

Diagnostic Accuracy of Fecal Calprotectin Assay in Distinguishing Organic Causes of Chronic Diarrhea from Irritable Bowel Syndrome: A Prospective Study in Adults and Children

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Background: Fecal calprotectin (FC) has been proposed as a marker of inflammatory bowel disease (IBD), but few studies have evaluated its usefulness in patients with chronic diarrhea of various causes. We evaluated the diagnostic accuracy of a FC assay in identifying “organic” causes of chronic diarrhea in consecutive adults and children.

Methods: We consecutively enrolled 70 adult patients (30 males, 40 females; median age, 35 years) and 50 children (20 males, 30 females; median age, 3.5 years) with chronic diarrhea of unknown origin. All patients underwent a complete work-up to identify the causes of chronic diarrhea. FC was measured by ELISA.

Results: In adult patients, FC showed 64% sensitivity and 80% specificity with 70% positive and 74% negative predictive values for organic causes. False-positive results (8 of 40 cases) were associated with the use of aspirin (3 cases) or nonsteroidal antiinflammatory drugs (1 case) and with the presence of concomitant liver cirrhosis (3 cases). False-negative results mainly included patients suffering from celiac disease (5 cases). Patients with IBD (9 cases) were identified with 100% sensitivity and 95% specificity. In pediatric patients, sensitivity was 70%, specificity was 93%, and positive and negative predictive values were 96% and 56%.

False-negative results (11 of 35 cases) were associated mainly with celiac disease (6 cases) or intestinal giardiasis (2 cases).

Conclusions: FC assay is an accurate marker of IBD in both children and adult patients. In adults, false negatives occur (e.g., in celiac disease) and false-positive results are seen in cirrhosis or users of nonsteroidal antiinflammatory drugs. Diagnostic accuracy is higher in children.

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Irritable bowel syndrome (IBS)⁵ is one of the most frequent causes of chronic diarrhea in adult patients, and previous studies have suggested that a careful standard medical history could suggest IBS diagnosis rather than an organic disease (1, 2). However, the diagnostic work-up of patients with diarrhea and suspected IBS includes a barium x-ray study of the colon or sigmoidoscopy, and colonoscopy is recommended for patients over the age of 50 (3). Consequently, to avoid invasive investigations, several noninvasive markers have been suggested to distinguish functional gastrointestinal disorders from “organic” diseases. Among these, fecal calprotectin (FC) concentrations have been shown to be a good marker of organic disease, being higher in patients with inflammatory bowel disease (IBD) (4) and colorectal carcinoma than in controls (5). Calprotectin constitutes ~60% of the soluble cytosol proteins in neutrophil granulocytes and plays a central role in neutrophil defense. Consequently, its concentration in

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Received January 10, 2003; accepted March 19, 2003.

⁵ Nonstandard abbreviations: IBS, irritable bowel syndrome; FC, fecal calprotectin; IBD, inflammatory bowel disease; and NSAID, nonsteroidal antiinflammatory drug.

stool correlates with the intensity of neutrophil infiltration of the intestinal mucosa and with the severity of inflammation. Furthermore, its *in vivo* and *in vitro* resistance to degradation allows fecal samples to be assayed for a reliable calprotectin determination (6). Most previous studies on the FC assay, however, were carried out on prediagnosed groups of patients, and its diagnostic accuracy in distinguishing organic from "functional" diarrhea has rarely been evaluated in adult patients (4, 7, 8) and never in children.

The aim of the present study was to evaluate the positive and negative predictive values of the FC assay in identifying the organic causes of chronic diarrhea in consecutive individuals referred as outpatients to gastroenterology centers both for adults and for children.

Patients and Methods

We enrolled all consecutive patients referred for chronic diarrhea to the outpatient clinics of the Division of Internal Medicine of the University Hospital and of the Pediatric Division of "Di Cristina" Hospital, both in Palermo, between January and June 2001. Thirty-eight individuals during the same period were recruited as controls; they were apparently healthy or were prediagnosed as having IBD.

STUDY DESIGN

The inclusion criterion was a history of chronic diarrhea of unknown origin, lasting for more than 4 weeks, with or without abdominal pain. Exclusion criteria included previous evaluation for chronic diarrhea, overt gastrointestinal bleeding, sigmoidoscopy or colonoscopy during the previous 2 years performed for any cause, familial adenomatous polyposis and hereditary nonpolyposis, colorectal cancer syndrome, and pregnancy.

For each participant in the study, data were collected about the use of nonsteroidal antiinflammatory drugs (NSAIDs), aspirin, and anticoagulants. The concomitant presence of other nongastroenterologic diseases, in particular, rheumatoid arthritis or other connective tissue inflammatory diseases, or respiratory or urinary tract inflammation/infection, was recorded to evaluate a possible influence of these diseases on FC concentration.

A full medical history was obtained from all adults enrolled in the study, and a physical examination was performed; each patient was evaluated by documentation of the Rome criteria, which are considered a guide for IBS diagnosis (9). In addition, all patients underwent first-step hematology and chemistry tests (including erythrocyte sedimentation rate, serum C-reactive protein, blood cell counts, electrolytes, and thyroid, liver, and renal function); serologic assays for suspected celiac disease (anti-gliadin IgA and IgG and anti-transglutaminase IgA); stool examination for occult blood, ova, and parasites; and a lactose-H₂ breath test. In accordance with the current diagnostic approach (3, 10), adult patients also under-

went sigmoidoscopy with biopsy if under 40 years of age or colonoscopy with biopsy if over 40. Patients with negative results for all of the examinations described above and with a clinical history indicative of IBS according to the Manning criteria (1) were considered to be suffering from IBS and began a therapeutic trial with amitriptyline or propantheline bromide plus bromazepam.

Pediatric patients underwent the same first-step examinations as the adults with the exception of the lactose-H₂ breath test, but particular attention was given to a possible diagnosis of cow's milk intolerance/allergy. This included an elimination diet without cow's milk and its derivatives, and the patients cured or improved on this diet underwent a subsequent double-blind cow's milk challenge as described previously (11). The disappearance of the diarrhea on elimination diet and its reappearance on food challenge was considered indicative of food intolerance. In addition, children with positive occult blood in the stool or with serum indices of inflammation (positive serum C-reactive protein, erythrocyte sedimentation rate >20, increased white blood cell or platelet count) underwent colonoscopy with biopsy. Pediatric patients negative for all the first-step examinations were considered to be suffering from IBS and began a therapeutic trial with trimebutine.

All participants with one or more positive examinations at the first-step evaluation, according to the judgment of the individual laboratory/instrumental data physicians, underwent a diagnostic work-up, which may have included esophago-gastro-duodenoscopy, colonoscopy, small intestine barium examination, abdominal ultrasonography, and/or computed tomography scan, transit test, and duodenal fluid microbiological evaluation.

Subsequent monthly follow-up for 17–23 months (median, 20 months) was performed to confirm the initial diagnoses. In particular, a diagnosis of IBS was confirmed if diarrhea disappeared or improved during treatment and when routine hematochemical analyses, evaluated every 6 months, remained within reference values. Other diseases were diagnosed using generally accepted conventional criteria.

For the adult patients, two control groups were selected: one composed of 10 patients suffering from active ileocolonic Crohn disease (5 males, 5 females; age range, 20–65 years; median, 35 years), diagnosed according to standard endoscopy and histology criteria, and the other composed of 10 healthy volunteers (5 males and 5 females; age range, 25–45 years; median, 30 years). Among the patients suffering from Crohn disease, four had an exclusive ileum localization, five an ileum-colon localization, and one an exclusive colon localization. Eight of these patients were studied during an acute phase of the disease, and the median Crohn Disease Activity Index (12) in these patients was 195 (range, 60–300; values <150 indicate nonactive disease).

Similarly, two control groups were enrolled for the pediatric patients: the first composed of 8 patients suffering from ileocolonic Crohn disease (5 males, 3 females; age range, 4–12 years; median, 10 years), diagnosed according to standard endoscopy and histology criteria; the second composed of 10 healthy children (5 males, 5 females; age range, 1–10 years; median, 3.5 years). The pediatric patients included in the Crohn disease control group were in an active phase of the disease, and all had an ileum-colon localization. The severity of their disease was assessed by the Lloyd–Still score (13), which ranged between 25 and 61 (values >75 indicate nonactive disease).

The protocol was approved by the ethics committee of the University Hospital of Palermo, and informed consent was obtained from the adult patients and the parents of the children involved in the study.

FC ASSAY

One stool sample for calprotectin estimation was collected and returned by each study participant within 1 week of the first visit. Laboratory personnel unaware of the clinical diagnoses or details of the patients' clinical histories stored the specimens at -20°C and assayed calprotectin within 4 weeks. After thawing, calprotectin concentrations were measured by a commercial ELISA system (Calprest; Eurospital) based on polyclonal antibodies against calprotectin, according to the method modified by Ton et al. (14). Two 100-mg fecal aliquots from a single stool sample from each participant were assayed, and the mean of the two measurements was recorded. Threshold values supplied by the manufacturer ($<50\ \mu\text{g/g}$ of stool = negative; $50\text{--}100\ \mu\text{g/g}$ of stool = borderline; $>100\ \mu\text{g/g}$ of stool = positive) were considered for statistical analysis. The intraassay CV for FC was 3.1% at a mean (SD) of $63\ (20)\ \mu\text{g/g}$ ($n = 25$), and the interassay CV was 10% at $76\ (21)\ \mu\text{g/g}$ ($n = 25$).

The gastroenterologists who made the final diagnoses were unaware of the FC results throughout the study.

STATISTICAL ANALYSIS

We followed the STARD checklist for studies on the diagnostic accuracy of tests (15).

The sensitivity and specificity of the FC assay and its positive and negative predictive values, along with their 95% confidence intervals, were calculated by standard statistical methods (16). The Mann–Whitney test was used to compare the FC values recorded in IBS patients and in the other groups studied. The Fisher exact test was used to compare the frequency of the association between false-positive results and drug use or presence of other associated diseases. The Pearson correlation coefficient was calculated to verify the correlation between individual FC concentration and the activity index of the Crohn disease.

Results

A total of 126 consecutive patients with chronic diarrhea were initially included in the study. Six patients (four adults and two children), all diagnosed as suffering from IBS after the first clinical/laboratory evaluation, were lost during the follow-up; consequently, their data were excluded from the final analysis. Thus a total of 70 adult patients (30 males, 40 females; age range, 18–72 years; median, 35 years) and 50 children (20 males, 30 females; age range, 8 months–10 years; median, 3.5 years) were enrolled.

The final diagnoses for the 120 patients with chronic diarrhea who completed the study, divided into adults and children, are shown in Table 1. None had more than one gastrointestinal disease. The final diagnosis was IBS in 55 patients (40 adults and 15 children), whereas in the other 65 patients (30 adults and 35 children), an organic cause of chronic diarrhea was found. In all cases, the clinical and laboratory follow-up confirmed these diagnoses.

The median and ranges of FC concentrations in patients with IBS, in patients with organic chronic diarrhea, and in the control groups are shown in Table 2. In adult patients, individuals with an organic cause of diarrhea had significantly higher FC concentrations than individuals with IBS ($P = 0.03$, Mann–Whitney test). The values of the age-matched healthy controls did not differ from those of the IBS patients and were significantly lower than those of the patients with organic diarrhea ($P = 0.002$, Mann–Whitney test). Age-matched control patients with Crohn disease had FC values higher than both IBS patients ($P < 0.0001$) and those with organic diarrhea ($P = 0.015$, Mann–Whitney test).

All of the consecutive adult patients with chronic diarrhea suffering from Crohn disease had high FC con-

Table 1. Number and percentage of pediatric and adult patients included in the study and final diagnoses of the individuals considered as controls.

	No. of cases (%)
Children	50
IBS	15 (30%)
Patients with organic diarrhea	35 (70%)
Cow's milk intolerance	15 (30%)
Celiac disease	13 (26%)
Multiple food intolerances	5 (10%)
Intestinal giardiasis	2 (4%)
Adults	70
IBS	40 (57%)
Patients with organic diarrhea	30 (43%)
Celiac disease	10 (14%)
Multiple food intolerances	2 (3%)
Colorectal cancer/adenomatous polyps	3 (4%)
Microscopic colitis	2 (3%)
Diverticulosis/diverticulitis	4 (6%)
Crohn disease	9 (13%)

Table 2. Median values and range of FC (expressed as $\mu\text{g/g}$ of stool) in patients with IBS, patients with chronic diarrhea from organic causes, and in the control groups, according to age.

	IBS	Diarrhea from organic causes	Crohn disease controls	Age-matched healthy controls
Adults				
n	40	30	10	10
Median	35	77.5	320	20
Range	10–210 ^{a,b}	15–400 ^{a,c,d}	155–450 ^{b,d}	10–40 ^c
Children				
n	15	35	8	10
Median	20	45	260	15
Range	10–130 ^{e,f}	20–200 ^{e,g,h}	160–350 ^{f,h}	10–40 ^g

^{a-d} Mann-Whitney test on results in adults: ^aIBS vs diarrhea from organic causes, $P = 0.03$; ^bIBS vs Crohn disease controls, $P < 0.0001$; ^cdiarrhea from organic causes vs age-matched healthy controls, $P = 0.002$; ^ddiarrhea from organic causes vs Crohn disease controls, $P = 0.015$.

^{e-h} Mann-Whitney test on results in children: ^eIBS vs diarrhea from organic causes, $P = 0.04$; ^fIBS vs Crohn disease controls, $P < 0.0001$; ^gdiarrhea from organic causes vs age-matched healthy controls, $P = 0.005$; ^hdiarrhea from organic causes vs Crohn disease controls, $P = 0.01$.

centrations (median, 320 $\mu\text{g/g}$; range, 180–400 $\mu\text{g/g}$), and FC concentrations correlated significantly with the Crohn Disease Activity Index ($r = 0.54$; $P < 0.01$). The ROC curve plotted from the data of the consecutive patients with final diagnoses of IBS or Crohn disease showed that the calprotectin value with the highest diagnostic accuracy was 170 $\mu\text{g/g}$: it was 100% sensitive and 95% specific in the diagnosis of Crohn disease. Furthermore, values above the cutoff limit of 50 $\mu\text{g/g}$ were recorded in 5 of the 10 patients suffering from celiac disease, in 2 of the 4 patients with diverticulosis/diverticulitis, in both of the patients with microscopic colitis, in 1 of the 3 patients with colorectal cancer or adenomatous polyps, and in neither of the 2 patients suffering from multiple food intolerances.

Regarding the 40 adult patients with a final diagnosis of IBS, we found FC values above the cutoff limit of 50 $\mu\text{g/g}$ in 8 cases. All of these patients had been previously diagnosed as suffering from other diseases: liver cirrhosis related to hepatitis C infection (three cases), ischemic coronary disease (two cases), chronic bronchitis (two cases), and chronic renal insufficiency (one case). Three of the eight were taking aspirin, and one was taking NSAIDs at the time of the study. Both the frequency of liver cirrhosis (three of eight false-positive cases) and the use of aspirin or NSAIDs (four of eight false-positive cases) were significantly associated with false-positive calprotectin results ($P = 0.026$ and $P < 0.023$, respectively, Fisher exact test).

In our group of consecutive patients with chronic diarrhea, 1 of the 9 (11%) patients with Crohn disease, 6 of the 21 (29%) with other organic diseases, and 30 of the 40 (75%) with IBS fulfilled the Rome criteria for IBS diagnosis. Furthermore, in the diagnosis of "organic causes of

diarrhea", C-reactive protein (upper limit of the reference interval, $< 1 \text{ mg/L}$) and erythrocyte sedimentation rate (upper limit of the reference interval, $< 12 \text{ mm/h}$) produced sensitivities of 40% and 66% and specificities of 90% and 63%, respectively.

In children with chronic diarrhea attributable to organic causes, FC concentrations above the cutoff of 50 $\mu\text{g/g}$ were found in 7 of the 13 patients with celiac disease, 13 of the 15 patients with cow's milk intolerance, 4 of the 5 patients with multiple food intolerances, but in neither of the 2 patients with intestinal giardiasis infection. Ten of the patients with a final diagnosis of cow's milk intolerance or multiple food intolerances underwent gastroscopy and/or sigmoidoscopy because they had positive occult blood in the stool. In all of these patients, duodenal or colon histology showed intense inflammatory infiltration of the mucosa by polymorphonuclear leukocytes (mainly eosinophils) and plasma cells. Only one of the children with a final diagnosis of IBS had an FC concentration above the cutoff limit of 50 $\mu\text{g/g}$. She was a patient with concomitant autoimmune hepatitis and also underwent colonoscopy and small bowel x-ray examination for a suspected associated IBD, but these examinations and histology of the colon and ileum were negative.

Children with organic diarrhea had significantly higher FC values than those with IBS ($P = 0.04$, Mann-Whitney test). The values of the healthy controls did not differ from those of the IBS patients and were significantly lower than in the patients with organic diarrhea ($P = 0.005$, Mann-Whitney test). Pediatric controls suffering from Crohn disease had FC values higher than both the IBS patients ($P < 0.0001$, Mann-Whitney test) and the patients with organic diarrhea ($P = 0.01$, Mann-Whitney test). However, the correlation between FC concentration and the Lloyd-Still activity score was not significant ($r = -0.26$; $P = 0.2$).

A cross-tabulation of the calprotectin results obtained in pediatric and adult patients, according to the final diagnoses, is shown in Table 3, and Table 4 summarizes the sensitivity, specificity, and positive and negative predictive values of the FC assay in distinguishing between organic causes of diarrhea and IBS calculated in the study

Table 3. Cross-tabulation of the calprotectin results in adult and pediatric patients with chronic diarrhea, according to the final diagnoses.

	IBS, n	Organic diseases, n	Total, n
Adults			
Positive tests	8	19	27
Negative tests	32	11	43
Total	40	30	70
Children			
Positive tests	1	24	25
Negative tests	14	11	25
Total	15	35	50

Table 4. Sensitivity, specificity, and positive and negative predictive values (and their 95% confidence intervals) for the FC assay in the diagnosis of chronic diarrhea attributable to organic causes.^a

	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
Cutoff of 50 $\mu\text{g/g}$				
All cases (n = 120)	66 (55–77)	84 (77–91)	83 (76–90)	68 (58–78)
Adults (n = 70)	64 (50–78)	80 (69–91)	70 (57–83)	74 (62–86)
Children (n = 50)	70 (55–85)	93 (86–100)	96 (90–100)	56 (37–75)
Cutoff of 100 $\mu\text{g/g}$				
All cases (n = 120)	46 (33–59)	93 (88–100)	90 (84–96)	59 (47–71)
Adults (n = 70)	44 (26–62)	93 (87–99)	81 (71–91)	69 (53–82)
Children (n = 50)	52 (32–72)	93 (86–100)	94 (87–100)	44 (23–65)

^a Consecutive patients with chronic diarrhea were studied. The analysis was performed with two different cutoff limits: 50 and 100 $\mu\text{g/g}$ of stool.

population according to two cutoff limits indicated by the manufacturer: 50 and 100 $\mu\text{g/g}$ of stool. The higher cutoff (100 $\mu\text{g/g}$) showed a higher positive predictive value but a lower negative predictive value and sensitivity than the lower cutoff value (50 $\mu\text{g/g}$). In general, the assay was more accurate in children than in adult patients.

Discussion

IBS has been reported to affect 6–20% of the general population (9), and it is probably the most frequent cause of chronic diarrhea in adults. Although simple clinical criteria based on medical history and physical examination are suggested for a positive diagnosis of IBS, a definitive diagnosis is still reached by excluding other diseases, thus following a more or less aggressive investigation plan (3, 9). To limit the numerous and cumbersome investigations, several noninvasive tests on blood or stool samples have been proposed (17–20). Recently, FC has been shown useful in identifying patients with chronic diarrhea from IBD (4, 7), but almost all of the studies published were performed in prediagnosed groups of patients (21–24) or in patients in whom the major clinical problem was to distinguish between Crohn disease (or colon inflammation) and IBS (4, 7). Consequently, these studies did not consider the wide spectrum of other organic causes of chronic diarrhea that must be distinguished from IBS. Furthermore, there are no prospective data on the calprotectin assay in consecutive children with chronic diarrhea.

Our data were collected according to a prospective study design, and the percentage of patients initially included and then excluded as lost during the follow-up was <5% (6 of 126 patients). The data obtained fully confirmed that the FC assay in adults can distinguish between IBD and IBS (4, 7). In fact, in the consecutive patients enrolled, we found that all individuals with a final diagnosis of Crohn disease had FC values higher than the highest cutoff chosen (100 $\mu\text{g/g}$). However, it must be emphasized that we studied a small control group of individuals with IBD; consequently, these data should not be overstated. FC values in the general group of patients with organic causes of chronic diarrhea were

lower than in the patients with Crohn disease, and in the diagnosis of organic diarrhea, the assay had 64% sensitivity and 80% specificity. This specificity is very close to other previously reported values (4, 8), but we also highlighted two factors associated with the frequency of false-positive results: the use of aspirin or NSAIDs and the presence of concomitant liver cirrhosis. The association between NSAID use and high fecal calprotectin concentrations has been reported previously (25–27), and although another study has shown conflicting data (4), we suggest that NSAID use should be discontinued some weeks before FC is assayed to assure higher specificity. The evidence that patients with liver cirrhosis can have false-positive calprotectin values is in agreement with the report of frequent mucosa abnormalities in the colons of patients with portal hypertension (28). Furthermore, false-positive FC results have been reported to be attributable to day-to-day biological variations in calprotectin excretion (29).

The FC sensitivity in our series of adult patients was lower (64%) than that reported previously. This is logical because in the other studies, patients with a strong suspicion of IBD were selected (4, 7), but it merits comment if we consider the 89% sensitivity reported by Tibble et al. (8) in a study that had a design similar to ours. In the present study, we found a high frequency of negative calprotectin results for patients with celiac disease, and as these diagnoses represented approximately one-third of the causes of "organic diarrhea", this led to a low FC sensitivity. Low FC sensitivity in patients with celiac disease has not been reported previously, but it is consistent with the typical intestinal histology of celiac disease, characterized by a prevalently lymphocyte infiltration of the mucosa, with few neutrophils (30). We must underline that the low sensitivity in our study may be attributable to a selection bias because our hospital is a tertiary care center for food intolerance (31, 32), and most of the patients we enrolled could have been preselected by their physicians. However, the frequency of celiac disease in the general population is known to be on the increase in both Europe and the US (33); consequently, an increase in

the "weighting" of celiac disease among the causes of chronic diarrhea should be considered.

Regarding the range of FC values in our study in patients with diarrhea from organic causes and in healthy adult controls, it is interesting to note that they are comparable to the values found in a population with colorectal neoplasia and in the corresponding healthy controls in another study performed with the same laboratory method (34).

Recently, FC has been reported in groups of infants with infantile colic and in children suffering from recurrent abdominal pain (35), and data from children highly suspected to be suffering from IBD have been published (36). However, no previous data on the usefulness of FC in consecutive children with chronic diarrhea have been published to date. We found that the calprotectin assay had a higher sensitivity and specificity in children than in adult patients. In fact, it would seem that false-positive results are very rare in children, probably because of the lower frequency of associated disease and the less-frequent use of drugs that could damage the intestinal mucosa. As in adult patients, the sensitivity of the calprotectin assay in children was limited by false-negative results in patients with celiac disease. Regarding the high positive predictive value of FC that we obtained for the pediatric patients (96%), we must emphasize that this is also linked to the high prevalence of organic diseases (70%) in our study group. It is possible that performing the FC assay other than in a Gastroenterology Unit situation could give a lower positive predictive value. However, it is interesting to note that children with chronic diarrhea attributable to cow's milk allergy or multiple food allergies had high calprotectin values in an increased percentage of cases (17 of 20). Because food allergy is one of the main causes of diarrhea in infants and children and it has been reported that cow's milk allergy affects up to 8% of the pediatric population (37), it is relevant that the FC assay was also a reliable marker for organic causes of diarrhea in these patients. The high sensitivity of FC in the set of food-intolerant patients is an important finding because it is known that skin prick tests and IgE antibodies do not detect allergies in most patients with cow's milk allergy (38, 39). Additional studies performed on larger groups of patients with suspected food allergies will be necessary to confirm the possible usefulness of FC in the laboratory work-up of this disease.

In conclusion, our study confirmed that the FC assay identifies IBD in both children and adult patients. However, when used in consecutive adult patients with chronic diarrhea, it can fail to identify some organic causes, such as celiac disease, and can give false-positive results attributable to concomitant extraintestinal diseases or drug use. In pediatric patients, the calprotectin assay had better sensitivity and specificity than in adult patients in identifying organic causes of chronic diarrhea. Although the negative predictive value of FC in children

(56%) in the present study does not permit this assay to be considered as a screening test to exclude organic causes of diarrhea, its high positive predictive value encourages its use in the evaluation of children with chronic diarrhea.

We are grateful to Carole Greenall for reviewing the English of this manuscript.

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