

Gray-Scale and Color Doppler Ultrasound Findings in Children With Cow's Milk Allergy

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OBJECTIVE. The objective of our study was to evaluate whether gray-scale and color Doppler ultrasound can reveal intestinal inflammation in infants with cow's milk allergy (CMA).

SUBJECTS AND METHODS. This study evaluated the clinical findings and gray-scale and color Doppler sonograms of 34 infants. Seventeen 0- to 6-month-old infants with suspected CMA and 17 nonsymptomatic age-matched infants were evaluated by a blinded investigator who determined the percentage of vessel density and the thickness of different parts of the bowel. Clinical and sonographic variables were evaluated in the same regions of bowel considering three time points: presentation, after 4 weeks of feeding only amino acid-based formula, and after challenge test. Likelihood ratios and receiver operating characteristic (ROC) curves were used to define a cutoff point for vascular density percentage.

RESULTS. The difference in the percentage of vessel density between patients with CMA (mean, 28.1%) and control infants (mean, 7.77%) was statistically significant. ROC analysis showed that a cutoff point of 18.7% could differentiate between patients with CMA and control infants with 81.8% sensitivity and 94.1% specificity. The area under the curve was 0.941. We found statistical differences in bowel wall thickness between control patients and patients with CMA.

CONCLUSION. There was a significant increase in vessel density in infants younger than 6 months with CMA compared with healthy age-matched infants. The most appropriate cutoff point for vessel density was 18.7%. The results of this study suggest that Doppler ultrasound could be used as a screening tool to diagnose CMA.

The prevalence of allergic diseases, including food allergies, has increased over the past decades [1]. Cow's milk allergy (CMA) is a common clinical problem in pediatric patients, with a frequency of 2.5–7.5% of the general population [2, 3]. In the pathogenesis of CMA, early exposure of the immature infantile intestine to cow's milk protein triggers an immunologic response and consequent intestinal inflammation of variable intensity [4], either IgE-mediated or non-IgE mediated [5]. Patients with CMA may present with several gastrointestinal disorders: chronic diarrhea and malabsorption, gastroesophageal reflux, constipation, vomiting, insufficient weight gain, anemia, or low digestive tract hemorrhage [6, 7]. However, the symptoms of CMA are often unspecific: irritability, colic, intense crying, and refusal to eat [8].

The variability of the clinical signs and symptoms and the lack of conclusive laboratory tests or specific histologic changes in

the intestinal mucosa make CMA difficult to diagnose [9]. Several medical specialization guidelines prescribe the use of an exclusion diet; symptoms improve in cases suggestive of CMA, and the allergy is confirmed using a challenge test a few weeks after clinical improvement [10, 11].

In pediatrics, gray-scale sonography and color Doppler sonography have been increasingly used to evaluate acute and chronic abdominal vascular disorders and intestinal inflammation [12–14]. Inflammatory and infectious intestinal diseases cause a thickening of the intestinal wall that can be detected using gray-scale ultrasound [15, 16]. Color Doppler ultrasound detects increases in vessel density in the intestinal wall when there is active inflammation [17, 18]. These imaging modalities have not been used to examine children with CMA. The aim of this study was to evaluate whether gray-scale and color Doppler ultrasound can reveal intestinal inflammation related to CMA.

Subjects and Methods

This study was divided into two phases with complementary designs. The first phase was a comparison of ultrasound findings in a group of infants with signs and symptoms suggestive of CMA and in a healthy control group. The second phase was a clinical trial that compared ultrasound findings in infants with signs and symptoms suggestive of CMA at three diagnostic time points: at presentation, after treatment, and after challenge test.

This prospective study evaluated clinical data and gray-scale and color Doppler ultrasound findings of 34 consecutive children divided into two groups. The study was approved by our institutional review board in April 2006. Parents or guardians signed informed consent forms.

The study group comprised 17 infants 0–6 months old who presented with signs and symptoms of CMA and were referred to the division of pediatric gastroenterology of our institution between May 2006 and July 2009. The control group enrolled 17 nonsymptomatic infants 0–6 months old who were seen for a monthly pediatric follow-up visit at the pediatrics service of our institution during the same time period.

Clinical and ultrasound variables were evaluated at three time points in the study group and only once in the control group (Fig. 1). Study group inclusion criteria were patients suspected of having CMA according to physical examination and history obtained during a visit to the pediatric gastroenterology outpatient service. Exclusion criteria were exclusive breastfeeding; the use of another type of complementary diet during the study time; the use of any medication; another gastrointestinal disease; birth at < 36 weeks; and weight below third or above 97th percentile according to World Health Organization charts [19].

A clinical questionnaire was applied always by the same investigator, and the following data were collected: patient age; weight gain in the past 30 days; duration of breastfeeding; and whether cow's milk formula had been introduced in the infant's diet and, if so, when.

Data collected for the study group infants were recorded as dichotomous variables (yes or no): vomiting, diarrhea, blood in stools, irritability, constipation, and skin lesions. No questionnaire has been validated for CMA. Therefore, signs and symptoms were defined according to the literature: constipation, defined as delay or difficulty to evacuate, dry stools for

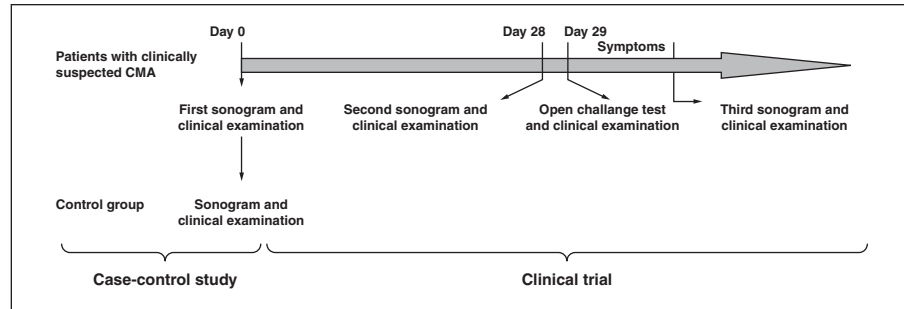


Fig. 1—Diagram shows study design (Neocate formula, Nutricia Advanced Medical Nutrition). CMA = cow's milk allergy.

2 weeks or more, and substantial patient discomfort [20]; diarrhea, defined as three or more loose or watery stools per day [21]; atopic urticaria or eczema, defined as maculopapular rash, diffuse erythema, or both; blood in stools; vomiting, defined as more than three episodes per day followed by discomfort; and irritability, defined as crying more than 3 hours per day during and after feeding with difficulty falling asleep and awakening several times during the night with intense crying.

All the symptoms were associated with the time when cow's milk formula was introduced and the later development of symptoms.

Ultrasound examinations were performed using linear transducers (C12–3 HD11 XE, Philips Healthcare). The gray-scale study measured the thickness of the wall of bowel loops in the jejunum, terminal ileum, and all colon segments. Measurements were made by placing one pointer at the mucosal layer and the other at the serosa in the same loop. Ultrasound examinations were performed by the same examiner, an experienced radiologist blinded to which group patients belonged.

The color Doppler test evaluated the quantity of peripheral mesenteric vessels in a 4-cm² area (2 × 2 cm). To examine the ileal and jejunal regions, five areas were examined in the right and five in the left side of the abdomen. All colored areas were classified as vessels. Vessel density in the surface was evaluated using image software, which we describe later in this article, that determined the percentage of colored areas in each image and the mean value.

Color Doppler gain was adjusted to eliminate the color artifacts but to retain vessel signals. Vessels were recorded as pulsating (artery) or continuous (vein) signal that persisted in a certain area. The ultrasound unit was adjusted to detect low-velocity blood flows, and velocity was standardized at 8 cm/s using a low wall filter. To avoid ob-

taining images with artifacts, imaging was not performed during patient movements or bowel loop peristalsis.

No patient in either group had, at the time of ultrasound examination, any other associated clinical condition that might affect intestinal vessels. The infants fasted for 3 hours before ultrasound examinations. The patients were first examined while lying on their back, and the examination was performed using a left and right flank approach or an anterior abdominal approach to avoid loops with gas contents. We tried to examine areas in which the bowel loops had no liquid, solid, or gas contents. If that was not possible, the examination was postponed 30–60 minutes to ensure that bowel loops were emptied. The average time of an ultrasound examination was 15 minutes.

After the ultrasound images were saved, public domain software (ImageJ, National Institutes of Health) was used for computer-assisted analysis. This software [22], available for free download, can provide the percentage of vessel density in the tissue sample under examination, the 4-cm² area described earlier, and can calculate the percentage of colored area on each image. Because the color image corresponded to blood vessels (pulsatile or continuous color in a certain pixel) in the images obtained, the vascularized area could be determined in each image, and a value from 0% to 100% was obtained. This number was called the percentage of vessel density (Fig. 2).

When the ultrasound examinations, questionnaire, and physical examinations were completed, the guardians of study group patients received instructions to feed the infants with only an amino acid–based formula (Neocate formula, Nutricia Advanced Medical Nutrition) for 4 weeks (Fig. 1).

After 4 weeks receiving amino acid–based formula, study group patients underwent another evaluation in the pediatric gastroenterology

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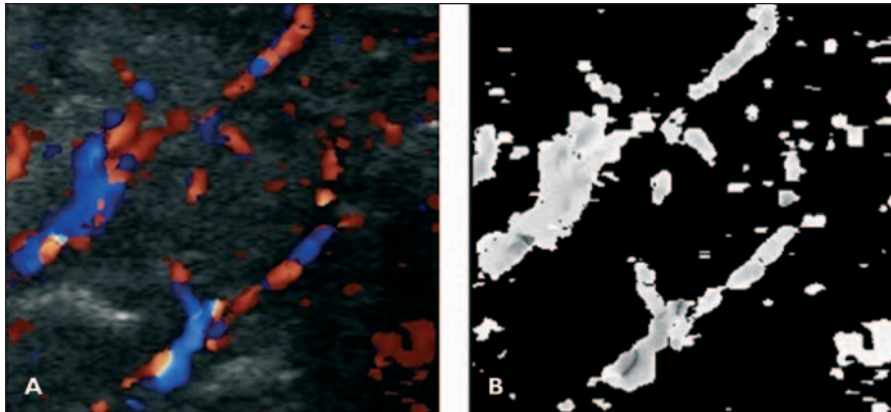


Fig. 2—Use of software to calculate vessel density. **A**, Color Doppler sonogram shows jejunal loops and vessel density in selected 4-cm² region of interest. **B**, Figure analyzed using ImageJ software (National Institutes of Health) [22] shows percentage of vessel density.

outpatient service using the questionnaire, clinical examination, and gray-scale and color Doppler ultrasound examinations.

After the ultrasound examinations, the study group patients underwent an open challenge according to guidelines [10]. The parents and pediatricians were aware that the infants would be exposed to cow's milk protein under medical supervision in a hospital. After a physical examination of the undressed infant, including skin examination, a drop of cow's milk formula was placed on the infant's lips. If no reaction occurred after 15 minutes, cow's milk formula was given orally, and the dose was increased stepwise (1, 5, 10, 20, 50–100 mL) every 30 minutes. Thereafter, the infant was observed for 3 hours and examined for skin or respiratory reactions before leaving the hospital. If no reaction occurred, parents were told to give the infant at least 250 mL of cow's milk for-

mula each day for the next week and observe the child for late reactions.

Another ultrasound examination, clinical control, and questionnaire were completed according to the same evaluation criteria described earlier if the patient had signs or symptoms that confirmed CMA or a week after the oral challenge test even when there was no clinical confirmation.

SPSS software (version 13.0, SPSS) was used for statistical analyses. The Student *t* test was used to compare symmetrically distributed continuous variables between groups and the Mann-Whitney test, for asymmetric distributions. The Pearson chi-square or Fisher exact test was used for categorical variables. Repeated-measures analysis of variance, followed by the Bonferroni test, was used to compare means of ultrasound variables in the three scans.

A cutoff point was defined for the percentage of vessel density according to quar-

tiles and the likelihood ratio (LR). The cutoff point was also assessed using the ROC curve. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used as aids in the calculation of the best cutoff point. The area under the curve (AUC) was also calculated together with 95% CI. The level of significance was set at 5% ($p < 0.05$).

Results

Thirty-four patients were enrolled in the study. Both groups were similar at baseline with no significant differences in sex or median age, weight, or length. Patients in the study group had a lower weight gain in the previous month than patients in the control group ($p = 0.003$). The infants in the study group had a shorter breastfeeding duration and earlier introduction of cow's milk formula ($p = 0.004$). Irritability (100%) and vomiting

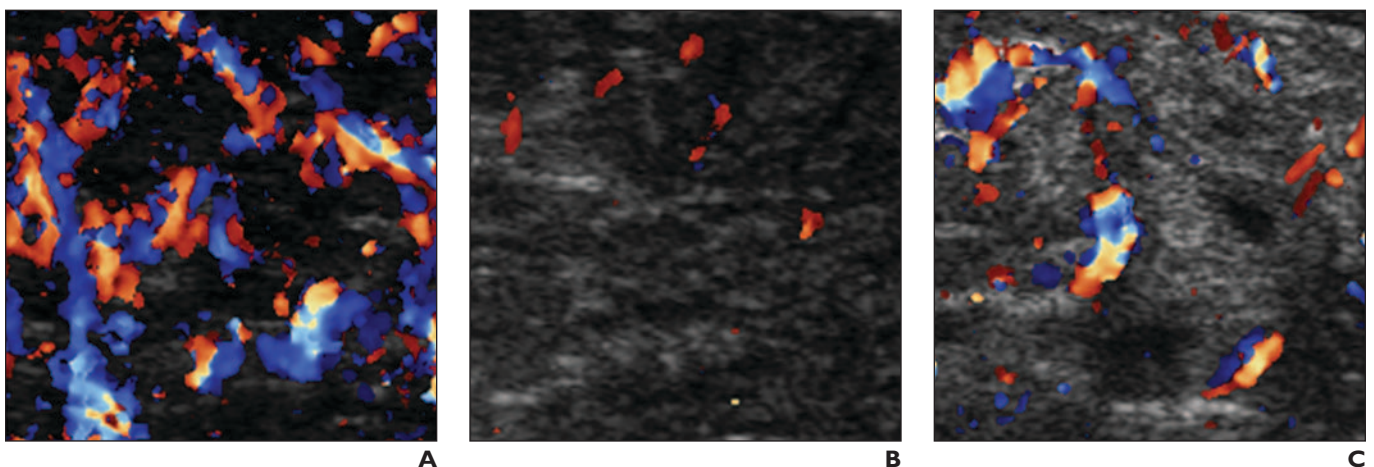


Fig. 3—6-week-old male infant with suspected cow's milk allergy. Allergy is suspected because patient presented with symptoms of irritability, vomiting, severe food refusal, and poor weight gain.

A, Doppler ultrasound image obtained before treatment shows increased vascularity.

B, Doppler ultrasound image obtained after infant was fed amino acid–based formula for 4 weeks shows complete improvement of symptoms.

C, Doppler ultrasound image shows important increase in vascularity 48–72 hours after oral challenge test. At this examination, symptoms were vomiting, food refusal, and intense irritability.

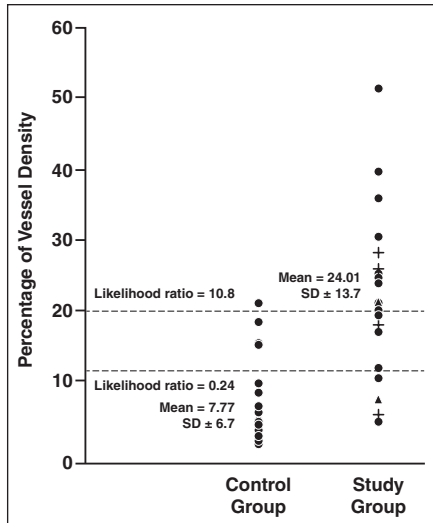


Fig. 4—Mean percentage vessel density for each patient in study group and control group based on 10 images ($p < 0.05$). Dotted lines show cutoff points according to 50th and 75th percentiles. ● = confirmed case of CMA, + = no confirmed case of CMA, ▲ = patients that dropped out before second ultrasound examination.

(70%) were the most frequent symptoms in the study group. Two of the 17 patients in the study group underwent only the first ultrasound test: The parent of one patient refused to provide consent for the challenge test and the parent of the other patient did not bring the infant back to continue follow-up. In the remaining 15 infants who completed the study and underwent three ultrasound examinations, 11 had CMA confirmed by the challenge test.

Figure 3 shows three Color Doppler images that correspond to three sample points of a 6-week-old infant who was suspected of having CMA. Figure 4 shows the percentage of mean vessel density for each patient in both groups at baseline. The mean percentage of vessel density in the study group (24.01%; SD, 13.7%) was greater than that in the control group (7.77%; SD, 6.7%) ($p < 0.05$).

The cutoff points for percentage of vessel density according to the 50th and 75th percentiles in this study were below 12% to rule out CMA, with an LR of 0.24 for the possibility of CMA diagnosis and NPV of 86.7%, and above 20% to confirm CMA, with an LR of 10.8 and PPV of 87.5% (Table 1 and Fig. 4).

Weight gain of the 11 patients with a confirmed diagnosis of CMA between the first and the second study time points—that is, after having received only amino acid-based formula for 4 weeks—was greater (mean, 0.88 kg; SD, 0.33) than the weight gain in

TABLE 1: Likelihood Ratio (LR) to Confirm Diagnosis of Cow's Milk Allergy (CMA) According to Percentage of Image Area With Blood Vessels on Ultrasound

Percentage of Vessel Density	No. (%)		LR	PPV (%)	NPV (%)
	Patients With Confirmed CMA ($n = 11$)	Control Subjects ($n = 17$)			
0 to < 12	2 (18.2)	13 (76.5)	0.24		86.7
12–20	2 (18.2)	3 (17.6)	1.03		
> 20	7 (63.6)	1 (5.9)	10.8 ^a	87.5	

Note—PPV = positive predictive value, NPV = negative predictive value.

^a $p < 0.001$ for group with percentage from 0% to < 12%.

the month before the first visit (0.53 kg; SD, 0.28) ($p = 0.036$).

The mean percentages of vessel density for patients with a confirmed diagnosis of CMA and control patients were significantly different ($28.1\% \pm 14.5\%$ vs $7.77\% \pm 6.07\%$, respectively) ($p = 0.001$); its progression for patients with a confirmed diagnosis of CMA is described in Figure 5.

ROC analysis was used to define the optimal cutoff point for percentage of vessel density and revealed that a cutoff point of 18.7% could differentiate between patients likely to have CMA and control patients; sensitivity was 81.8%; specificity, 94.1%; and PPV and NPV, 90.0% and 88.9%, respectively. The AUC was 0.941 (95% CI, 0.860–1.000) (Fig. 6).

The thicknesses of the intestinal segments in the control group and at the three time points in the study group are shown in Table 2. The comparison of intestinal wall thickness between control group patients and patients with confirmed CMA indicated that intestinal wall thickness was greater among the latter group ($p < 0.05$). However, the second

and third scans showed the same difference in wall thicknesses in only the jejunal and ileal segments (Table 2).

Discussion

For this study, we evaluated the use of gray-scale and color Doppler ultrasound as a diagnostic tool in pediatric patients with clinical findings suggestive of CMA. This method was compared with the oral test challenge, which is considered to be the reference standard test for CMA diagnosis. The percentage of mesenteric vessel density according to color Doppler scans in patients with signs and symptoms suggestive of CMA was significantly greater than in healthy infants, and the value increased when only the patients with a diagnosis confirmed by the challenge test were included in the analysis.

Moreover, the value of vessel density from the second (after 4 weeks of exclusion diet) to the third (after the challenge test) ultrasound examination in patients with a confirmed diagnosis of CMA increased from 12.7% to 16.5%, but this increase was not statistically

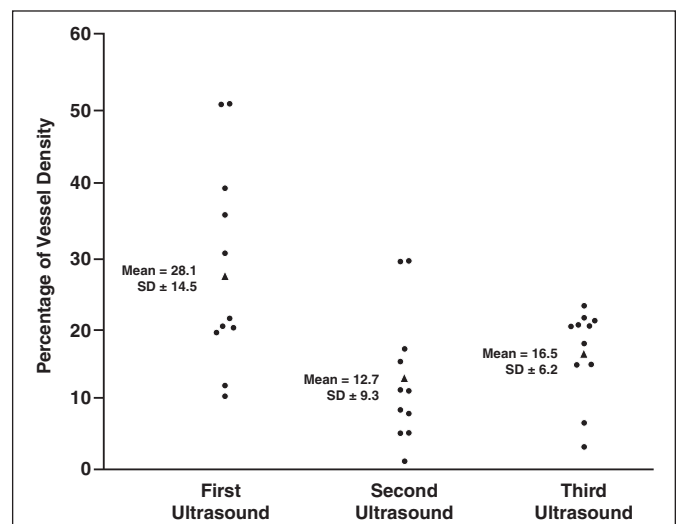


Fig. 5—Mean values of three color Doppler sonograms for 11 patients (●) with confirmed cow's milk allergy. Values of first sonogram were greater than values of second ($p = 0.003$) and third ($p = 0.024$) sonograms; values for second and third sonograms did not differ from each other ($p = 0.519$). ▲ = mean.

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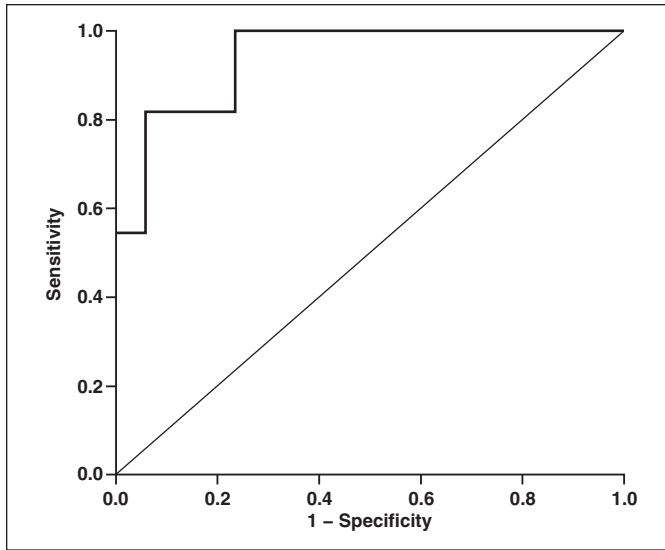


Fig. 6—Sensitivity and specificity of percentage of vessel density for disease according to receiver operating characteristic (ROC) curve analysis. Area under curve is 0.941; 95% CI, 0.860–1.000; cutoff point, 18.7%; sensitivity, 81.8%; specificity, 94.1%; positive predictive value, 90.0%; and negative predictive value, 88.9%.

significant. This finding may be attributed to the fact that the ultrasound examination was conducted immediately after symptoms appeared. Because it was an open test, parents were asked to report the appearance of any symptom and it would not be ethical to keep cow's milk feeding only to reach a more significant increase of inflammation in the digestive tract.

The cutoff percentages for vessel density were below 12% and above 20%, which matched the 50th and 75th percentiles, respectively, in the sample. These values also show an LR of 0.24 and 10.8, respectively, to rule out or confirm the diagnosis of CMA.

The power of percentage of vessel density as a test to diagnose CMA was assessed using the area under the ROC curve. The percentage of vessel density was a significant predictor of CMA because the area under the ROC curve was 0.941. Sensitivity, specificity, PPV, and NPV were above 88%.

Ultrasound findings, particularly vessel density, seem to be associated with inflammatory changes in the intestinal wall [13, 17, 23]. The physiopathologic mechanism of CMA in infants is usually associated with intestinal inflammation [24], and ultrasound may, therefore, detect these changes. When associated with clinical parameters, ultrasound may be useful to suggest the diagnostic hypothesis of CMA.

Color Doppler imaging may detect an increase in intestinal vessels in other intestinal inflammatory diseases. Our results showed that similar results may also be found in infants with CMA. This increase in vessel density may be used as a noninvasive tool to help to establish a diagnosis and to follow up infants suspected of having CMA.

As expected, patients with signs and symptoms suggestive of CMA had a lower weight gain than healthy patients, as well as a shorter duration of breastfeeding and

earlier introduction of cow's milk formula. The most frequent symptoms were irritability (100%) and vomiting (70%). Symptoms of CMA are unspecific, and the diagnosis, therefore, is difficult.

We used an amino acid-based formula (Neocate) because it is completely free of allergens and would ensure a safer response to the exclusion diet [25–27]. According to the literature, the oral challenge test can be used to confirm the diagnosis of CMA. However the challenge test can reveal false-negative results that must be taken into account [28, 29]. Therefore, tolerance may have developed during the 4 weeks of the exclusion diet. Two infants of the study group underwent only the first ultrasound examination. The parent of one infant refused to give consent for the oral challenge test and the parent of the other did not bring the infant back for follow-up. However, both infants were using amino acid formula. The remaining 15 infants of the study group completed the study and underwent three ultrasound examinations each; 11 had CMA confirmed by the oral challenge test and four did not.

Two of the four infants in whom the CMA diagnosis was not confirmed after oral challenge test have had symptoms highly suggestive of CMA, such as bloody stools and diarrhea. These infants' symptoms have improved after the use of amino acid-based formula. The vessel density measurement was above the cutoff point; this finding might be explained because tolerance to the allergic protein could have developed.

The intestinal wall thickness measured in the four segments under study using gray-scale ultrasound was significantly different between control patients and patients with CMA. However, this difference remained significant on the second and third ultrasound scans in only the ileum and the jejunum. Because the difference in intestinal wall thickness between the control group and the patients with CMA was small, this parameter seems to have little value for clinical practice.

The main symptom for four of the infants with a confirmed diagnosis of CMA was blood in stools. The mean wall thickness in those infants on the first ultrasound examination was 2.97 mm in the left colon, which was significantly greater than the mean wall thickness in the control group. This difference suggests that the assessment of submucosal wall thickening in infants with CMA associated with blood in stools may help to establish a diagnosis and to monitor clinical progression.

TABLE 2: Intestinal Thickness on Ultrasound for Control Subjects and Patients With Confirmed Cases of Cow's Milk Allergy (CMA)

Intestinal Segment Measured	Wall Thickness (mm) (mean ± SD)				p_{group}^a	$p_{\text{ultrasound}}^b$
	Control Subjects	Patients With Confirmed Cases of CMA				
		First Ultrasound	Second Ultrasound	Third Ultrasound		
Left colon	1.18 ± 0.38	1.99 ± 1.17	1.33 ± 0.40	1.72 ± 0.77	0.049	0.123
Right colon	1.05 ± 0.10	1.55 ± 0.52	1.15 ± 0.33	1.35 ± 0.48	0.011	0.117
Ileum	1.86 ± 0.52	2.28 ± 0.44 ^b	1.77 ± 0.69 ^a	2.10 ± 0.46 ^{a,b,c}	0.038	0.006
Jejunum	1.29 ± 0.28	1.72 ± 0.57 ^b	1.15 ± 0.46 ^a	1.52 ± 0.46 ^{a,b,c}	0.037	0.004

^aComparison of control group with confirmed cases in first ultrasound using the Student *t* test.

^bComparison of three ultrasound examinations of confirmed cases using repeated-measures analysis of variance.

^cNo difference according to Bonferroni test.

For practical and ethical reasons, amino acid–based formula was not given to asymptomatic patients and ultrasound examinations were not repeated in these patients.

In many previous studies, investigators have counted the number of vessels in the intestinal wall to define the intensity of circulation [13, 30, 31]. However, considering that the small-bowel loops are tortuous and overlapping, the identification of individual vessels may be difficult. Therefore, we decided to evaluate vessel density of the intestinal wall by capturing images of several ($n = 10$) segments and calculating a mean value for the areas with the most and the fewest vessels. This method may be adequate for clinical practice because it uses free image software.

Two of the limitations of this study were the small number of patients and the fact that the sample was clinically heterogeneous. Patients had CMA symptoms of varying degrees of severity, which might be a sign of different clinical presentations. Even so, our results showed significant differences. Another possible limitation was that the same radiologist performed all ultrasound studies. To minimize this limitation, the examiner was blinded and acquired 10 digital images of each infant that were then quantitatively analyzed by ImageJ software.

This study may open an interesting line of research about CMA, an important disease with a high incidence among infants. The free computer-assisted image analysis method used in this study is innocuous to patients, is easy to perform by the medical team, and has good applicability.

Conclusions

There is an important difference in intestinal vessel density in infants younger than 6 months with CMA compared with healthy infants matched according to age. The results of this study suggest that Doppler ultrasound could be used as a tool to screen for CMA. An 18.7% cutoff point for vessel density should be adopted: Results below the cutoff would indicate that the likelihood of CMA is low and above it, that it is high.

References

- Hill DJ, Firer MA, Shelton MJ, et al. Manifestations of milk allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986; 109: 270–276
- Sicherer SH. Food allergy. *Lancet* 2002; 360:701–710
- Wershil BK, Butzner D, Sabra A, et al. Allergy and immunologic disease: Working Group Report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2002; 35(suppl 2): S74–S77
- Sampson H. Update on food allergy. *J Allergy Clin Immunol* 2004; 113:805–819; quiz, 820
- Walker-Smith J. Cow's milk allergy: a new understanding from immunology. *Ann Allergy Asthma Immunol* 2003; 90[suppl 3]:81–83
- Magazzu G, Scoglio R. Gastrointestinal manifestations of cow's milk allergy. *Ann Allergy Asthma Immunol* 2002; 89[suppl 1]:65–68
- [No authors listed]. American Gastroenterological Association medical position statement: guidelines for the evaluation of food allergies. *Gastroenterology* 2001; 120:1023–1025
- Jakobsson I, Lindberg T. Cow's milk proteins cause infantile colic in breast-fed infants: a double-blind crossover study. *Pediatrics* 1983; 71: 268–271
- Ewing WM, Allen PJ. The diagnosis and management of cow milk protein intolerance in the primary care setting. *Pediatr Nurs* 2005; 31: 486–493
- Vandenplas Y, Koletzko S, Isolauri E, Hill D, Oranje A, Brueton M. Guidelines for the diagnosis and management of cow's milk protein allergy in infants. *Arch Dis Child* 2007; 92:902–908
- Niggemann B, Beyer K. Diagnosis of food allergy in children: toward a standardization of food challenge. *J Pediatr Gastroenterol Nutr* 2007; 45: 399–404
- Quillin SP, Siegel MJ. Gastrointestinal inflammation in children: color Doppler ultrasonography. *J Ultrasound Med* 1994; 13:751–756
- Dietrich CF, Jedrzejczyk M, Ignee A. Sonographic assessment of splanchnic arteries and the bowel wall. *Eur J Radiol* 2007; 64:202–212
- Epifanio M, Baldisserotto M, Spolidoro J, Gaiger A. Gray-scale and color Doppler sonography in the evaluation of children with suspected bowel inflammation: correlation with colonoscopic and histologic findings. *Clin Radiol* 2008; 63:968–978
- Canani RB, Horatio L, Terrin G, et al. Combined use of noninvasive tests is useful in the initial diagnostic approach to a child with suspected inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2006; 42:9–15
- Baud C, Saguinthaah M, Veyrac C, et al. Sonographic diagnosis of colitis in children. *Eur Radiol* 2004; 14:2105–2119
- Spalinger J, Patriquin H, Miron MC, et al. Doppler US in patients with Crohn disease: vessel density in the diseased bowel reflects disease activity. *Radiology* 2000; 217:787–791
- Scholbach T, Herrero I, Scholbach J. Dynamic color Doppler sonography of intestinal wall in patients with Crohn disease compared with healthy subjects. *J Pediatr Gastroenterol Nutr* 2004; 39:524–528
- World Health Organization Website. The WHO child growth standards. www.who.int/childdgrowth/en/. Accessed March 8, 2011
- Constipation Guideline Committee of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Evaluation and treatment of constipation in infants and children: recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2006; 43: e1–e13
- King C, Glass R, Bresee J, Duggan C; Centers for Disease Control and Prevention. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep* 2003; 52:1–16
- ImageJ: image processing and analysis in Java Website. rsb.info.nih.gov/ij/index.html. Accessed March 8, 2011
- Ruess L, Blask AR, Bulas DI, et al. Inflammatory bowel disease in children and young adults: correlation of sonographic and clinical parameters during treatment. *AJR* 2000; 175:79–84
- Troncone R, Discepolo V. Colon in food allergy. *J Pediatr Gastroenterol Nutr* 2009; 49[suppl 2]: S89–S91
- Hill DJ, Murch SH, Rafferty K, et al. The efficacy of amino acid-based formulas in relieving the symptoms of cow's milk allergy: a systematic review. *Clin Exp Allergy* 2007; 37:808–822
- De Boissieu D, Dupont C. Allergy to extensively hydrolyzed cow's milk proteins in infants: safety and duration of amino acid-based formula. *J Pediatr* 2002; 141:271–273
- Niggemann B, Binder C, Dupont C, Hadji S, Arvola T, Isolauri E. Prospective, controlled, multi-center study on the effect of an amino-acid-based formula in infants with cow's milk allergy/intolerance and atopic dermatitis. *Pediatr Allergy Immunol* 2001; 12:78–82
- Niggemann B, Beyer K. Pitfalls in double-blind, placebo-controlled oral food challenges. *Allergy* 2007; 62:729–732
- Caffarelli C, Petroccione T. False-negative food challenges in children with suspected food allergy. *Lancet* 2001; 358:1871–1872
- Siegel MJ, Friedland JA, Hildebolt CF. Bowel wall thickening in children: differentiation with US. *Radiology* 1997; 203:631–635
- Faingold R, Daneman A, Tomlinson G, et al. Necrotizing enterocolitis: assessment of bowel viability with colour Doppler US. *Radiology* 2005; 235:587–594