

Persistent cow's milk protein intolerance in infants: the changing faces of the same disease

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Summary

Background Recent research has shown that cow's milk protein intolerance (CMPI) often persists beyond 4 years of age.

Aims To evaluate the clinical and immunological characteristics of a group of infants with persistent CMPI.

Patients and methods Twelve infants (6 m, 6 f) with persistent CMPI were followed up from birth until a median age of 5 years. The patients underwent CMP challenge each year to evaluate CMP-tolerance. As controls we followed 26 infants (12 m, 14 f) with CMPI that resolved within 1–2 years.

Results A family history of atopic disease was found in 10/12 patients with persistent CMPI and in 10/26 controls ($P < 0.01$). Clinical presentation changed over time: at onset symptoms were prevalently gastrointestinal, while at the end of the study there was an increased frequency of wheezing and constipation and a higher frequency of delayed reactions to CMP-challenge than at study commencement (9/12 vs 2/12; $P < 0.007$). 11/12 infants with persistent CMPI and 3/26 controls ($P < 0.0001$) presented multiple food intolerance. During the observation period 9/12 infants with persistent CMPI and 2/26 controls showed atopic disease: asthma, rhinitis, eczema ($P < 0.0001$).

Conclusions Persistent CMPI forms are characterized by: (a) considerable importance of familial atopic disease; (b) change in CMPI manifestations over time and more prolonged delay between CMP consumption and manifestation of symptoms; (c) very high frequency of multiple food intolerance and allergic diseases.

Keywords: cow's milk protein intolerance, symptoms, total IgE, specific IgE to cow's milk antigens, IgG antibetalactoglobulin, skin-prick tests, cow's milk challenge

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Introduction

Cow's milk protein intolerance (CMPI) is a common food intolerance, but the data on CMPI natural history differ greatly: some authors assert that symptoms tend to regress within three years [1–3], while others underline that a considerable percentage of patients remain symptomatic beyond 4 years of age [4,5].

In the present descriptive study we collected the clinical and laboratory data of a group of selected patients with persistent CMPI, diagnosed according to the criteria of the

European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) [6], over a mean observation period of 4 years. We compared them with patients with CMPI which resolved within 2 years. The results obtained underlined how clinical manifestations of CMPI in the same patient surprisingly changed over time.

Patients and methods

In June 1997, we have reviewed the clinical charts of the children suffering from CMPI, followed in the day-hospital of the II Division of Pediatrics of the Di Cristina Hospital, during the previous 7 years. From these, we selected the

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subjects followed up from birth by the chief investigator (G.I.) (first examination within 15 days after birth) and regularly observed at least every 2 months either at a private clinic or in hospital, until the age of 5 years, at least; so that we had exact details of the natural history of CMPI in these subjects. In this way, we included in the study 38 infants (18 m, 20 f) with CMPI; of these patients, 12 infants (6 m, 6 f), age range 5–7 years (median 5 years) at the moment of the study, showed persistent CMPI. All the other 26 infants (12 m, 14 f; age range 5–9 years, median 6 years) with CMPI which resolved after 9–12 months of CMP-free diet, within the 2nd year of life, were included in the study as controls.

In all cases the clinical suspicion of CMPI was confirmed by a double-blind diagnostic challenge performed in hospital, within 4–6 weeks after onset of symptoms, after the patients had followed a CMP-free diet. In the patients with diarrhoea or colic, a possible lactase deficit was excluded by non-improvement during a lactose-free diet. The CMP-challenge was postponed until after 6 months of age in only one case, patient G.S., who on onset of CMPI had presented anaphylactic shock (see Table 1). Other CMP-challenges were later performed yearly, all in our Hospital and we always evaluated the results in a double-blind manner.

In the group of persistent CMPI patients, when the disease was suspected, eight patients began feeding with soy milk formulas, two with a semi-elemental formula containing hydrolyzed casein, and one ass's milk; the only patient still being breast fed continued this and his mother began a CMP-free diet. In the control group 19 received soy milk formulas, two a semi-elemental formula, two ass's milk and three continued breast-feeding.

On diagnosis of CMPI, family history of atopic disease was accurately recorded; in particular, we considered as suffering from atopic disease those patients' parents who presented bronchial asthma, rhinitis, dermatitis or conjunctivitis and who had the following characteristics: (a) positive skin-prick tests and/or RASTs for food or environmental antigens (tests performed in our laboratory); (b) no evidence of other aetiological factors for their diseases; (c) they were successfully treated with steroids and/or antihistaminic drugs.

During the follow-up we paid particular attention when diagnosing the onset of atopic diseases; in this respect, we diagnosed allergic rhinitis when the patient suffered from a serous nasal secretion for more than 4 weeks, without signs of infection, and microscopic examination of the nasal mucus smear showed an eosinophil count higher than 20% of the total cells. We diagnosed bronchial asthma when the patient suffered from expiratory dyspnoea associated with coughing, in absence of fever or other manifestations of infection, the physical examination of the thorax showed

wheezing, and the symptoms were seasonal and/or linked to a precise antigen exposure. We diagnosed allergic eczema in presence of erythematous lesions excoriated or tending to spread on the face (more frequently around the mouth) or on the body and limbs.

Milk challenge procedure

The challenges were performed when the infants were completely asymptomatic following a CMP-free diet.

The double-blind procedures and precautions were the same as previously described [7]. In brief, challenges were performed using a CM-formula or a placebo formula containing hydrolyzed proteins. A computer generated randomization scheme was used to determine the challenge sequences so that both parents and doctors were unaware of the nature of the challenge formula. In fact, the patients received several feeds of milk coded A or B by the dispensary of the Hospital; for infants aged < 15 months, bottles contained a formula based on cow's milk or a formula based on hydrolyzed proteins; for infants aged > 15 months feeds contained commercially available whole cow's milk or hydrolyzed protein-based milk. The challenge was performed beginning with an initial quantity of 5 mL and reaching the total equivalent of a full feed in 3 h. If no reaction was observed, the patients were discharged and continued the challenge at home. The challenge period was one week for both the placebo and the CM-formulas; during this week the parents recorded any clinical symptoms and especially skin, respiratory and gastroenterological symptoms. The challenge was stopped if a clinical reaction occurred. Patients were re-examined in hospital in the case of any adverse reaction and/or at the end of the challenge period. In the cases without a clear clinical relapse within the period of the double-blind challenge, and/or when there were symptoms attributable to diseases other than CMPI after challenge, the test was repeated to exclude the possibility that non-immediate reactions might be the result of intervening infection or accidental exposure to other environmental challenges. In addition, more than one diagnostic challenge was performed in three cases presenting CMPI symptoms considered as not 'classical': constipation, gastroesophageal reflux and colic; in these patients the reactions to challenge, the disappearance of symptoms on a CMP-free diet, pre and postchallenge laboratory examinations and, where performed, pre and postchallenge intestinal biopsy, left no doubt of a CMPI diagnosis. More precise details on the study design for these patients have been previously published [7–9].

When there were no clinical reactions to challenge, the patients were left on a free diet and followed up for 1 month with more frequent examinations (every 7–15 days) to observe any eventual delayed manifestations.

Furthermore, at the first, diagnostic, CM-challenge, the variations in a number of laboratory parameters were recorded: increase in circulating polymorphonuclear leucocytes greater than $3,500/\text{mm}^3$ (6 h after CM-challenge), eosinophils in the faecal and nasal mucus, occult blood in stools.

Finally, at the moment of the first challenge, five infants were exclusively breast-fed and in these cases their mothers, after a CMP-free diet, began to assume the milk feeds coded as A or B, always according to a double-blind procedure.

Adverse reactions to non cow-milk foods

In all the study patients new foods were introduced with great care. Each food was introduced singly, with a time lapse between the introduction of two new foods of at least 15 days. If after the introduction of a new food the parents noted the appearance of symptoms, the infant was examined by the chief investigator and the food excluded from the diet. If the symptoms disappeared during the elimination diet, a challenge was performed in hospital to confirm that the food in question had caused the food intolerance, using the method described for the CMP-challenge.

Laboratory examinations

A series of tests evaluated the immunological status of the patients both at first diagnosis of CMPI and at the end of the study. In particular, at diagnosis serum levels of total IgE and IgG antibetalactoglobulin were assayed and RASTs were performed for whole cow's milk, beta-lactoglobulin, alpha-lactalbumin and casein. At the end of the study these tests were repeated and RASTs performed also for other food and environmental allergens. In addition, skin prick tests were carried out with food and environmental antigens.

Serum levels of total IgE were assayed using a commercial Kit (Phadebas IgE paper radio-immunosorbent test Kit, Pharmacia Diagnostics, Uppsala, Sweden); the upper normal limit for serum IgE varied according to age-dependent cut-offs (for each age class the cut-off was equal to the mean value + 2 SD observed in our laboratory in healthy infants of the same age range: up to 4 months old the cut-off was 6 IU/mL, between 5 and 12 months 25.5 IU/mL, between 1 and 5 years 31.2 IU/mL, between 6 and 10 years 37.7 IU/mL). Serum IgG antibetalactoglobulin levels were determined by an enzyme-linked immunosorbent assay (Beta-lactotest, Eurospital, Trieste, Italy); values over 36% higher than a control standard (up to 20%), were considered positive. The threshold value of 36% had the greatest diagnostic accuracy in a large study performed in our laboratory on more than 200 healthy control subjects and more than 200 patients with CMPI [10]. Skin-prick tests (PRICKs) were performed using a commercial Kit (Lofarma

Diagnostic, Milan, Italy); any wheal diameter that exceeded that of the control and was more than one-fourth the size of the histamine wheal was regarded as positive (a planimeter was used to measure the area of the papule). IgE antibodies to specific antigens were measured with the Phadebas RAST assay (Pharmacia Diagnostic) and expressed as PRU/mL; values greater than 1.43 PRU/mL were considered positive. As regards the laboratory examinations performed during the CM-challenge, the test for eosinophils in the nasal mucus was considered positive if the percentage of eosinophils was greater than 10% in the cells of a given area of the smear; the fecal eosinophil test result was considered positive if single cells were clearly present; occult blood in the stools was evaluated using a commercial kit (BM test colon albumin; Boehringer Mannheim, Germany).

Results

Table 1 shows the clinical characteristics of the 12 infants with persistent CMPI compared with the 26 controls. The infants with persistent CMPI had been born between 38 and 42 weeks into pregnancy and birth weight was appropriate for the gestational age. Only two were born by Caesarian section, dizygote twins (S.A. and M.A.); the group also included two other pairs of siblings (A.S. and G.S., P.P. and F.P.). In only one case (A.S.) was breast-feeding continued beyond 6 months, while in the remaining cases it was suspended within the third month. All the bottle-fed patients were on various commercial formulas derived from cow's milk. No difference was observed between patients and controls for frequency of acute infectious enteritis prior to the onset of CMPI (Fisher's test) gestational age (Student's *t*-test), *in utero* development (Student's *t*-test), age at onset of CMPI (Mann-Whitney *U*-test), feeding type (chi square test) and frequency of symptoms (chi square test). In contrast, family history of allergic disease was significantly more frequent in the patients than in the controls (10/26 cases); $P < 0.01$ (Fisher exact test).

In all the studied subjects symptoms regressed during CMP-free diet. After 4–6 weeks of CMP-free diet, the diagnostic CM-challenge showed the relapse of all patients, with the same symptoms recorded as at the first observation, within 24 h after commencement of the CM-challenge. Furthermore, in all patients there were alterations in one or more of the laboratory tests performed on challenge (occult blood in stools in 34 cases, increase in circulating polymorphonuclear leucocytes in 18 cases, positive eosinophils in stools in four cases, positive eosinophils in nasal mucus in eight cases).

Table 2 shows clinical reactions to CMP challenge, performed after 9–12 months of CMP-free diet. In 5/12 patients with persistent CMPI the symptom observed after challenge was different to the one presented at diagnosis.

Table 1. Clinical characteristics of the 12 patients with persistent CMPI and of the control group

Case	Patient	Sex	Age	Feeding at birth	Age at onset (months)	Symptoms	Family history of allergy
1	P.R.F.	M	5	breast	3	diarrhoea	yes
2	S.S.	F	5	mixed	3	diarrhoea	yes*
3	A.B.	F	5	breast	2	diarrhoea, vomit	yes
4	A.S.	M	5	breast	3	dermatitis	yes
5	G.S.	M	6	bottle	15 days	shock	yes
6	L.S.	M	6	bottle	9	constipation	yes
7	G.A.	M	5	bottle	8	wheezing	yes
8	P.P.	F	7	bottle	1	GER	yes
9	F.P.	F	5	bottle	3	diarrhoea	yes
10	D.L.B.	M	5	mixed	1	colic	no
11	S.A.	F	5	breast	3	dermatitis	yes*
12	M.A.	F	5	breast	3	dermatitis	yes*
CONTROLS							
		12M	5–9 (range)	10 breast	1–12 (range)	diarrhoea 9 cases	10/26 cases
		14F		6 mixed 10 bottle		vomit 6 cases dermatitis 6 cases GER 4 cases bronchospasm 1 case	

*Family history of atopic disease in both parents.

However, a further challenge in these five patients within 7 days confirmed the relationship between symptoms and CMP consumption. None of the control group infants showed persistent CMP intolerance to this challenge;

follow-up confirmed that all these infants were symptom-free on CMP-diet. Subsequently, in patients with persistent CMPI the clinical reaction to CMP changed greatly over time and there was no correspondence in any study patient

Table 2. Symptoms of CMPI at diagnosis compared with symptoms and time of the onset of symptoms at first reintroduction challenge (9–12 months after the beginning of the CM-free diet) and at end-study challenge, in patients with persistent CMPI

Case	Symptoms at diagnosis	Symptoms at first challenge	Time in hours	Symptoms at end study	Time (h)
1	diarrhoea	wheezing	>48	wheezing	>48
2	diarrhoea	diarrhoea – vomit	<6	constipation	>48
3	diarrhoea – vomit	diarrhoea – vomit	<6	constipation	>48
4	dermatitis	wheezing	6–24	wheezing	6–24
5	shock	wheezing	6–24	wheezing	6–24
6	constipation	constipation	>48	food refusal	>48
7	wheezing	diarrhoea – vomit	6–24	diarrhoea	6–24
8	GER	diarrhoea – vomit	6–24	dermatitis	>48
9	diarrhoea	diarrhoea – vomit	6–24	wheezing	>48
10	colic	diarrhoea	6–24	dermatitis – constipation	>48
11	dermatitis	dermatitis	6–24	wheezing – constipation	>48
12	dermatitis	dermatitis	6–24	wheezing – constipation	>48

Table 3. Immunological data at diagnosis and at the end of the study in 12 infants with persistent CMPI and in 26 controls (values are given in parentheses). The following values were considered elevated: IgE (kallikrein U/L) age-dependent cut-offs; IgG anti-betalactoglobulin > 36%; RAST > 1.43 PRU/ML

Case	Elevated IgE (IU/mL)		Elevated IgG anti-betalactogl. (%)		RASTs positivity to CM antigens (SU/ML)	
	Diagnosis	End	Diagnosis	End	Diagnosis	End
1	no (17)	yes (102)	yes (49)	no (18)	no	No
2	no (20)	no (28)	yes (68)	no (20)	no	No
3	no (9)	no (21)	yes (72)	no (12)	no	No
4	yes (166)	yes (186)	yes (60)	no (24)	yes (22.4)	yes (10.2)
5	no (14)	yes (221)	yes (110)	no (17)	no	No
6	no (21)	no (18)	yes (82)	no (7)	no	No
7	yes (182)	yes (452)	yes (64)	no (21)	yes (8.4)	yes (10.7)
8	no (14)	no (21)	yes (78)	no (16)	no	No
9	no (18)	yes (208)	yes (81)	no (22)	no	No
10	yes (148)	yes (221)	yes (49)	yes (48)	yes (16.2)	yes (10.1)
11	yes (170)	yes (421)	yes (58)	no (16)	yes (4.9)	yes (2.2)
12	yes (188)	yes (368)	yes (178)	no (8)	yes (19.9)	yes (16.2)
CONTROLS	Diagnosis	End	Diagnosis	End	Diagnosis	End
Number of positive cases	6/26	2/26	25/26	1/26	6/26	0.26
Range values	4–194	8–188	30–149	12–56	0–21.6	//

Statistical analysis: at entry to the study no significant difference was observed between patients and controls; at end-study the following were significantly higher in patients than in controls: serum IgE values ($P < 0.005$, Mann–Whitney U test), frequency of elevated serum IgE ($P < 0.001$, Fisher's exact test) and frequency of positive RASTs for milk antigens ($P < 0.005$, Fisher's exact test).

between the symptoms observed at the final challenge and those presented at CMPI onset. In particular, there was a clear increase over time in the number of patients with wheezing ($P < 0.03$ Fisher test). Moreover, there was a growing tendency over time for reactions to be delayed during challenge (> 48 h); in fact, only 2/12 had delayed reactions at the first diagnostic challenge, compared with 9/12 during the final challenge ($P < 0.007$; Fisher test).

Table 3 shows immunological data of the study subjects at diagnosis and at the end of the study. At diagnosis, IgE mediated CMPI was more frequent in patients than in controls, but not significantly. At the end of the study both the frequency of positive serum IgE ($P < 0.001$, Fisher's exact test) and the comparison of the IgE values in patients and in controls ($P < 0.005$, Mann–Whitney U -test) showed a significantly greater activation of IgE-mediated mechanisms in subjects with persistent CMPI. The end-study frequency of RASTs positivity for milk antigens was also significantly higher in patients than in controls ($P < 0.005$, Fisher's exact test). The skin prick tests for milk antigens,

performed only at the end of the study, were positive in 8/12 cases.

During the CMP-free diet the study patients presented intolerance towards other foods; in these patients, cutaneous, respiratory or gastrointestinal symptoms appeared after eating a specific food and the diagnostic procedure confirmed food intolerance. For this reason, in the patients with persistent CMPI soy milk was substituted with a semielemental formula containing hydrolyzed proteins or ass's milk in three cases. In the control group two patients showed soy intolerance and were fed with a semi-elemental formula, without any further disturbances. Other foods caused intolerance during the study period; in patients with persistent CMPI we observed intolerance to egg (10 cases), tomato (three cases), banana (3 cases), orange (two cases), chicken (two cases) and cocoa (one case). The adverse reactions to these foods were: dermatitis (11 cases), diarrhoea (eight cases), wheezing (six cases), constipation (two cases) (more than one symptom could be present in each subject). In all these cases the symptoms disappeared when the

suspected food was excluded from the diet and subsequently the patients underwent a double-blind food challenge in hospital; the results of the challenges confirmed the intolerance to each specific food. In total 11/12 patients with CMPI showed intolerance to other foods. In the controls the frequency of multiple food intolerance was 3/26 cases: two patients were, in fact, intolerant to soybean and one to egg (persistent CMPI vs controls: $P < 0.0001$; Fisher's exact test).

During the study, atopic disease was observed in 10/12 children with persistent CMPI (bronchial asthma eight cases, rhinitis one, eczema one). In these infants RASTs and skin-prick tests for the most common environmental allergens showed hypersensitivity to *acari* (four cases), *parietaria* (four cases) and cat's fur (four cases); in these cases antihistaminic drugs were frequently given as well as beta-stimulants and occasionally corticosteroids. In controls only 2/26 infants showed atopic disease during the follow-up (persistent CMPI vs controls $P < 0.0001$; Fisher exact test).

Growth of the patients with persistent CMPI was on the whole satisfactory (median weight/height ratio equal to 30th centile, range 5th–80th) and did not differ from that of the control group (median weight/height ratio 35th centile, range 10–90th). The patients with persistent CMPI also received oral supplements of vitamins and minerals.

Discussion

Although it is agreed that CMPI symptoms generally disappear within the first 2–3 years of life, recent prospective studies have shown that 33–44% of these infants are still intolerant at 4 years of age [4,5]. The present retrospective study describes the clinical histories of 12 patients with symptoms of CMPI which persisted until at least the 4th year, to determine eventual clinical and/or immunological characteristics which can be considered typical for these patients. The first important difference between patients with persistent CMPI and controls was the frequency of positive family history. In fact, 10/12 patients with persistent CMPI had a least one parent with atopic disease. This was considerably higher than the frequency recorded in other Italian studies (23–42%) [8,9,11] and it can be supposed that a genetic predisposition has a really important role in determining whether CMPI will persist after the first few years of life.

As regards the immunological tests, it is interesting that total IgE values increased over time in patients with persistent CMPI, and at the end of the study there was a higher number of subjects with total IgE above normal limits. This result is probably attributable to the development of hypersensitivity to other antigens, which contributed to increasing total IgE. During the course of the clinical history of these

patients there was in fact a clear tendency towards multiple food allergy (in 11/12 patients) with a frequency greater than in the control group or those reported in other studies [11,12].

However, the most interesting aspect of this study is certainly the pathomorphism of CMPI in patients with persistent disease. In all patients the clinical manifestations observed at the end of the study were different to those at commencement and there was a progressive tendency towards a longer time lapse between the CMP challenge and appearance of symptoms. In particular, at onset gastrointestinal manifestations were most frequent (7/12 cases), whereas during the final study challenge we prevalently recorded respiratory symptoms (6/12 cases).

It was, however, very complex to attribute a particular symptom to CMPI as over time the delay between the reintroduction of CMPI and clinical relapse tended to exceed 48 h. Delayed reactions to CMP challenge at the end of the study were observed in 9/12 cases, with a frequency statistically higher than at the beginning of the study. In addition, the delay was even 2–3 weeks in two cases and, obviously, these subjects were being openly CM-fed; thus the possibility that the reactions to CM might represent a conditioned response cannot be excluded. However, even in these cases the repetition of CMP challenges performed 2–3 times over a short time period confirmed the relationship between symptoms and CMPI consumption; furthermore 5/9 patients with delayed reactions suffered from constipation and were also included in a study with a double-blind administration of CM or soy milk over 2 weeks: in all cases their reaction to CM was unmistakable, with constipation reappearing after 2–5 days. To explain the longer delay between CM-challenge and symptom reappearance during the end-study challenge in comparison with the first CM-challenge, we hypothesize that the CMP-intolerant patients may acquire over time a certain degree of food tolerance which permits them to tolerate small amounts of antigen load. In other words, we think that food tolerance/intolerance is not a 'black or white phenomenon', but that there is a wide 'grey zone' in which a small quantity of food antigens does not determine allergic reactions and clinical symptoms, but a greater quantity of antigens does determine the reaction. It is probable that in the children with very delayed clinical reactions we observed, a great antigen load was "necessary" to determine a clinical reaction. In these children a study of the cellular-mediated immune reactions would be very interesting.

It is evident that several questions remain without a precise answer from our study; furthermore, as this is a descriptive study and the sample size of the patients groups included was small, further prospective studies are needed involving a large cohort of CMPI infants to confirm our observations.

However, the following conclusions can be suggested which remain to be confirmed in subsequent studies: (a) genetic predisposition seems to have a very important role, and is certainly greater in the forms of CMPI which persist beyond the first few years of life, (b) clinical manifestations of CMPI change in the same patient over time and the reaction to reintroduction of CMP tends to be delayed; (c) a very high percentage of cases presented multiple food intolerance and tended to develop atopic disease which prevalently affected the respiratory system.

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