Cow's Milk–Associated Gastrointestinal Symptoms Evaluated Using the Double-Blind, Placebo-Controlled Food Challenge

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ABSTRACT

Objective: The aim of this study was to evaluate the suspicion of cow's-milk allergy in infants with unspecific gastrointestinal (GI) symptoms using the double-blind, placebo-controlled food challenge.
Methods: A prospective cohort study, which recruited 57 consecutive children with gastrointestinally manifested symptoms suspected of cow's-milk allergy. All patients underwent a 5-day double-blind, placebo-controlled food challenge for cow's milk.
Results: The median age of the patients was 8.7 months. None had measurable cow's-milk–specific IgE. The food challenge was positive in 18 (32%) cases, with symptoms manifesting within 48 hours in 17 of 18 cases. The only symptom that correlated with the positive challenge was loose stools, reported as a presenting symptom in 78% of challenge-positive and in 46% of challenge-negative children ($P = 0.043$). During active challenge, the respective proportions were 82% and 2% ($P < 0.0001$). No serious adverse effects were manifested during the challenges. In the challenge-negative group, significant placebo reactions occurred in 18 (46%) patients. In the challenge-negative children, adult-type hypolactasia genotype CC frequency was higher (31%, $P = 0.033$) than national prevalence of 18%.
Conclusions: In an infant with unspecific GI symptoms suspected of cow's-milk allergy, this diagnosis is seldom confirmed. Other reasons for the troublesome GI symptoms should also be identified.

Key Words: adult-type hypolactasia, cow's-milk allergy, double-blind placebo-controlled food challenge

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C ontroversy exists regarding whether gastrointestinal (GI) symptoms related to cow's-milk protein (CMP) in infancy can be diagnosed as CMP allergy (CMPA) in the absence of cow's-milk–specific IgE (1,2). In infancy, the most common GI symptoms that clinicians associate with CMPA include diarrhea/loose stools, vomiting or gastroesophageal reflux disease, and colicky crying. Researchers have estimated the occurrence of colicky crying between 5% and 20%, depending on the diagnostic criteria and the study setting (3). Although gastroesophageal reflux symptoms are common in infancy, their association with CMPA remains controversial (1,4). The occurrence of CMPA in young children younger than 2 years is only 1.5% to 2.2% (5–7). CMPA may manifest itself in the GI tract in 20% to 50% of patients, and such presentations are usually non-IgE mediated (6,7). The most severe form of gastrointestinally manifested CMPA is food protein–induced enterocolitis syndrome (FPIES), the prevalence of which was estimated recently at 0.34% (8). In present-day clinical work, however, the majority of CMPA suspicions are based on less severe symptoms. Suspicion of CMPA is high: up to 3-fold compared with physician-confirmed diagnoses (9,10). GI manifestations of suspected CMPA impose a significant burden on families with young children. There is evidence that the psychosocial distress is connected to the fear of possible adverse reactions and the need to avoid particular foods, in addition to the actual symptoms (11,12). Unnecessarily avoiding particular foods may be protracted, even after a negative food challenge test, because of parental anxiety (13,14). Avoiding CM is the only known remedy for CMPA. In the above-mentioned functional GI disorders, such diet restrictions are not evidence based. Studies addressing the diagnostics of GI-manifested CMPA (in comparison with the more researched IgE-mediated CMPA) are lacking. The double-blind, placebo-controlled food challenge (DBPCFC) is considered the criterion standard for food allergy, but its use and detailed protocols in CMPA are not standardized (15).

We hypothesized that by combining clinically available laboratory testing with a well-documented DBPCFC, we could identify parameters associated with gastrointestinally manifested CMPA. In this way, the diagnosis of CMPA and the need for strict CM avoidance could be ascertained. This article describes the characteristics of our prospective cohort of infants with GI symptoms undergoing DBPCFC for CM.

METHODS

Study Protocol

We prospectively recruited children who had been referred to the Helsinki University Central Hospital during the year 2010 for suspicion of CMPA with predominantly GI symptoms. All of the children and lactating mothers had eliminated CM from their diet a minimum of 2 weeks before the challenge. We only included

patients whose symptoms had improved as a result of this diet. Skin manifestations were not an exclusion criterion as long as the main symptom was GI. Each child underwent skin prick tests (SPTs) to common food allergens (including CM and cereals). Blood and stool specimens were collected at baseline (during CM-elimination diet), and after both active and placebo challenges. All of the children underwent a full clinical examination before the DBPCFC. One pediatrician (L.M.-S.) supervised the DBPCFCs. We contacted the families a minimum of 6 months after the challenge to ascertain the CM tolerance and address other possible health issues. We invited all of the symptomatic and DBPCFC-positive patients for a personal visit. Those without symptoms at the time of the 6-month follow-up were interviewed by telephone.

DBPCFC

Challenge formulas were prepared at the clinic by a ward assistant who was not involved in patient care. As a placebo, we used the tolerated hypoallergenic formula that the patient used during the elimination period. The CMP-containing active formula was prepared by mixing a CM-based formula (NAN1, by Nestle Finland, Espoo) into placebo formula powder, with a ratio of 1:2. The first day's dose was 100 mL. For challenge days 2 to 5, the powders were stored in 4 bags, with each bag containing powder for 600-mL milk. The bags containing either active or placebo formula powder were labeled either A or B. The code for the blinding was kept in a sealed envelope labeled with the child's identification. The parents were given verbal and written instructions concerning the dilution and use of the study formulas.

The babies with suspicion of CMA symptoms elicited through breast milk, who were still exclusively (±solids) breast-fed (n = 9), had first a trial of an extensively hydrolyzed formula to ascertain tolerance and acceptability (unless an amino acid formula had been tested and tolerated before). During the challenge procedure, the amount of study milk actually given to a breast-fed child was 2 to 3 dL/day, well above the amount of possible residual CMP in breast milk. Mothers expressed and stored breast milk during the challenge, to minimize the risk of weaning. The 2 infants refusing the bottle were withdrawn from the study.

On day 1, we orally gave 10 mL of formula A to the patients at the outpatient clinic. After 60 minutes, if the child was well, 50 to 100 mL of formula A was given orally. After that, the child was followed up on for a minimum of 2 hours before discharge. The next day, the parents were instructed to give 3 to 6 dL of study formula to the child every day for the next 4 consecutive days. This same procedure was repeated with formula B after 1 to 2 weeks, with a minimum of 7-day symptom-free time in between the formulas. We instructed the parents to contact us in case the formula had to be discontinued because of symptoms. Postprovocation laboratory testing was done either 4 to 6 days after formula had been started, or else on the day the challenge formula was discontinued, if that happened earlier. The code was broken by the supervising pediatrician during the after-challenge appointment if either formula had provoked meaningful symptoms in the child. If no symptoms occurred, the code was not broken except for research purposes, and the challenge deemed negative. The challenge-negative participants were instructed to reintroduce CM formula (lactating mothers could use CM in their own diet) gradually, starting with a mix of 1:1 hypoallergenic formula and CM formula. The challenge-positive participants were instructed to continue the CM-elimination diet, although in the absence of immediate severe symptoms in the DBPCFC we recommended conducting rechallenges at home with small amounts of CM after 3 months.

Control Group

A control group of 22 children was recruited from patients (age 0–4 years) attending the Allergology Clinic for any other reason but atopic diseases or food allergy. Typical diagnoses were suspected antibiotic allergy and nonatopic wheezing. The median age in this group was 13.2 months (range 4.8–40).

Laboratory Tests

We determined serum CM-specific IgE and total IgA, full blood counts and hemoglobin, and lactase CC_{13910} genotyping using routine, commercially available methods. The milk protein IgG and IgA antibodies were measured by the enzyme-linked immunosorbent assay technique using an adapted infant formula to coat the microtiter plates. Values are expressed as percentage of the standard with an extremely high titer of CM antibodies. The major antigen in the formula was casein (16).

SPTs were carried out on the volar aspect of the forearm with positive control (histamine hydrochloride 10 mg/mL, ALK-Abello, Hørsholm, Denmark), negative control (buffer solution, ALK-Abello) and CM formula. The SPTs were read after 15 minutes. The wheal's longest and shortest perpendicular axes were measured, and the results were expressed as the mean wheal diameter in millimeters. Reactions with a mean wheal diameter of ≥3 mm were considered positive.

Statistical Analyses

We analyzed categorical data between 2 groups, such as CMPA symptoms, using Fisher exact χ^2 test. Comparisons among ≥3 groups were analyzed using χ^2 test. Proportional data, such as the prevalence of the lactase genotype, were analyzed using a proportional χ^2 test. We conducted all of the analyses using GraphPad Prism software (version 5.0 for Mac; GraphPad Software Inc, San Diego, CA).

The Helsinki University Hospital ethics committee approved this study. Both legal guardians signed a written informed consent before the child was enrolled in the study.

RESULTS

We recruited 68 patients of which 57 underwent the DBPCFC within the study protocol. Eighteen (32%) children had a positive DBPCFC result (Table 1). At the time of the DBPCFC, the patients' median age was 8.7 months (range 2.4–40.8). CMP had been eliminated from the child's and lactating mother's diet for a median 2.5 months before the DBPCFC, with perceived positive response. Other demographic data are presented in Table 1. The results from laboratory parameters associated with CMPA are listed in Table 2 (16). None of the patients were positive for CM–specific IgE. Eleven noncompliers withdrew from the study for various reasons: 3 ingested CM at home/day care without any symptoms recurring, 2 infants refused the bottle and the challenge had to be discontinued, 1 withdrew after symptomatic A formula (active challenge), and for the remaining 6, the DBPCFC was not concluded within the study setting because of the refusal to undergo further blood tests or difficulties in scheduling the test dates.

In the DBPCFC-positive group, the only presenting symptom reported significantly often as leading to the suspicion of CMPA was loose stools, in 14 of 18 (78%) children (Table 3). Loose stools were reported during the active CM challenge in all of them. During the placebo challenge, loose stools were reported only for 1 patient in the positive challenge group (with lower stool frequency than during the active challenge), whereas in the challenge-negative

TABLE 1. Characteristics of study patients undergoing the double-blind, placebo-controlled food challenge for cow's milk

	All patients n = 57	Challenge negative, n = 39	Challenge positive, n = 18	P^*	Controls (with no food allergies), n = 22
Age (median, range), mo	8.7 (2.4–40.8)	8.7 (2.5–25.6)	8.4 (2.4–40.8)	NS	13.2 (4.8–30)
Boys, n (%)	28 (50)	19 (50)	9 (50)	NS	13 (59)
Maternal age (median, range), y	33 (22–41)	33 (22–41)	32.5 (25–39)	NS	33 (22–41)
Maternal education (college), n (%)	27 (47)	21 (54)	6 (33)	NS	ND
Firstborns, n (%)	24 (42)	19 (49)	5 (28)	NS	ND
No. using extensively hydrolyzed formula[†] (%)	39 (68)	27 (70)	12 (66)	NS	NA
No. using amino acid formula[†] (%)	11 (19)	8 (20)	3 (17)	NS	NA
No. using soy formula[†] (%)	7 (12)	4 (10)	3 (17)	NS	NA
Duration of cow's-milk–free diet before challenge (median, range), mo[‡]	2.5 (0.5–35)	2.4 (0.5–14)	2.5 (0.5–35)	NS	NA
Cow's milk protein not tried at 6-month follow-up[§], n (%)	14 (25)	2 (5)	12 (67)	<0.01	NA

NA = not applicable; ND = not determined; NS = not significant.
[*] Statistical difference between the challenge-negative and -positive groups.
[†] Including those exclusively breast-fed before the challenge (n = 3 in the challenge-positive group, n = 4 in the challenge-negative group).
[‡] Retrospective data, based on parental recall at the time of the double-blind, placebo-controlled food challenge.
[§] All cow's-milk protein excluded from diet, including bakery products and cooked food.

group during placebo, loose stools were reported for 10 of 39 (25%) children. Vomiting/spitting up occurred with similar rates in the challenge-negative group during placebo (7/39, 18%), and in the challenge-positive group during active challenge (3/18, 17%). The hypoallergenic formula used during the elimination period did not affect the outcome of the DBPCFC. The referring doctor had started an amino acid formula for a CM-elimination diet for 11 (19%) children; 3 of them reacted to the active challenge, the same proportion as in the entire cohort. Eight children were receiving soy-based formula and 4 of them reacted to the active challenge.

We performed adult-type hypolactasia genotyping in 52 patients. In Finland, the prevalence of the adult-type hypolactasia CC genotype (which is associated with low lactase levels) is 18% (17). In the DBPCFC-negative group (35 analyses), 11 (34%) patients had the CC genotype. This difference in population frequency is significant ($P = 0.038$). In the DBPCFC-positive group, the CC genotype was present in 4 of 17 (24%) patients (Table 2).

The DBPCFC protocol that we used was feasible and safe. The observed symptoms were milder than expected. Only 1 child reacted by vomiting within 30 minutes of the provocation (on placebo); there were no other immediate reactions among the studied children. Reactions to CMP occurred within 48 hours, except in 1 patient in whom symptoms were reported after 6 days (on active challenge).

Significant placebo reactions occurred in 18 of 39 (46%) patients with negative DBPCFC, all within 48 hours (Table 3). The placebo reactions were unpredictable: The reactions could be directly attributed to concurrent infection in only 3 patients. The most common placebo symptoms in the challenge-negative group were increased irritability or crying/fussiness (15 patients), vomiting (7 patients), and loose stools (10 patients). Upper respiratory symptoms were reported during both the placebo and the active challenges, but parents seldom associated them with concurrent GI symptoms.

TABLE 2. Laboratory findings for patients undergoing double-blind, placebo-controlled food challenge for cow's milk and for controls

	DBPCFC-negative patients, n = 39	DBPCFC-positive patients, n = 18	Controls,[†] n = 21	P
No. cow's-milk–specific IgE >0.35 kU/L	0	0	0	NS
No. adult-type hypolactasia CC genotype	11/35 (31%)[*]	4/17 (24%)	4 (22%)	[*]0.0387 (challenge negative vs national prevalence)
No. cow's-milk–specific IgA above reference[§]	2	3	2	NS
No. cow's-milk–specific IgG above reference[§]	1	1	4	NS
No. total IgA <0.06	1	1	0	NS
No. blood eosinophils >0.4 × 10⁹ cells/L	11	2	4	NS
No. skin prick test for cow's milk ≥3 mm[‡]	2	2	0	NS
No. fecal hemoglobin positive (after active challenge)	3 (1)	2 (0)	0	NS

NS = not significant.
[*] $P \leq 0.05$. P values reported for χ^2 test, except for adult-type hypolactasia, in which proportional χ^2 test was used, against 18% population prevalence in Finland.
[†] Controls with no suspicion of food allergy.
[‡] Wheal size exceeding the negative control by ≥3 mm.
[§] The reference values reported for cow's-milk–specific IgA and IgG (16).

TABLE 3. Characteristics of pediatric patients undergoing a 5-day, double-blind, placebo-controlled food challenge for cow's milk

	Symptoms at suspicion of cow's-milk allergy		Symptoms reported for the challenge positive, n = 18		Symptoms reported for the challenge negative, n = 39	
	Challenge positive, n = 18	Challenge negative, n = 39	During active challenge, 18/18 (100%)	During placebo challenge, 6/18 (33%)	During active challenge, 10/39 (25%)	During placebo challenge, 18/39 (46%)
Crying/fussiness (%)	11 (56)	30 (77)	8 (44)	4 (22)	2 (5)	15 (38)
Vomiting/spitting up (%)	8 (44)	19 (49)	3 (17)	2 (11)	0	7 (18)
Loose stools (%)	14* (78)	18* (46)	15* (83)†	1 (6)†	1 (2)†	10 (25)†
Flatulence (%)	4 (22)	10 (26)	5 (28)	0	2 (5)	3 (8)
Constipation (%)	2 (11)	10 (26)	0	0	0	0
Skin manifestations (%)	5 (28)	10 (26)	2 (11)	3 (17)	2 (5)	2 (5)
Upper respiratory symptoms reported during challenge			5 (28)	5 (28)	7 (18)	6 (15)

*P = 0.0432.
†P < 0.0001.

Reintroduction of CMP to the diet after negative challenge was mainly successful. Thirty-seven (95%) of the DBPCFC-negative patients used CMP-containing dairy products, even though at the 6-month follow-up some of them still refused to drink CM as such. The 2 patients who had not been given CMP by 6 months time tolerated CMP when encouraged to try it right after the follow-up appointment. Feeding-related problems (refusal to eat, vomiting/gagging, other diet restrictions without food allergy diagnosis) persisted after the negative challenge result in 6 patients (15%), and also in 6 patients (33%) in the challenge-positive group. In the DBPCFC-positive group, 12 of 18 patients had not been given any CMP by the 6-month follow-up, despite exhibiting mild symptoms during the challenge. The 6 challenge-positive patients who were given small amounts of CMP tolerated it well. Symptoms did not persist in patients who were taking extensively hydrolyzed formula, and thus we did not need to start any of these patients on an amino acid formula after the challenge.

DISCUSSION

To the best of our knowledge, this study is the largest published study on a prospective cohort of patients with suspected GI manifestations of CMPA only. Vanto et al (18) reported on 301 children suspected of having CMPA who underwent a 5-day DBPCFC, with 34% of the patients manifesting GI symptoms. Unfortunately, they did not report the outcome of the DBPCFC for the GI-manifested group alone. We performed the 5-day DBPCFC in 57 infants. The proportion of positive active challenge reactions noted in our study was 32%, the same range as in other previous studies using the DBPCFC (19,20). Based on our clinical experience, the study patients were representative of present-day CMPA suspicions in a Finnish allergy clinic. On average, the time frame from the beginning of the symptoms to the DBPCFC was relatively short, with a median duration of cow's-milk–free diet of 2.5 months. It may be argued that natural tolerance had developed during this time. The median age of our patients was 8.7 months; tolerance to CM has been shown to develop in the majority of patients with CMPA only after the age of 1 year (30). In fact, the 2 patients with the longest durations of CM-free diet were DBPCFC positive. Although the nature and severity of symptoms with these infants do not match those described usually with food allergy–linked gastroenteropathies, the parents believed that the problem was significant enough that they agreed to the time-consuming and demanding protocol of the DBPCFC. Therefore, mild forms of the disease do not explain the large number of suspected cases with CMPA that were later rejected. Considering the frequency and nature of the placebo reactions (eg, reported loose stools in 25% on placebo), it is likely that some of the DBPCFC-positive reactions we observed may in fact be false. This also is in accordance with previous reports (19,20).

The pathology behind non–IgE-mediated GI manifestations of CMPA remains elusive. The universally used theory involves T-cell–mediated immunological responses (21). The paucity of confirmative data may be because of the fact that previous studies have either not been done among prospectively recruited, DBPCFC-confirmed patients or that they have pooled together both IgE-mediated and non-IgE CMPA as well as possible placebo reactions. In our study, in both the DBPCFC-negative and -positive groups, SPTs for CM were positive (wheal size 3–4 mm) in 2 children with no associated skin manifestations. Regarding the SPT wheal size in CMA in children, the 95% positive decision point corresponds with wheal size 12.8 mm; for wheal size 3 to 4 mm, the predictive probability is estimated just <50% (22). FPIES is the most severe GI-related manifestation of CMPA (8). The symptoms of FPIES manifest within a few hours with severe repetitive

vomiting and lethargy, followed by diarrhea. None of our patients presented with FPIES-like symptoms. Our clinical suspicion is that this more severe form of non–IgE-mediated CMPA has in fact become rare; the reasons for this remain speculative.

The GI symptoms that parents in this study associated with suspicion of CMPA were grouped in the following way: crying/fussiness, loose stools, vomiting/spitting up, flatulence, and constipation. Parental perception of ''excessive'' crying or fussiness during infancy is extremely subjective (3), and the other described symptoms also overlap considerably with normal infant GI physiology. We prospectively collected detailed symptom data on charts both 1 week before and during the provocations. Only the parental report on ''loose stools,'' which we use here to denote stools that parents perceived as liquid with increased frequency, applied significantly more often to the DBPCFC-positive group compared with the DPCFC-negative group. Because loose stools were in fact reported in 25% of the DBPCFC-negative group during placebo challenge, even this symptom cannot be reliably interpreted in open challenges. Rectal bleeding has been associated with CMPA, but none of our patients presented with hematochezia, in accordance with other reports (23,24). Constipation in an infant may be worsened by abundant CMP, but the immunological (or allergic) nature of this phenomenon is unproven (1). In our cohort, the 5-day CMP challenge did not provoke constipation. Failure to thrive in the few patients in our cohort was in fact associated with dietary restrictions and feeding problems, rather than with persisting CMPA symptoms.

Placebo reactions were so remarkable in nearly 50% of challenge-negative children that the parents identified the placebo milk period as the more symptomatic. We observed similar rates for vomiting/spitting up occurring during placebo challenge in the DBPCFC-negative group and during active challenge in the DBPCFC-positive group. Thus, care should be taken if relying on vomiting/spitting up only as a CMPA symptom. Also excessive crying/fussiness as perceived and reported by parents did not agree with DBPCFC results, in concordance with a previous study (9). Our results clearly show that in gastrointestinally manifested CMPA suspicion, open food challenges should not be used.

The use of an amino acid formula was frequent (19%) in our cohort, possibly reflecting the diagnostic challenges in gastrointestinally manifested CMPA. In a randomized prospective trial, DBPCFC-proven allergy to extensively hydrolyzed formula occurred in 2.2% of CMPA patients, 69% of whom were negative for CM-specific IgE (25). In the present study, the use of amino acid formula did not raise the likelihood of a positive DBPCFC reaction. In the challenge-positive group, none of the patients on extensively hydrolyzed formula had persisting symptoms. It should be noted that most extensively hydrolyzed hypoallergenic formulas contain lactose, whereas the only amino acid formula available in Finland is lactose-free. Our challenge protocol was not designed to test lactose intolerance.

The more frequent adult-type hypolactasia CC_{13910} genotype in children with GI symptoms suspected of CMPA deserves attention. Because of the study size, this finding may relate to a low number of patients; however, in adults, the CC_{13910} genotype frequency was higher than expected in those experiencing unexplained GI pain, even when controlled for CM consumption (26). The lactase activity levels in CC genotype children are individual, but they start to decline after preschool age in the majority of white children (27). Thus, a low level of lactase related to the genetic trait is unlikely, although not excluded, in our patients. Interestingly, in colicky infants, abnormal breath hydrogen testing may occur, indicating possible carbohydrate malabsorption in such patients (28). The mechanisms for regulation of lactase enzyme activity levels in infants and the presentation of intestinal symptoms in children with CC_{13910} allele are incompletely understood (29).

The strengths of this study include its prospective setup, its detailed challenge, and follow-up data. Our clinic is a secondary referral center for pediatric outpatients within an urban area with a population of >1 million. In our clinic, the DBPCFC is in routine use, especially in non-IgE CMPA. Thus, those who refused to participate did so because of the laboratory tests involved in the study or because of the study DBPCFC schedules. One pediatrician and nurse supervised all of the study DBPCFCs, which adds significantly to the consistency of the results. The nearly 100% rate of infants using CMP without symptoms after negative DBPCFC confirms the negative challenge results.

The limitations of this study include its size and the DBPCFC protocol. The 5-day duration of the DBPCFC was designed to provoke the delayed reactions that occur within 72 hours of ingestion. The eventual percentage of positive active challenges was lower than we expected. Therefore, the power of this study to recognize the subtypes of CMP intolerance (inflammatory/allergic, lactose-associated, functional or false-positive) is not sufficient. We did not search for proof of possible concurrent microbial infections which could cause symptoms similar to CMP-provoked GI symptoms. We did not believe it was prudent to re-perform the DBPCFC in the CMPA-positive group, because of preexisting feeding problems and parental opposition. Because the symptoms noted during the challenge were relatively mild, we instructed the parents to perform re-challenges at home after 3 months; however, this was done infrequently, even though we always discussed with the parents the possibility of false-positive challenges.

In conclusion, we have reported findings for a cohort of 57 patients undergoing DBPCFC for suspicion of GI-manifested CMPA. The diagnosis was confirmed in only one-third of the patients; CM-specific IgE was negative in all. Placebo reactions were common and can easily lead to biased interpretation in an open challenge. In terms of the suspicion of CMPA with GI symptoms, a trial of extensively hydrolyzed formula is reasonable; but also other causes for the GI symptoms should be identified. Further studies are needed to assess the association of suspected GI symptoms with the adult-type hypolactasia CC genotype.

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