Utility of Fecal Lactoferrin in Identifying Crohn Disease Activity in Children

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ABSTRACT

Objectives: Fecal lactoferrin (FL) is a noninvasive biomarker that is elevated in Crohn disease (CD) compared to irritable bowel syndrome. The purpose of this study was to evaluate FL in identifying children with active versus inactive CD.

Patients and Methods: Fresh stool samples were collected from children with CD scheduled for endoscopy or a clinic visit, and from new outpatients who were scheduled for colonoscopy. FL was determined using a polyclonal antibody-based enzyme-linked immunosorbent assay. Physical global assessment, endoscopic findings, erythrocyte sedimentation rate (ESR), and the Pediatric CD Activity Index (PCDAI) were recorded for patients with CD. The PCDAI scores symptoms, laboratory parameters, physical examination, and extraintestinal manifestations. A score of $\leq$10 is inactive disease, 11 to 30 is mild active, and $\leq$31 is moderate to severe active.

Results: Of 101 study patients (4- to 20-year-old, 66 boys), 31 had active CD, 23 had inactive CD, and 37 had noninflammatory bowel disease (non-IBD) conditions. Four patients with ulcerative colitis and 6 patients with polyposis were excluded from analysis. FL was significantly elevated in CD versus non-IBD ($P < 0.001$) and in active versus inactive CD ($P < 0.001$). The PCDAI and ESR were higher in active CD than in inactive CD (both $P < 0.001$). Using an FL cutoff of 7.25 μg/g, FL has 100% sensitivity and 100% negative predictive value in detecting active CD. Using an FL cutoff level of 60 μg/g, FL had 84% sensitivity, 74% specificity, 81% positive predictive value, and 77% negative predictive value for detecting active CD.

Conclusions: FL is a promising biomarker of active CD and may be more practical to use when it is not feasible to obtain all of the necessary clinical information for the PCDAI.

Key Words: Crohn disease, irritable bowel syndrome, lactoferrin, pediatric inflammatory bowel disease

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Patients with inflammatory bowel disease (IBD) are often subjected to repeated invasive or cumbersome diagnostic examinations, not only at initial diagnosis but also on subsequent monitoring of clinical status or evaluation of recurrent gastrointestinal (GI) symptoms. The oscillation between active and inactive disease states presents challenges with identifying symptoms generated by intestinal inflammation compared to functional disorders such as irritable bowel syndrome (IBS). Early and specific diagnosis of active IBD is essential for optimal treatment and avoidance of surgery. There has been increasing interest in nonendoscopic methods to aid in the diagnosis of IBD, monitor symptoms, evaluate response to therapy, and predict relapse. Laboratory markers such as the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and clinical activity indices have variable diagnostic accuracies and do not correlate well with endoscopy and histology (1). Radiologic examinations such as computed tomography enteroclysis produce superior images compared with barium small bowel radiograph, but many patients find the transnasal intubation of the duodenum uncomfortable (2). Tagged leukocyte scans are helpful but expensive and subject patients to radiation (3). The wireless capsule endoscopy may show active small bowel disease not demonstrated by radiographic examinations but may be interpreter dependent and may not be readily available in pediatric centers.

The mucosal barrier is altered in intestinal inflammation and allows white blood cells to cross the intestinal wall. Activated leukocytes infiltrate the mucosa and can be detected in feces because of shedding in the intestinal lumen (4,5). Proteins present in these activated neutrophils are measurable in feces and thus may serve as surrogate markers of inflammation (6). Two are now commercially available in the United States: calprotectin and lactoferrin. The others being investigated are fecal polymorphonuclear neutrophil elastase (7), fecal S100A12 (8,9), and fecal M2-pyruvate kinase (10).

Lactoferrin is an iron-binding protein that is secreted in an iron-free form from many epithelial cells into most exocrine fluids, particularly milk (11). It is also a major component of the secondary granules of neutrophils and is released during degranulation upon neutrophil activation. During inflammation, lactoferrin levels of the biological fluids increase dramatically. For instance, in blood, lactoferrin can increase from 1 to 200 μg/mL with systemic bacterial infection. In intestinal inflammation, fecal lactoferrin (FL) has also been found to be markedly elevated and shown to be a sensitive and specific marker of intestinal inflammation (5).

FL is a noninvasive biomarker that has been shown to be elevated in patients with Crohn disease (CD) compared with patients with IBS. The purpose of this study was to further evaluate the utility of FL in identifying children with active versus inactive CD. To our knowledge, there has not been a study evaluating lactoferrin levels in children with CD using endoscopic data.

PATIENTS AND METHODS

This prospective study was approved by the Indiana University–Purdue University at Indianapolis Clarian institutional review

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board. Consecutive pediatric patients with CD who were scheduled for endoscopy or a clinic visit, and consecutive patients scheduled for colonoscopy for diagnostic evaluation of chronic GI symptoms, were invited to participate. Informed consent was obtained before sample and data collection. Patient enrollment and sample collection started in October 2003 and concluded in July 2007.

Data Collection and Laboratory Analysis

Each patient submitted a single fresh stool sample that was immediately frozen at −20 °C. FL concentration was determined using a quantitative polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (IBD-SCAN, Tech Lab, Inc, Blacksburg, VA) and results were reported as microgram per gram of stool. A level ≥7.25 μg/mL was considered elevated. Briefly, each specimen was weighed and serially diluted and then tested against a standard curve using purified human lactoferrin. ELISA optical densities of patient specimens that fell on the linear portion of the standard curve were used to generate a protein concentration (Bradford method).

CD activity was based on physician's global assessment of active or inactive disease and endoscopic findings, when available. Endoscopic findings of erosions, aphthae, and ulcerations consistent with CD were recorded. The physician's global assessment was considered to be "active" when there were gross endoscopic abnormalities suggestive of active CD. ESR and the Pediatric CD Activity Index (PCDAI) were recorded for patients with CD. The PCDAI scores symptoms (abdominal pain, stools, subject well-being), laboratory parameters (hematocrit, ESR, albumin), physical examination (weight, height, velocity, abdominal examination findings, perirectal disease), and extraintestinal manifestations (12). A PCDAI score of ≤10 indicates inactive disease.

Statistical Methods

Comparisons of FL, PCDAI, or ESR between groups of patients, CD active, CD inactive, and IBS or others, were performed using nonparametric Mann-Whitney *U* test.

Univariate and multiple logistic regression analyses were used to perform sensitivity–specificity analyses and construct receiver operating characteristic (ROC) curves with area under the curve (AUC) as a measure of diagnostic accuracy. Tests were conducted as 2-sided at the $\alpha = 0.05$ significance level for all analyses. SAS 9.1.3 software (SAS Institute, Inc, Cary, NC) was used to perform all of the analysis.

RESULTS

Of 101 study patients (4- to 20-year-olds, 66 males), 31 had active CD, 23 had inactive CD, and 37 had non-IBD conditions (Table 1). The non-IBD conditions were IBS = 14, constipation = 6, hemorrhoid = 3, anal fissure = 3, functional abdominal pain = 3, gastroesophageal reflux disease = 2, lactose intolerance = 2, feeding dysfunction = 1, failure to thrive = 1, mild acute proctitis = 1, and giardiasis = 1. Four patients with ulcerative colitis and 6 patients with polyposis were excluded from the analysis. Endoscopic findings in the 22 patients with active CD included erosions, aphthae, ulcers in the upper GI, terminal ileum and colon (n = 3), upper GI tract and colon (n = 5), upper GI tract and terminal ileum (n = 1), terminal ileum and colon (n = 6), terminal ileum only (n = 1), and colon only (n = 6).

The median levels of FL were 280 μg/g (range 13–1170 μg/g) for active CD, 22 μg/g (range 0.1–759 μg/g) for inactive CD, and 0.56 μg/g (range 0.01–67.42 μg/g) for non-IBD patients (Table 1). The ROC curve for FL and the endoscopic presence or absence of intestinal inflammation in active CD, inactive CD, and non-IBD patients is shown in Figure 1.

FL was significantly elevated in CD than in non-IBD ($P < 0.001$) and in active CD than in inactive CD ($P < 0.001$). The PCDAI was higher in active CD (median 23, range 0–60) than in inactive CD (median 0, range 0–13), $P < 0.001$. ESR was higher in active CD (median 32, range 1–70) than in inactive CD (median 8, range 0–39), $P < 0.001$.

The ROC curves for PCDAI, ESR, and FL are presented in Figures 2 to 4. The AUC of each parameter's ability in detecting active CD are PCDAI = 0.93, FL = 0.86, and ESR = 0.86. The addition of lactoferrin to the PCDAI does not improve the diagnostic accuracy because the PCDAI by itself has high sensitivity and specificity. The addition of ESR to lactoferrin also did not improve diagnostic accuracy. Using the reference laboratory FL cutoff level of 7.25 μg/g, FL had 100% sensitivity, 43% specificity, 70% positive predictive value, and 100% negative predictive value for a correct diagnosis of CD. Using a FL cutoff level of 60 μg/g, FL had 84% sensitivity, 74% specificity, 81% positive predictive value, and 77% negative predictive value for detecting active CD.

DISCUSSION

Our study supports the use of lactoferrin in differentiating active CD from inactive CD and non-IBD patients. Our non-IBD patients were a heterogeneous group reflective of a pediatric clinical practice rather than a pure IBS group as reported in previous adult studies of lactoferrin. This heterogeneity likely explains why some

TABLE 1. Patient characteristics

	Active CD	Inactive CD	Non-IBD
N	31	23	37
Male	20	15	23
Female	11	8	14
Mean age ± SD	13.1 ± 3.9	13.9 ± 3.6	10.8 ± 3.9
No. underwent endoscopy	22	2	37
Lactoferrin median (range)	280 μg/g (13–1170 μg/g)	22 μg/g (0.1–759 μg/g)	0.56 μg/g (0.01–67.42 μg/g)
PCDAI median (range)	23 (0–60)	0 (0–12.5*)	—
ESR median (range)	32 (1–70)	(0–39)	—

CD = Crohn disease; ESR = erythrocyte sedimentation rate; IBD = inflammatory bowel disease; PCDAI = Pediatric Crohn Disease Activity Index; SD = standard deviation.

* One patient with inactive CD had an elevated ESR, which contributed to the elevated PCDAI = 12.5.

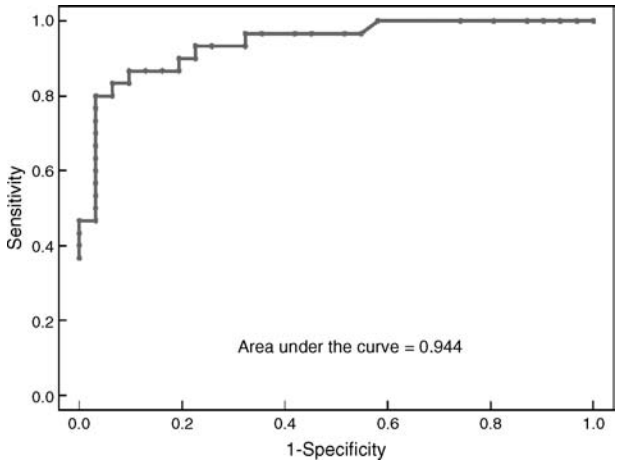

FIGURE 1. Receiver operating characteristic of fecal lactoferrin: endoscopic presence or absence of intestinal inflammation.

non-IBD patients had slightly elevated FL. For example, the 16-year-old patient with diarrhea-predominant IBS was found to have lymphoid hyperplasia in her colon, which likely explains her FL of 67.3 μg/g. A 6-year-old with an FL of 38.1 μg/g had alternating constipation-diarrhea and was found to have nonspecific, mild acute inflammation on random colon biopsies of an endoscopically normal-appearing colon. Although stool studies for infectious organisms were negative for these patients, the microscopic findings of mild inflammation in grossly normal-appearing colons make it plausible that there was a self-limited infectious trigger that precipitated the patients' symptoms.

Two of the non-IBD patients were found to have mild reflux esophagitis on endoscopy. It is possible that lactoferrin was not elevated in these cases because lactoferrin is thought to be degraded in an acidic gastric pH (13). One non-IBD patient had giardia with a normal duodenal biopsy and normal FL. A child with persistent IBS was found to have mild, acute, nonspecific proctitis on colonoscopy. This patient had a modestly elevated FL (29.3 μg/g).

We excluded 3 patients who were found to have juvenile polyps at the time of colonoscopy. Histological examination of a juvenile polyp typically reveals a dense inflammatory infiltrate (14)

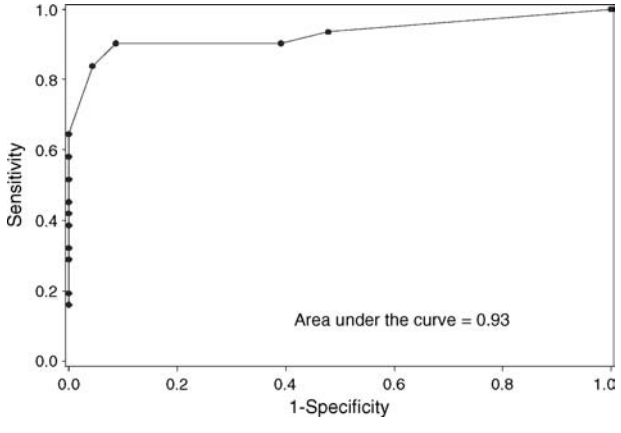

FIGURE 2. Receiver operating characteristic of pediatric Crohn disease activity index: active versus inactive Crohn disease.

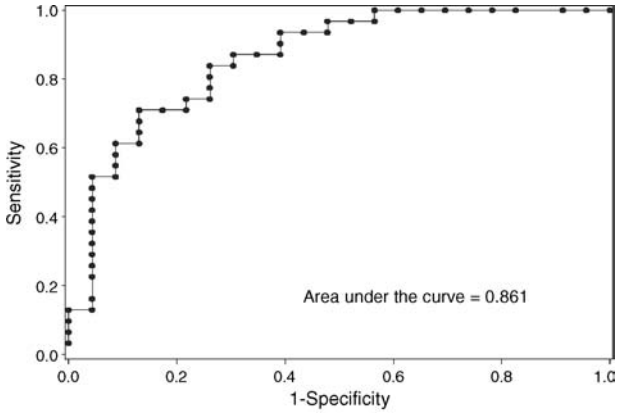

FIGURE 3. Receiver operating characteristic of fecal lactoferrin: active versus inactive Crohn disease.

that intuitively will increase the FL. Our 3 patients with juvenile polyps did have elevated FL levels (22.6–953 μg/g). We were unsure of what effect an adenomatous polyp would have on FL. Hence, we also excluded the 3 patients found to have adenomatous polyposis coli because an adenoma, although typically not considered inflammatory in nature, has increased levels of cyclooxygenase-2 and/or prostaglandins (15).

FL was elevated in 8 patients with inactive CD. Two of them developed disease exacerbation 4 months to 2 years following this study. This study was not designed to follow FL levels over time, although it would have been interesting to determine whether the FL progressively increased and at what FL level did patients become symptomatic. Sipponen et al (16) demonstrated that FL and fecal calprotectin were more sensitive surrogate markers identifying endoscopically active CD compared to the CD activity index or CRP. We can infer from their data that fecal biomarkers are reflective of mucosal inflammation much earlier before clinical symptoms manifest. Conversely, FL also may be used as a marker of endoscopic remission as shown in patients receiving anti-tumor necrosis factor-α therapy whose FL fell from 105 μg/g pretreatment to 2.7 μg/g posttreatment (17).

All of the patients with active CD had FL levels above the reference cutoff level of 7.25 μg/g consistent with the high

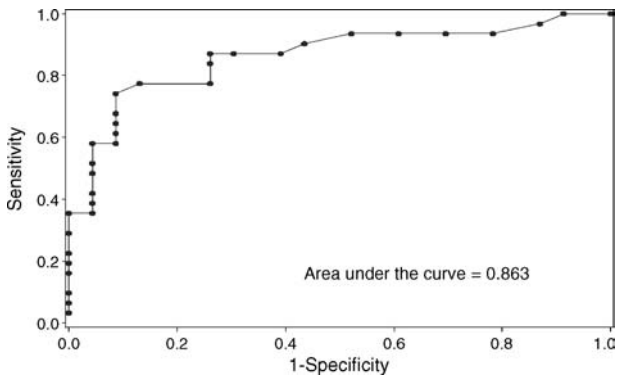

FIGURE 4. Receiver operating characteristic of erythrocyte sedimentation rate: active versus inactive Crohn disease.

sensitivity and negative predictive value of FL. To increase its specificity for differentiating active from inactive CD, we suggest using a higher FL level of 60 μg/g. In the study by Kane et al (5), they also found that patients with inactive IBD still had significantly elevated FL compared with healthy control subjects and patients with IBS (4), although it was not the intent of their study to determine a cutoff FL level that would differentiate active from inactive IBD.

CONCLUSIONS

We have shown that FL is elevated in patients with active CD compared with inactive CD. Endoscopically active CD was associated with elevated FL. FL is a promising biomarker of active CD and may be more practical to use when it is not feasible to obtain all of the necessary clinical information for the PCDAI.

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