

Immunodeficiency and other clinical immunology

Gastroesophageal reflux and cow's milk allergy in infants: A prospective study

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Background: Recent reports have suggested that gastroesophageal reflux in pediatric patients may be caused by food allergy.

Objective: The aim of our study was to determine the frequency of the association of gastroesophageal reflux with cow's milk protein allergy in patients in the first year of life.

Methods: We studied 204 consecutive patients (median age, 6.3 months) who had been diagnosed as having gastroesophageal reflux on the basis of 24-hour continuous pH monitoring and histologic examination of the esophageal mucosa.

Results: Clinical history suggested diagnosis of cow's milk allergy in 19 infants, and 93 others had positive test results (serum IgE anti-lactoglobulin, prick tests, circulating or fecal or nasal mucus eosinophils) but did not have symptoms indicating cow's milk allergy. The cow's milk-free diet and two successive blind challenges confirmed the diagnosis of cow's milk allergy in 85 of the 204 patients with gastroesophageal reflux. The clinical presentations of the infants with gastroesophageal reflux alone were different, in view of the greater frequency of diarrhea ($p < 0.0001$) and atopic dermatitis ($p < 0.0002$). In all, gastroesophageal reflux was associated with, and probably caused by cow's milk allergy, in 85 of 204 cases (41.8%).

Conclusions: Considering the frequency of this association, patients younger than 12 months old with symptoms of gastroesophageal reflux should be carefully examined to determine whether this disorder is primary or caused by cow's milk allergy. (*J ALLERGY CLIN IMMUNOL* 1996;97:822-7.)

Key words: Cow's milk allergy, gastroesophageal reflux, infants, pH monitoring, anti- β -lactoglobulin, esophagitis

Cow's milk allergy (CMA) and gastroesophageal reflux (GER) are very common diseases in the first year of life.¹⁻³ It is not always easy to distinguish between these two pathologic conditions on the basis of their clinical presentation, because many symptoms are common to both GER and CMA (e.g., vomiting, infantile colic, and failure to thrive). Furthermore, a recent report by the Working Group on Gastroesophageal Reflux Disease of

Abbreviations used

CM:	Cow's milk
CMA:	Cow's milk allergy
GER:	Gastroesophageal reflux

the European Society of Paediatric Gastroenterology and Nutrition indicated that GER can be caused by food allergy, and identification of cases of secondary GER is very important in making therapeutic decisions.⁴ However, to our knowledge, there has been only one study in the literature on the CMA-GER association,⁵ but there are no prospective studies to confirm the existence of this association and its frequency.

The aims of this study were: (1) to determine the

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existence and frequency of the CMA-GER association in patients in the first year of life who were under observation in our department for symptoms compatible with a diagnosis of GER, and (2) to observe any eventual differences in clinical presentation and pH monitoring characteristics in a group of patients with GER and CMA and in a second group with GER only.

METHODS

We included in the study all patients who were breast-fed or fed a formula containing cow's milk (CM), observed in our department over the last 3 years, for symptoms compatible with a diagnosis of GER. Patients with one or more of the following symptoms were included in the study: vomiting, regurgitation, growth disorders, wheezing, infantile colic (fits of crying for a total duration of >3 hours per day in unweaned infants <3 months of age), food refusal (daily food intake <50% of the normal calorie requirement for age of patient), apneic episodes (respiratory arrest for >20 seconds), hypochromic anemia (hemoglobin level <9 gm/dl). During the first observation, clinical history was recorded prospectively, and if it supported the diagnosis of suspected GER, the patient underwent 24-hour pH monitoring and esophagoscopy, and a biopsy of the esophageal mucosa was performed. Those infants with a total percentage reflux time greater than 5.2% over the 24 hours⁶ and histologic findings compatible with the diagnosis of esophagitis, according to Block et al.,⁷ were considered to have GER. In regard to the severity of the esophagitis, we considered the findings of the endoscopic and histologic examination of the esophagus, which we have recently published.⁸ In this way, 204 patients (97 boys and 107 girls) were selected; they had a median age of 6.3 months (range, 1 to 12 months). Of these, 60 were breast-fed or breast- and formula-fed, and 164 were formula-fed. These patients contemporaneously underwent screening tests for concomitant CMA; cutaneous tests were therefore performed with whole CM, lactalbumin, and β -lactoglobulin (Lofarma Diagnostic, Milano, 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. The test result 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. The level of eosinophils in the peripheral blood was positive if these elements were greater than 400/mm³. The reference values for the normal limits of the tests were obtained in our laboratory with a large population of healthy subjects.

IgG anti- β -lactoglobulin levels (Betalactotest; Eurospital, Trieste, Italy) were also assayed: values greater than 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 by us with more than 200 healthy control subjects and more than 200 patients with CMA and gastroenterologic symptoms (unpublished data).

All of the patients with at least one positive test result or with clinical signs suggesting CMA associated with GER (e.g., alterations in bowel habits, rhinitis, atopic dermatitis) began an exclusion diet, in which CM was replaced with a formula containing hydrolyzed proteins. After a period of at least 3 months, the patients with suspected CMA underwent a double-blind CM challenge, and both the clinical reactions and variations in a number of laboratory parameters were recorded: increase in circulating polymorphonuclear leukocytes greater than 3500/mm³ (6 hours after challenge), peripheral eosinophils, eosinophils in the fecal and nasal mucus, occult blood in stools. In addition, 24 hours before and 24 hours after challenge all of these patients underwent intestinal biopsy in accordance with the commonly accepted diagnostic criteria for CMA.⁹⁻¹¹ Biopsy specimens were obtained just distal to the ligament of Treitz with a pediatric-size Watson modification of the Crosby capsule, positioned by means of fluoroscopy. The histologic evaluation of the intestinal mucosa was performed in blinded fashion, with the pathologist unaware of whether the sample had been taken before or after the administration of CM. The challenges were performed in the hospital, and each reaction was evaluated by doctors unaware of whether the formula given was a CM derivative or a formula containing hydrolyzed protein. In all cases during the challenge, the infants received an infusion of NaCl 0.9% solution to facilitate immediate intervention in case of anaphylactic shock. The challenge was performed beginning with an initial quantity of 10 ml and reaching the total equivalent of a full feed in 3 hours. The patients with a negative challenge response were fed with a CM formula, whereas those with signs of hypersensitivity during challenge were continued on the diet of formula containing hydrolyzed proteins; the latter patients underwent CM challenge once again after a mean period of 3.5 months (range, 3 to 9 months). During this second challenge only the clinical reactions and laboratory parameters were recorded, and no intestinal biopsies were performed. Parents gave informed consent, and the study was approved by the ethics committee of our faculty.

Twenty-four-hour pH monitoring

Monitoring of pH values was performed by passing a flexible miniature electrode with a pH-sensitive tip through the nose and along the esophagus, where it was positioned at a point equal to 87% of the distance from the nose to the lower esophageal sphincter, calculated with Strobel's formula.¹² Before this, the mini-electrode was connected to a portable recording system and standardized at pH values of 1 and 7. The portable recording system, composed of a computer monitor (Proxima LIGHT; Sensor Medics, Milano, Italy), recorded variations in intraesophageal pH over 24 hours

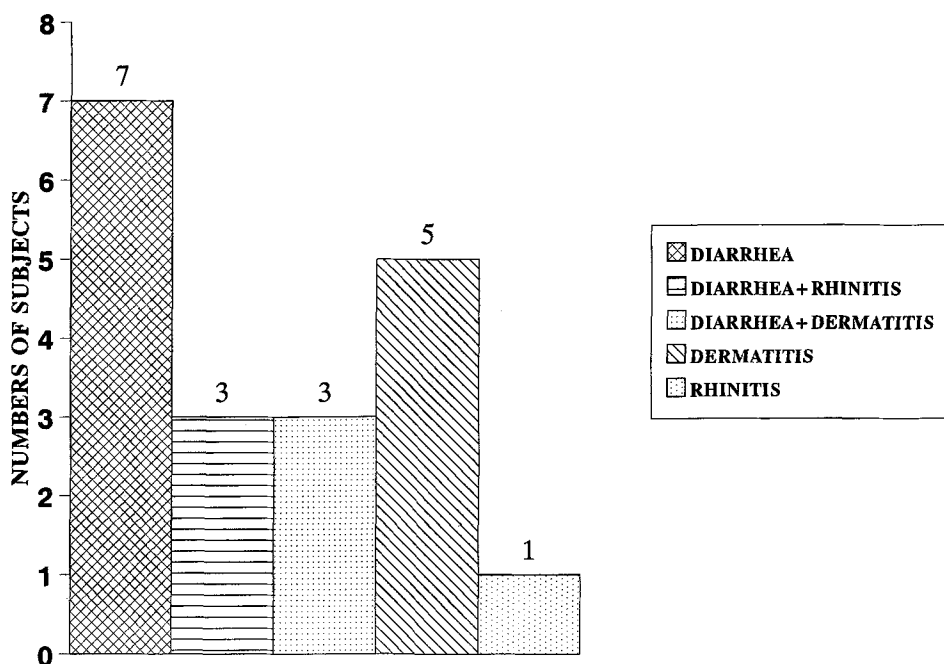


FIG. 1. Symptoms of suspected CMA in 204 consecutive infants diagnosed with GER.

and analyzed them in the form of a graphic pH tracing. After the electrodes were positioned, the mothers of the children were requested to note the times of meals and the sleeping-waking rhythm of the patients. The monitoring was well tolerated, and most of the children spent the 24-hour monitoring period at home to avoid causing changes in their daily routines. At the end of the monitoring period, the computer provided numerical data relative to the following parameters: (1) monitoring time, (2) reflux episode time, (3) percentage and length of time during which pH was less than 4, (4) number of reflux episodes, and (5) duration of the longest single reflux episode. Finally, the Jolley score¹³ was calculated, with respect to the reference values in healthy infants reported in the tables devised by Johnson and De Meester.¹⁴ This score evaluates the >2 hour postprandial period; a total score was calculated for each patient with an upper normal limit of 64; we determined for each subject—while awake, asleep, upright, and supine—the following parameters: number of GER episodes, number of GER episodes lasting longer than 5 minutes, longest GER episode, and percentage of time pH was less than 4 (the resulting 16 variables comprise the Jolley score). This score has also recently been used in other studies¹⁵ and appears to be particularly sensitive in identifying GER-induced apparent life-threatening events.^{16, 17}

Statistical analysis

The Mann-Whitney U test was used to compare the pH metric data and the Jolley score values recorded at the time of diagnosis in patients with GER and CMA and in those with GER only. The range and median

values were calculated for all of the pH metric data we recorded.

The chi square test was also used to determine the frequency of the various symptoms at the time of diagnosis and the differences in distribution for sex, age, family history of atopy, and severity of esophagitis.

RESULTS

In the group of 204 patients diagnosed with GER, detailed clinical history showed that 19 subjects had concomitant symptoms of suspected CMA, different from the inclusion criteria for the study. Fig. 1 shows that the most frequent symptom was diarrhea with presence of mucus in the feces; in addition, these patients had alterations in at least one of the laboratory parameters.

Among all of the patients with a clinical history that was not indicative of CMA, we detected 93 cases in which at least one of the laboratory parameters was outside normal limits. Fig. 2 summarizes the number of positive test results recorded in the whole study group of 204 unweaned infants; all of the patients with positive prick test results or with increased values of circulating fecal or nasal eosinophils also had abnormal serum levels of IgG anti-lactoglobulin. On the basis of the clinical and laboratory data, 112 patients were consequently started on a diet free of CM. After a period ranging from 3 to 7 months, these patients were hospitalized to undergo a double-blind CM

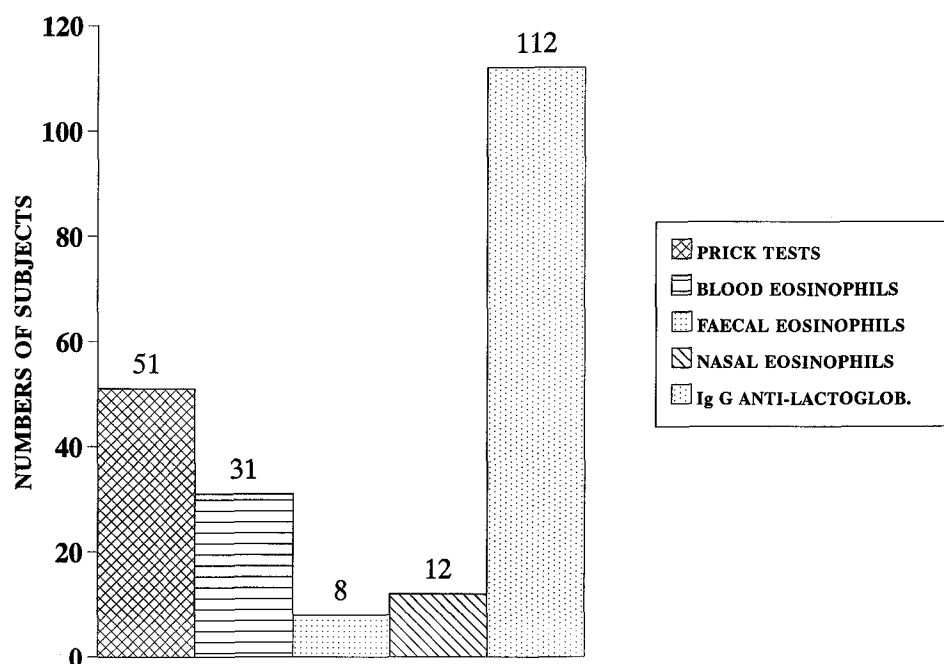


FIG. 2. Number of positive tests indicating a suspected CMA recorded in 204 infants diagnosed with GER.

challenge. Intestinal biopsy, performed 24 hours after the challenge, showed partial atrophy of the villi in 85 infants (41 boys and 44 girls), whereas there was no change in intestinal histologic findings in the remaining 27 infants initially started on the CM-free diet. In these latter patients, moreover, there were no changes in the laboratory parameters, nor were there clinical signs to support a diagnosis of CMA. The 85 patients with a positive challenge result were again started on a CM-free diet and challenged in a double-blind fashion once again after a mean period of 3.5 months. On this second occasion, the patients showed symptoms and laboratory data alterations that confirmed the diagnosis of CMA (Fig. 3). It is worth emphasizing that the diagnosis of CMA was confirmed in all of the 19 patients who already had signs of suspected CMA when the diagnosis of GER was made. The remaining 119 patients with GER, in whom a CMA diagnosis had been ruled out, were followed up for a mean time of 8 months (range, 3 to 24 months), and no symptoms of CMA were observed in this period.

The frequency of the GER-CMA association in the study infants younger than 1 year old was therefore 41.8% (85 of 204 cases). No differences in distribution were seen for sex, age, or type of feeding in the patients with GER and CMA (41 boys and 44 girls; mean age, 4.6 ± 2.8 months; 25

breast-fed or breast- and formula-fed infants) and the subjects with GER only (56 boys and 63 girls; mean age, 5.0 ± 3.0 months; 35 breast-fed or breast- and formula-fed infants). Clinical family history showed atopy in parents or siblings of 36 of 85 subjects with GER and CMA and in 31 of 119 subjects with GER alone. In regard to the severity of esophagitis, we observed 63 cases of grade I and 22 cases of grade II esophagitis in the group of subjects with GER and CMA and 80 cases of grade I and 39 cases of grade II esophagitis in the group of subjects with GER but not CMA (difference not significant).

In regard to the frequency of gastroenterologic or respiratory symptoms at the time of GER diagnosis in the two patient groups, we found a significant association for two of the typical CMA symptoms in the group of patients with GER and CMA, but not in the patients with GER only: diarrhea ($p < 0.0001$) and atopic dermatitis ($p < 0.0002$). There was no difference in the frequency of the other symptoms (rhinitis, vomiting, regurgitation, growth disorders, wheezing, fits of crying, food refusal, apneic episodes, and anemia).

Table I shows the pH monitoring data in the 85 patients with GER and CMA (group A) and in the remaining 119 subjects with GER only (group B) at the time of diagnosis. It must be stressed that at the time of diagnosis, at the start of the study,

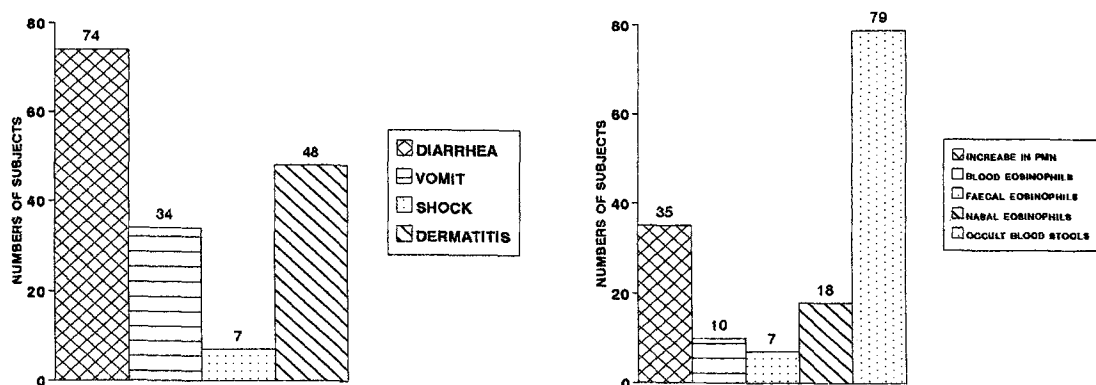


FIG. 3. Symptoms and laboratory data alterations in 85 patients with suspected CMA on second challenge.

TABLE I. pH monitoring data (range and median values) in 85 infants with GER and CMA (group A) and in 119 infants with GER only (group B) at time of diagnosis

	Group A	Group B
Total reflux time (%)	6-13 9	6-71 9
No. of reflux episodes per day	12-115 45	7-224 55
Longest reflux episode (min)	4-438 28	3-177 21
No. of reflux episodes >5 min	0-31 5	0-34 4
Jolley score	91-1141 250	66-884 167

there was no significant difference in the pH monitoring parameters between the two groups, with the exception of the Jolley score, which was significantly higher in the patients with GER and CMA than in the patients with GER only (Mann-Whitney U test: $p < 0.008$).

Finally, because the IgG anti- β -lactoglobulin assay proved to be the most useful of the immunologic tests we considered in confirming a diagnosis of CMA, we determined its sensitivity and specificity in the study population (Table II).

DISCUSSION

The growing prevalence of CMA^{1,2} and the coincidence of a number of characteristic symptoms common to GER and CMA led us to investigate the relationship between these two pathologic conditions, especially in view of the dietary-therapeutic implications imposed by a diagnosis of CMA. In accordance with the criteria outlined by the European Society for Paediatric Gastroenter-

TABLE II. IgG anti- β -lactoglobulin assay results

	β -lactotest		
	Positive	Negative	Total cases
Patients with GER and CMA	85	0	85
Patients with GER only	27	92	119
Sensitivity	85/85 (100%)		
Specificity	92/119 (78.1%)		

ology and Nutrition we considered those patients who had clear clinical reactions after CM consumption accompanied by alterations in at least one immunologic test result, which confirmed hypersensitivity to CM protein, as having CMA.

Our data show that there is a high, and perhaps unexpected, frequency of the GER-CMA association during the first year of life. Indeed, as many as 41.8% of the patients with GER we observed consecutively were shown to have CMA.

We are unable to give an exact calculation of the prevalence of CMA in the general population of our region, but the data reported in the literature indicate a range between 3% and 5%.^{1,2} The very high prevalence of CMA in patients with GER cannot therefore be attributed to the frequency of this disease in the general population.

In our study it was possible to suspect the association between GER and CMA by simply carefully recording clinical history in just over a quarter of the cases; 19 of the 85 patients with GER and CMA, in fact, had diarrhea, rhinitis, or atopic dermatitis at the time of hospitalization. However, these symptoms were not overtly manifest, and the parents had

consulted our department because of the presence of other symptoms, including some of those we considered as selection criteria for our study. It is therefore very important to emphasize that a precise clinical history may be sufficient to indicate a suspected diagnosis of CMA and GER. In all of the other cases, the diagnosis of CMA was made on the basis of positive laboratory test results including, above all, the results of IgG anti-lactoglobulin assay, which proved to be very sensitive, although aspecific, in screening for suspected CMA. Considering the high percentage of GER-CMA association observed in this study, we suggest that all infants with pathologic GER in the first year of life should be carefully observed for possible concomitant CMA.

In regard to the pH monitoring characteristics of GER in the two study groups, we would like to underline the significant difference in Jolley scores at the time of diagnosis: the score was much higher in the patients with GER and CMA than in those with GER only. This is most probably not an occasional result, but rather the expression of a specific characteristic, which we observed in most of the pH monitoring tracings in the patients with GER and CMA. In fact, we noted that these infants almost always had normal esophageal pH during feeding and immediately after a feeding; however, after this period, there was an evident, constant, and progressive fall in esophageal pH, and prolonged periods of pH less than 4 were recorded. Therefore the Jolley score, excluding the two postprandial hours in its calculation, fully confirmed our impression of the characteristics of reflux in the patients with CMA. Further studies are also required to fully explain this observation, but we suspect that the gastric emptying mechanism, although of controversial importance in the pathogenesis of GER,¹⁸⁻²⁰ may play an important role in causing this phenomenon.

In conclusion, our study appears to affirm that: (1) in just under half of the cases of GER in infants under the age of 1 year, there is an association with CMA; (2) the frequency of this association should induce physicians to screen for a possible concomitant CMA in all infants with GER in this age group; (3) further studies are necessary to determine whether a 24-hour pH-monitoring pattern, which is indicative of GER caused by CMA can be identified.

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