Background: Fecal calprotectin (FC) has been proposed as a noninvasive surrogate marker to determine the degree of intestinal inflammation and predicting relapse in patients with inflammatory bowel disease (IBD). The aim was to compare FC levels in IBD and healthy controls, to correlate FC levels with clinical disease activity, and to assess whether FC levels can be used to predict clinical relapse in children with IBD.

Methods: Enzyme-linked immunosorbent assay (ELISA) determined levels of FC were measured in more than 1 stool samples (n) from 32 IBD patients (n = 97) and from 34 healthy controls (n = 37). Disease activity was assessed by the Harvey–Bradshaw index in Crohn’s disease (CD) and by Physician’s Global Assessment (PGA) in both CD and ulcerative colitis (UC). Clinical events were recorded up to 9 months following stool collection in CD patients. Wilcoxon rank sum test and Fisher’s exact tests were used to compare FC levels in IBD patients and in control. Kaplan–Meyer analysis was used to determine a risk of clinical relapse in relation to FC levels.

Results: The IBD group had higher FC levels (range 17–7500 g/g) compared with control (16–750 g/g, \( P < 0.0001 \)). FC levels were higher during relapse (CD, 3214 ± 2186; UC, 2819 ± 1610) compared to remission (CD, 1373 ± 1630; UC, 764 ± 869; \( P < 0.0001 \)). Among those with clinical relapse, 90% had FC levels more than 400 \( \mu \)g/g in CD. Eighty-nine percent of CD encounters with FC levels less than 400 \( \mu \)g/g remained in clinical remission.

Conclusions: FC levels differentiate active IBD from controls. Among children with CD and in remission, FC levels may be useful in predicting impending clinical relapse.

Key Words: calprotectin, IBD, disease activity, children

Fecal Calprotectin Is Useful in Predicting Disease Relapse in Pediatric Inflammatory Bowel Disease

Dorota Walkiewicz, MD,* Steven L. Werlin, MD,* Daryl Fish, MD,* Mathew Scanlon, MD,* Patrick Hanaway, MD,† and Subra Kugathasan, MD*

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From the: *Department of Pediatrics, Medical College of Wisconsin, Children’s Hospital of Wisconsin, Milwaukee, Wisconsin, †Genova Diagnostics, Asheville, North Carolina.

Reprints: Subra Kugathasan, MD, Department of Pediatrics, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee WI 53226 (e-mail: skuga@mcw.edu).

Calprotectin, a calcium-binding protein released by myelomonocytic cells, plays a regulatory role in the inflammatory processes and functions in both antimicrobial and antiproliferative activities.1–5 Calprotectin is found in stool and can be measured quantitatively. It has been utilized for research and clinical use as a marker of inflammation.5–10 Fecal calprotectin (FC) is elevated in adults and children with inflammatory bowel disease (IBD).11–14 Since IBD is characterized by active disease (relapse) with quiescent periods (remission), several studies have attempted to correlate FC levels with disease activity to determine whether FC could be used as a noninvasive surrogate marker of disease activity. Although FC levels fluctuate during the course of IBD, it is persistently and significantly elevated during the disease relapse in both adults11,12,15,16 and children.13,14,17–19 Increased FC level in adults with IBD who are in clinical remission may be predictive of disease relapse.20–23 Despite many studies of FC in adults, the clinical correlation of FC and IBD and its ability to predict relapse in children with known IBD has not been well studied. We therefore attempted to clarify further the role of FC in pediatric IBD. The aims of our study were to compare FC levels in IBD and healthy controls, to correlate FC levels with clinical disease activity, and to assess whether FC levels can be used to predict clinical relapse in IBD in children.

MATERIALS AND METHODS

Patient Characteristics

The Children’s Hospital of Wisconsin Institutional Review Board (IRB) approved this study. Thirty-two IBD children and 36 controls were recruited from the Gastroenterology Clinic at the Children’s Hospital of Wisconsin. The diagnoses of Crohn’s disease (CD) and ulcerative colitis (UC) were confirmed by standard clinical, radiological, endoscopic, and histopathology findings as previously described. All CD patients had documented evidence of ileal only (15%), colonic (39%), or ileocolonic (48%) involvement at diagnosis. Similarly, 20 of 21 UC patients had pancolonic involvement at the time of diagnosis. Gender distribution was equal in both CD and UC groups.

The control groups were recruited through IRB-approved advertisement and mainly comprised healthy children.
of hospital staff. These children were healthy, without any medical problems.

All patients submitted 1 or more stool specimens over a 14-month period. A total of 134 stool specimens were obtained (37 from healthy children, 76 from CD patients, and 21 from UC patients). Each stool sample \( (n) \) was considered 1 clinical encounter for the purpose of this study. Patient characteristics are described in details in Table 1.

**Assessment of Disease Activity**

Disease activity was assessed by review of the charts retrospectively by 2 methods: 1) Harvey–Bradshaw Index (HBI) in CD patients and 2) Physicians Global Assessments (PGA) in both CD and UC patients during the clinic visit, based on physicians’ documentation in the medical record. HBI assesses disease activity in CD patients.\(^{24} \) It is based on clinical symptoms that include general well-being, abdominal pain, the number of liquid stools per day, and specific findings on physical examination (abdominal mass, perianal disease, arthritis, or rash). Previous studies in adults and children have shown that an HBI \( \geq 4 \) is usually associated with disease relapse.\(^{25–29} \) When evaluating disease activity by PGA it can be categorized as no disease activity or mild, moderate, or severe disease activity based on physician’s overall assessment during the clinical encounters based on the clinical symptoms and examination findings.\(^{30} \) For the purpose of this study, we defined remission if the patients had no disease activity and relapse if they had mild, moderate, or severe disease activity by PGA. For those who had good documentation, clinical events were recorded up to 9 months following the initial stool collection. Erythrocyte sedimentation rate (ESR) was available on 70 clinical encounters coinciding with stool collection.

**Fecal Calprotectin Measurement**

Stool specimens were coded and sent to Genova Diagnostics (Asheville, NC) for quantitative FC measurement by ELISA as previously described.\(^{18,31} \) An improved assay for calprotectin, previously described by Ton et al,\(^ {31} \) was used for analysis.

**Statistical Analysis**

Mean, median, and standard deviations were calculated. The Wilcoxon rank sum test was used to compare FC levels between IBD and healthy controls and between IBD patients with and without clinical disease activity. Simple regression analysis was used to calculate correlation coefficient between FC levels and the HBI in CD patients as well as for looking at correlations between ESR and calprotectin in CD and UC encounters. The Kaplan–Meyer survival analysis and log rank test were used to calculate the FC level that can be predictive of an impending clinical relapse in asymptomatic CD patients. Fisher’s exact test was used to calculate percentage of clinical encounters having clinical relapse in relation to FC values.

**RESULTS**

**Fecal Calprotectin in IBD and Healthy Controls**

A total of 134 samples (76 CD, 21 UC, and 37 healthy controls) were collected. In 41 encounters involved in our study clinical disease activity could not be assessed because of missing data or poor recording by the physicians. The disease activity was only available in 56 clinical encounters with IBD for analysis. Distribution of age, median, and mean levels for FC in each study group are shown in Table 1. Box plots in Figure 1 show the median, 25th, and 75th percentile FC levels as well as the lowest and the highest value of calprotectin for each group. FC level is expressed in g/g. As a group, IBD patients had higher FC levels, ranging from 17–7500 g/g compared with healthy controls (16–750 g/g) (\( P < 0.0001 \)). Patients with active IBD had significantly higher FC concentrations (\( P < 0.0001 \)).

**Fecal Calprotectin and Disease Activity in IBD**

There were 19 CD encounters and 4 UC encounters during disease relapse and 25 CD and 8 UC encounters in disease remission. Disease activities on the remaining clinical encounters were not available. Mean HBI during the disease relapse in CD was 4.3 ± 2.3 (median 5.0, range 1–9). Mean

<table>
<thead>
<tr>
<th>Disease Group</th>
<th>( N )</th>
<th>Age Range in Years</th>
<th>Median Age in Years</th>
<th>Median FC (( \mu g/g ))</th>
<th>Mean FC ± SD (( \mu g/g ))</th>
<th>FC Range (( \mu g/g ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37</td>
<td>5–16</td>
<td>10</td>
<td>43</td>
<td>88 ± 152</td>
<td>16–750</td>
</tr>
<tr>
<td>CD/remission</td>
<td>25</td>
<td>8–19</td>
<td>14</td>
<td>1293</td>
<td>1373 ± 1630</td>
<td>17–7500</td>
</tr>
<tr>
<td>CD/relapse</td>
<td>19</td>
<td>8–19</td>
<td>12</td>
<td>2557</td>
<td>3214 ± 2186</td>
<td>838–7500</td>
</tr>
<tr>
<td>UC/remission</td>
<td>8</td>
<td>8–20</td>
<td>13</td>
<td>517</td>
<td>764 ± 869</td>
<td>58–2596</td>
</tr>
<tr>
<td>UC/relapse</td>
<td>4</td>
<td>8–18</td>
<td>11</td>
<td>2491</td>
<td>2819 ± 1610</td>
<td>1295–4999</td>
</tr>
</tbody>
</table>

\( N \), number of clinical encounters corresponding to each stool sample; SD, standard deviation.
HBI in remission was 0.72 ± 1.0 (median 0, range 0–3). Three CD encounters in disease relapse had an HBI score > 7. The mean FC levels for this group was 2220 ± 564.8 g/g (median 1975, range 1819–2866 g/g). Forty-one CD encounters had an HBI score > 7. The mean FC in this group was 2164 ± 2154 g/g, (median 1587, range 17–7500 g/g). The correlation coefficient between the HBI and FC levels in patients with CD was 0.36 (P < 0.05). The mean FC level in patients with CD and UC in remission was higher in disease relapse than remission (P < 0.0001).

The mean ESR in CD encounters in relapse was 20 (range 8–49) and those in remission was 7 (range 1–10). Mean ESR in UC encounters in relapse was 26 (range 7–80) and those in clinical remission was 15 (range 3–35). The correlation coefficient between ESR and calprotectin was 0.32 (P = 0.007) in 70 clinical encounters (both CD and UC).

Utility of FC in Predicting Clinical Relapse

In the CD group, 25 clinical encounters were in clinical remission. During 9 months following stool samples collection, 10 episodes of clinical relapse were documented. Using the Kaplan–Meier method and log rank test, an FC level of 400 μg/g was significantly associated with clinical relapse in asymptomatic CD patients within 9 months of stool sample collection (P = 0.03) (Fig. 2). Using the predetermined FC level of 400 μg/g as a threshold, Fisher’s exact analysis showed that 9 out of 16 (56.2%) of clinical encounters with FC levels >400 g/g had clinical relapse within 9 months (95% confidence interval [CI] 30%–80%), but only 1 out of 9 (11%) of clinical encounters resulting in clinical relapse had FC levels <400 g/g (95% CI 55.5–99.8). Among clinical encounters of CD patients with FC levels < 400 g/g, 8 (89%) had no clinical relapse within 9 months (95% CI 51.8–99.7).

DISCUSSION

Our study demonstrated many important points pertaining to children with IBD and FC that will have broader clinical implications. As previously shown in adults, FC levels are higher in children with IBD compared to controls regardless of disease activity. In addition, FC is significantly elevated in children with IBD who are in relapse compared with those whose disease was in clinical remission. More important, our study has shown that FC values of 400 μg/g or more may predict an impending clinical relapse within the next 9 months in patients with quiescent CD. Conversely, since 89% of CD patients with FC levels of less than 400 μg/g stayed in remission, this cutoff value may predict those who will stay in clinical remission.

FC measurement has been found to be a useful, noninvasive test to differentiate patients with active IBD from healthy children and adults. The results of previous studies support the use of FC in clinical gastroenterology practice. However, the majority of the studies on FC have been done in adults. Although the available data from the limited pediatric studies are similar to that in adults, they are incomplete and the exact reference range for normal FC in children is still not defined.

Compared to healthy controls, adults with IBD have significant elevation of FC. FC concentrations also vary in...
relation to disease activity and are higher in disease relapse when compared to remission. Based on previous adult studies the cutoff value for FC level associated with mild intestinal inflammation in adults is between 50–100 μg/g and increases in relation to severity of inflammation. Roseth et al reported a correlation between low FC levels (<50 μg/g) and histologic findings of absence or mild disease activity in adults. They also reported that FC released from neutrophils found in gastrointestinal mucosa during bleeding episodes (gastrointestinal or swallowed blood during nasal bleeding) may be elevated as well. It has also been shown that nonsteroidal antiinflammatory drugs induced enteropathy and further FC elevation. Based on previous studies in adults with IBD, FC >150 μg/g predicted relapse in clinically asymptomatic patients over the following 12 months.

To our knowledge, there have been only a few studies of FC in children. Berni et al reported that the median FC in healthy children was 28 μg/g (range 1–113 μg/g). Olafsdottir et al found that the mean FC level in 24 healthy children was 40 ± 28 μg/g. Fagerberg et al analyzed FC levels in healthy children after excluding children with nasal or menstrual bleeding, abdominal pain, diarrhea, or nonsteroidal antiinflammatory drug intake. The overall median FC concentration in that study was 13.6 μg/g in 117 children ages 4 to 17 years. There were no differences in FC concentration attributable to age or sex. These findings were consistent with previously published adult data. Bunn et al reported that children with moderately active IBD had higher FC concentrations than those with mildly active disease. Disease activity in that study was based on a modified Lloyd–Still score that included clinical, radiologic, histologic, and laboratory examination. They also found elevated FC levels in clinically asymptomatic IBD children with mild bowel inflammation. In an attempt to study the utility of noninvasive planar white cell scans, Poullis et al demonstrated fecal calprotectin and planar scans correlated well with reduced inflammatory activity in IBD patients.

The Olafsdottir et al study on IBD children is worth a detailed discussion. They found that FC levels were higher in Norwegian children with IBD compared to healthy controls; however, only 19 IBD patients were studied and the relationship of FC level to disease activity was not evaluated. The mean FC level was 293 ± 218 μg/g in the IBD group. The FC range was not reported. Based on the graphical description of the FC values in relation to the different study groups, an overlap of FC levels in healthy and IBD group can be observed. Similarly, Berni et al reported a higher concentration of FC in children with IBD compared to healthy children, but the median, mean, and range of FC in children with IBD were not reported. Based on the graphical presentation of the study results we concluded that despite a significant difference in FC concentrations between children with active IBD and healthy controls, some IBD children in remission showed FC values comparable to controls.

Our study evaluated the utility of FC in North American children. Although the median FC level was comparable to findings in other studies from Europe, the healthy controls in our study had higher variability of FC than previously reported. Differences in FC values may be related to different techniques for calprotectin extraction. The study published by Bunn et al and most studies reported before 2003, evaluated FC using an earlier stool extraction process. Our study used a newer extraction method (PhiCal ELISA kit) available after 2003. As noted by Ton et al, the extraction yield of total calprotectin is much higher (average 78%) compared to 28%–30% with the old method, giving an overall 5-fold increase compared to the original method. The increased calprotectin yield with the new method is mainly due to dissociating agents in the extraction solution and an increased ratio between extraction solution and feces. Contrary to healthy controls in the Fagerberg et al study, our healthy controls were chosen without specific exclusion criteria. Since no exclusion criteria were used in the study, our population is more representative of a typical clinic population seen by gastroenterologists who face diagnostic dilemmas with varying gastrointestinal complaints.

As previously shown in European studies, we found increased FC in a mixed group of children with IBD, including those both in remission and in flare, when compared to healthy children. We also found no overlap in FC concentration between IBD children in disease flare and healthy controls. However, in contrast to previous studies we found an overlap in FC levels in children with clinically inactive IBD and healthy controls, suggesting that FC cannot be used to differentiate all patients with inactive IBD from controls.

Assessment of disease activity in our study was purely clinical, based on the history from the patients and the PGA. We did not use histologic findings in assessing disease activity since these were not available at the time of data collection, but we were able to use ESR and correlate this biochemical marker with calprotectin level in 70 clinical encounters. The statistical analysis showed a positive correlation between ESR and calprotectin, meaning that with increased ESR value the calprotectin level was increased as well, confirming the theory that calprotectin levels rise with increased inflammation. Despite missing histologic findings in our patients, those with clinically asymptomatic disease and elevated level of calprotectin could potentially have histological inflammation or macroscopic inflammation that resulted in elevated FC levels. Based on our findings we may conclude that FC levels less than 400 μg/g in asymptomatic CD patients may predict a longer remission rate as opposed to those with an FC value higher than 400.

Our study has several limitations. First, missing data points prevented us from calculating disease activity in each
clinical encounter within IBD patients. This study would have been stronger if the FC values were calculated along with disease activity, biochemical markers, and endoscopic evaluations prospectively. We also used a single nonvalidated disease activity measure, the PGA, in our UC patients. More important, the small sample size prevented us from performing positive and negative predictive values of FC in our patient population, which would have given a better idea about the utility of FC in clinical practice. Despite these limitations, repeated FC levels in the same subject during the clinical course of IBD and the ability to predict the clinical relapse based on FC cutoff values while they were in clinical remission is the main strength of our study.

We conclude that FC differentiates healthy children from those with active IBD and plays a role in predicting the length of clinical remission in asymptomatic children with IBD in relation to FC level. FC does not discriminate between healthy children and children with IBD who are in remission. Since FC differentiated patients with active IBD from healthy controls, FC levels can be used as a screening tool to differentiate between children with IBD and those with other noninflammatory gastrointestinal disorders, such as functional abdominal pain, if proven by further adequately powered, prospective, clinical studies in children.

REFERENCES