protein infant formulae and follo-on formulae: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2006;42:352-61.
33. National Institute of Environmental Health Sciences, National Institutes of Health. Draft NTP brief on soy infant formula. 2010. 47p. http://ntp-server.niehs.nih.gov/?objectid=716A5EEB-F1F6-975E-781CA5472900FCAA. Access: 01/04/2010.
34. Solé D, Silva LR, Rosário Filho NA, Sarni RO, Sociedade Brasileira de Pediatria e Associação Brasileira de Alergia e Imunopatologia. Consenso Brasileiro sobre alergia alimentar 2007. Rev Bras Alerg Imunopatol. 2008;31:65-89.

Correspondence:
Maria das Graças Moura Lins
Rua Cap. Sampaio Xavier, 205/1801 - Graças
CEP 52050-210 - Recife, PE - Brazil
Tel.: +55 (81) 3427.3170, +55 (81) 3223.5052
E-mail: gracaml@gmail.com