Comparison of Fecal Inflammatory Markers in Crohn’s Disease

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Background: Fecal biomarkers are used increasingly to monitor Crohn’s disease (CD). However, the relative accuracy of different markers in identifying inflammation has been poorly evaluated. We evaluated fecal calprotectin (FC), lactoferrin (FL), and S100A12 (FS) using endoscopic validation in a prospective study of the progression of CD after intestinal resection.

Methods: Data were collected from 135 participants in a prospective, randomized, controlled trial aimed at preventing postoperative CD recurrence. Three hundred nineteen stool samples were tested for FC, FL, and FS preoperatively and 6, 12, and 18 months after resection. Colonoscopy was performed at 6 and/or 18 months. Endoscopic recurrence was assessed blindly using the Rutgeerts score. C-reactive protein (CRP) and Crohn’s Disease Activity Index (CDAI) were assessed.

Results: FC, FL, and FS concentrations were elevated preoperatively (median: 1347, 40.9, and 8.4 μg/g, respectively). At 6 months postoperatively, marker concentrations decreased (166, 3.0, 0.9 μg/g) and were higher in recurrent disease than remission (275 versus 72 μg/g, P < 0.001; 5.7 versus 1.6 μg/g, P = 0.007; 2.0 versus 0.8 μg/g, P = 0.188). FC > 135 μg/g, FL > 3.4 μg/g, and FS > 10.5 μg/g indicated endoscopic recurrence (score ≥ 2) with a sensitivity, specificity, and negative predictive value (NPV) of 0.87, 0.66, and 91%; 0.70, 0.68, and 81%; 0.91, 0.12, and 71%, respectively. FC and FL correlated significantly with the presence and severity of endoscopic recurrence, whereas FS, CRP and CDAI did not.

Conclusions: FC was the optimal fecal marker for monitoring disease activity in postoperative CD and was superior to CRP and CDAI. FL offered modest sensitivity for detecting recurrent disease, whereas S100A12 was sensitive but had low specificity and NPV.

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Key Words: calprotectin, lactoferrin, S100A12, postoperative, biomarkers

Diagnosis and monitoring of Crohn’s disease (CD) activity is based on a combination of clinical assessment, biochemical markers of inflammation and endoscopy. However, there is often insufficient correlation between these tests to engender confidence in their routine use.1–3 Endoscopy is widely accepted as the gold standard for detecting and quantifying bowel inflammation, but is expensive, labor intensive, inconvenient for the patient and carries some risk.4 The correlation between clinical scoring systems, such as the Crohn’s Disease Activity Index (CDAI) and the Harvey–Bradshaw Index, and endoscopic findings in CD is poor.5,6 The correlation between serum biochemical markers of inflammation, such as C-reactive protein (CRP), and endoscopic findings in CD is also inconsistent,6,7 with more than one-third of patients with clinically or endoscopically active disease having a normal CRP.5,9

In recent years, there has been considerable interest in the use of fecal biomarkers in the diagnosis, monitoring, and management of inflammatory bowel disease (IBD). However, few studies have compared the performance of these biomarkers in large well characterized populations with routine endoscopic validation. This has led to some confusion about which biomarker is optimal. To date, fecal calprotectin (FC), lactoferrin (FL), and S100A12 (FS) have not been compared in patients with CD.

Calprotectin is a member of the S100 family of calcium-binding proteins (calgranulins). It constitutes 60% of the neutrophil cytosolic protein and is abundant in all body fluids in proportion to the degree of inflammation present.10 FC has been shown consistently to reflect endoscopic disease activity in CD.1,5,7,11–14 FC seems to be more sensitive than CDAI or CRP at detecting endoscopic inflammation15 and may be a reliable surrogate marker of mucosal healing in patients with CD.16,17 Increased FC concentrations are associated with an increased risk of clinical relapse18–27 and can be used to diagnose the presence of recurrent endoscopic CD after intestinal resection.28

Lactoferrin is an iron-binding 80-kD glycoprotein that is found in many body fluids. It is a major component of neutrophil...
secondary granules and is released during neutrophil degradation. FL has been used as a marker of active IBD and for monitoring a patient’s response to treatment. Small studies have shown that FL may reflect clinical recurrence after ileocolonic resection in CD, however, whether or not it reflects endoscopic recurrence is unknown. S100A12, like calprotectin, is a calcium-binding S100 calgranulin protein and is expressed as a cytoplasmic protein in activated neutrophils, with expression more restricted to granulocytes. FS can distinguish IBD from irritable bowel syndrome and also seems to reflect inflammatory activity in IBD. Its role in the postoperative setting has never been evaluated.

FC and FL correlate well with each other and seem to be comparable as reliable surrogate markers of mucosal involvement and treatment response in CD, and as predictors of relapse in IBD. The Postoperative–Crohn’s Endoscopic Recurrence (POCER) study has shown that FC is sufficiently sensitive to monitor for postoperative CD recurrence and is superior to both CDAI and CRP as a surrogate marker for recurrent mucosal lesions in the neoterminal ileum and anastomosis. A FC >100 μg/g indicates which patients require colonoscopic assessment with a sensitivity 0.89, specificity 0.58, positive predictive value (PPV) 53%, and negative predictive value (NPV) 91%. The aim of this study was to evaluate the accuracy of 3 markers, FC, FL, and FS, for the diagnosis of postoperative endoscopic CD recurrence. Using endoscopic validation, this was a prospective study of the progression of CD after “curative” resection. Data collected as part of the POCER study were used for this analysis.

MATERIALS AND METHODS

The Clinical Postoperative Recurrence Study

The POCER study has been described previously. In short, this was a prospective, randomized, controlled trial that assessed the value of postoperative endoscopic assessment and treatment intensification, or “step-up,” for early mucosal recurrence after resection of all macroscopic disease. Patients were treated with metronidazole, thiopurine, or adalimumab if thiopurine intolerant according to risk of recurrence and the endoscopic results of monitoring. Patients may have had previous upper gut disease, but to be included in the study, no residual upper gut disease was present at the time of surgery. Patients were permitted to be on proton pump inhibitor therapy for symptomatic reflux disease. Patients were not permitted to be on aspirin or non steroid anti-inflammatory drugs (NSAIDs) therapy and were instructed to avoid these during the study.

Colonoscopy was undertaken in two-thirds of patients at 6 months and all patients at 18 months postoperatively. Endoscopic remission was defined as Rutgeerts’ score i0 or i1 and recurrence defined as i2, i3, or i4.

One hundred seventy-four patients were included at 17 hospitals in Australia and New Zealand. Stool samples were taken preoperatively when a patient joined the study (baseline), and at 6, 12, and 18 months postoperatively. CDAI was calculated and serum CRP measured at these time points.

All patients provided written informed consent. Ethical approval for the study was obtained from the Human Research Ethics Committees of the participating hospitals (Clinical Trial Registration: NCT00989560).

Endoscopic Visual Assessment

At ileocolonoscopy, mucosal recurrence at the anastomosis and neoterminal ileum was assessed according to the Rutgeerts score by the endoscopist, who was not blinded to patient treatment. Photographs of the anastomosis and neoterminal ileum were, however, scored again by 2 senior investigators (P.D.C. and M.A.K.) blinded to the endoscopist’s score and the patient’s identity and treatment. A final consensus score was determined by the 2 blinded assessors.

For the 6 and 18-month colonoscopies, endoscopic remission was defined as Rutgeerts score i0 (no lesions) or i1 (<5 aphthous lesions) and recurrence as i2 (>5 aphthous lesions or larger lesions confined to anastomosis), i3 (diffuse ileitis), or i4 (diffuse inflammation with large ulcers and/or narrowing). Two secondary measures of endoscopic disease activity were also calculated: the Crohn’s Disease Endoscopic Index of Severity (CDEIS) and the Simple Endoscopic Score for Crohn’s Disease (SES-CD), to ensure robustness of the Rutgeerts score.

Stool Collection and Storage

Stool samples were stored at −80°C until the conclusion of the clinical study, at which time all samples were analyzed. For each biomarker, all samples were batched for a single analysis. Patients were instructed to collect stool samples no more than 3 days before the study visit, or if colonoscopy was to be performed, 3 days before colonoscopy before commencing bowel preparation. Samples were stored at −20°C in patients’ home freezer, transported on ice, and stored at −80°C at study centers until the conclusion of the clinical study. A time of day for stool collection was not stipulated.

Fecal Biomarker Assays

FC was measured by a quantitative enzyme immunoassay (ICAL; Bühlmann, Schonenbuch, Switzerland) as per manufacturer’s instructions. FL was measured by a quantitative enzyme immunoassay (IBD-SCAN; Techlab, Blacksburg, VA) as per manufacturer’s instructions. S100A12 was measured by an in-house quantitative enzyme immunoassay using a previously published protocol. All concentrations were expressed as microgram/gram of stool. All testing was performed without knowledge of patient data.

The upper limit of the reference range in patients without gut inflammation is well defined as <50 μg/g for FC, <7.25 μg/g for FL, and <10 μg/g for FS.
Statistical Analysis

For the clinical POCER study, the sample size was based on an alpha value of 0.05 (1-sided), 80% power, and expected endoscopic disease recurrence at 18 months for standard care of 60% and for active care of 35%, based on previous studies. Allowing for a 31% dropout of patients 170 patients (113 active and 57 standard care arms) were needed. The sample size was based on the clinical study design comparing 2 management strategies to prevent disease recurrence. The fecal biomarker component of the study, presented here, was not separately powered.

Data were analyzed using STATA12 (StataCorp, College Station, TX). Associations between categorical data were assessed using either Chi-square or Fisher exact test. Associations between endoscopic disease and FC, FL, FS, CDAI, and CRP were assessed by logistic regression analysis for binary outcomes and by the determination of Spearman rank correlation coefficient (r) for nonparametric correlations. The optimal cutoff values for FC concentration for assessment and prediction of endoscopic recurrence were determined using receiver operator characteristic analysis.

Three cohorts were used for analysis:

Cross-sectional Analysis

This analysis allowed for median FC, FL, and FS concentrations at all time points (preoperative, 6, 12, and 18 mo) to be calculated.

Endoscopic Validation Analysis

The patients included in this analysis are shown in Table 1. This analysis included FC, FL, and FS measurements taken at 6 or 18 months, at which time an endoscopic assessment was also performed. FC, FL, FS, CRP, and CDAI data from 6 and 18-month time points were correlated with endoscopic recurrence (Rutgeerts scores i2, i3, or i4) and scored endoscopic severity (i0–i4).

Longitudinal Analysis

Patients were included if they had provided ≥2 fecal samples during the period of postoperative follow-up, with at least 1 fecal sample matched to an endoscopic assessment performed at the same time point. This allowed determination of the relationship between FC, FL, and FS and disease behavior, escalation of medical therapy, and response to treatment step-up over time.

RESULTS

Patient demographics are shown in Table 1. The demographics of the endoscopic validation cohort closely reflected those of the wider study population. Baseline patient demographics were similar for the 3 analysis groups. The number of patients and samples that contributed to each analysis and the rates of endoscopic recurrence are detailed in Table 2.

Fecal Biomarker Concentration in Relation to Surgery, Mucosal Recurrence, and Remission

A total of 319 fecal samples from 135 patients (44% men, median age 36 yr; interquartile range [IQR], 26–47 yr) were studied. At 6 months, 92 patients underwent colonoscopy and of these 31 (34%) had endoscopic recurrence. At 18 months, 108 patients underwent colonoscopy (the remainder withdrew from the study before colonoscopy) and of these 45 (42%) had endoscopic recurrence.

FC, FL, and FS concentrations fell significantly after surgery (Table 3).

One hundred thirty-seven fecal samples from 99 patients could be paired with endoscopic assessments performed at 6 and 18 months. Median FC in those with recurrent disease was 330 µg/g for those in endoscopic remission (P < 0.001). For FL, figures were 6.6 versus 1.4 µg/g (P = 0.001) and for FS were 1.7 versus 0.8 µg/g (P = 0.043) for endoscopic recurrence and remission, respectively (Fig. 1A–C).

Area under the receiver operator characteristic (AUROC) curve analysis was used to determine the optimal cutoff for FC, FL, and FS and endoscopic recurrence. Combined 6 and 18-month observations (paired fecal biomarker and endoscopy results) were used for this analysis (Fig. 2A–C). AUROC values for FC, FL, and FS and endoscopic recurrence were 0.763, 0.683, and 0.379, respectively. The optimal cutoffs for FC, FL, and FS as predictors of endoscopic recurrence for combined 6 and 18-month observations are shown in Table 4. Optimal cutoffs for FC, FL, and FS were determined using receiver operator characteristic analysis. The optimal cutoffs for FC and FL were 0.117 g/g and 2.25 µg/g, respectively, while the optimal cutoff for FS was 0.54 g/g.

<table>
<thead>
<tr>
<th>TABLE 1. Patient Demographics</th>
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<tr>
<td></td>
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<tr>
<td>Sex (male)</td>
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<tr>
<td>Median age (IQR)</td>
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<tr>
<td>Preoperative CDAI, median (IQR)</td>
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<tr>
<td>Preoperative CRP, median (IQR)</td>
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<table>
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<tr>
<th>TABLE 2. Analysis Cohorts and Endoscopic Recurrence</th>
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<tr>
<td>Analysis Cohort</td>
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<tr>
<td>--------------------------</td>
</tr>
<tr>
<td>Cross-sectional</td>
</tr>
<tr>
<td>Endoscopic validation</td>
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<tr>
<td>Longitudinal</td>
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</tbody>
</table>

1088 | www.ibdjournal.org
Table 3. Median Fecal Biomarker Concentrations Were Elevated Preoperatively and Fell Dramatically by 6 Months Postoperatively

<table>
<thead>
<tr>
<th>Fecal Biomarker</th>
<th>Preoperative, µg/g</th>
<th>Postoperative, µg/g</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>1347</td>
<td>166</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FL</td>
<td>40.9</td>
<td>3.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FS</td>
<td>8.4</td>
<td>0.9</td>
<td>&lt;0.001</td>
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</table>

Fecal Biomarker Concentration, CDAI, CRP, and Endoscopic Findings

One hundred thirty-six patients had matched endoscopic, fecal biomarker, CRP, and CDAI results available, which were included in a correlation analysis. FC and FL correlated with both endoscopic recurrence ($r = 0.42$, $P < 0.001$ and $r = 0.306$, $P = 0.008$) and scored endoscopic severity (Rutgeerts score) ($r = 0.44$, $P < 0.001$ and $r = 0.384$, $P < 0.001$) respectively, but FS did not ($r = 0.176$, $P = 0.937$ and $r = 0.168$, $P = 1.000$). CRP and CDAI did not correlate with FC, FL, FS, endoscopic recurrence, or endoscopic severity (Table 5).

There was no statistically significant difference in FC concentration between those with CDAI $<150$ versus CDAI $\geq 150$ ($P = 0.085$).

AUROC values for CRP, CDAI, and endoscopic recurrence were 0.568 and 0.541, respectively (Fig. 2D, E).
Not all patients with endoscopic remission had normal fecal biomarker results, although a higher proportion of those with Rutgeerts i0 had a FC <100 µg/g (previously defined at the optimal cutoff for detecting postoperative endoscopic recurrence) when compared with those with Rutgeerts score i1 (70% versus 49%, respectively, P = 0.045). Median FC for those with i0 was significantly lower than i1 (49 versus 112 µg/g, respectively, P = 0.046).

### Fecal Biomarker Concentration in Response to Treatment

FC decreased significantly in response to intensification of drug therapy. In patients in endoscopic remission at 6 months who did not step up medical therapy, median FC concentration remained elevated at 6, 12, and 18 months at 129, 153, and 178 µg/g, respectively. In patients with endoscopic recurrence at 6 months who stepped-up treatment, the median FC concentration...
at 6 months fell from 324 to 180 mg/g at 12 months (P = 0.005) and 109 mg/g at 18 months (P = 0.004). In patients with endoscopic recurrence at 6 months who stepped-up treatment, median FL and FS concentrations were elevated (5.7 and 2.0 mg/g, respectively) but fell at 12 months (FL 1.81 mg/g and FS 1.1 mg/g) and 18 months (FL 0.78 mg/g and FS 0.9 mg/g). This decrease was statistically significant for FL between 6 and 12 months (P = 0.002) but not between 12 and 18 months (P = 0.825). The decreases seen in FS between 6 and 12 months and 12 and 18 months did not reach statistical significance (P = 0.147 and P = 0.329, respectively). In those in endoscopic remission at 6 months, who did not step up medical therapy, the FL and FS were 1.96 and 0.3 mg/g, respectively, and rose at 12 months (3.23 and 1.3 mg/g) with median values at 18 months of 2.51 and 1.5 mg/g. Changes over time in this group of patients were not found to be statistically significant for either FL or FS.

**Correlation Between the 3 Fecal Biomarkers**

A statistically significant correlation was found between all 3 fecal biomarkers, although FC appeared to correlate best with FL (r = 0.840, P < 0.001) (Table 4).

**DISCUSSION**

FC, FL, and FS have been shown to be significantly and consistently increased in both adult and pediatric patients with IBD versus non-IBD.14,39,51–61 FC has been studied the most extensively in patients with CD. Numerous small studies have

### TABLE 4. Sensitivity, Specificity, PPV and NPV, and AUROC of FC, FL, and FS in Identifying Endoscopic Recurrence (Rutgeerts ≥i2)

<table>
<thead>
<tr>
<th>Fecal Biomarker, μg/g</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV, %</th>
<th>NPV, %</th>
<th>AUROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC (n = 137)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated best cutoff = 135</td>
<td>0.87</td>
<td>0.66</td>
<td>56</td>
<td>91</td>
<td>0.763</td>
</tr>
<tr>
<td>50</td>
<td>0.96</td>
<td>0.38</td>
<td>45</td>
<td>94</td>
<td></td>
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<tr>
<td>100</td>
<td>0.89</td>
<td>0.58</td>
<td>53</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>0.77</td>
<td>0.68</td>
<td>55</td>
<td>85</td>
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<tr>
<td>200</td>
<td>0.71</td>
<td>0.74</td>
<td>59</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>FL (n = 137)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated best cutoff = 3.4</td>
<td>0.70</td>
<td>0.68</td>
<td>53</td>
<td>81</td>
<td>0.683</td>
</tr>
<tr>
<td>2.5</td>
<td>0.72</td>
<td>0.62</td>
<td>50</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.57</td>
<td>0.73</td>
<td>53</td>
<td>77</td>
<td></td>
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<tr>
<td>7.5</td>
<td>0.44</td>
<td>0.80</td>
<td>54</td>
<td>73</td>
<td></td>
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<tr>
<td>10</td>
<td>0.30</td>
<td>0.84</td>
<td>50</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>FS (n = 137)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated best cutoff = 10.5</td>
<td>0.91</td>
<td>0.12</td>
<td>35</td>
<td>71</td>
<td>0.379</td>
</tr>
<tr>
<td>5</td>
<td>0.78</td>
<td>0.22</td>
<td>35</td>
<td>66</td>
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<tr>
<td>7.5</td>
<td>0.84</td>
<td>0.14</td>
<td>34</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.90</td>
<td>0.12</td>
<td>35</td>
<td>69</td>
<td></td>
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<tr>
<td>12.5</td>
<td>0.91</td>
<td>0.11</td>
<td>35</td>
<td>70</td>
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</table>

**TABLE 5. Correlation Between FC, FL, and FS and Endoscopic Recurrence (Rutgeerts i2–i4), Scored Endoscopic Severity, CRP, and CDAI**

<table>
<thead>
<tr>
<th>Fecal Biomarker</th>
<th>Endoscopic Recurrence (Rutgeerts Score i2, i3, or i4)</th>
<th>Scored Endoscopic Severity (i0–i4)</th>
<th>CRP</th>
<th>CDAI</th>
<th>FS</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>FC</td>
<td>0.419</td>
<td>&lt;0.001</td>
<td>0.439</td>
<td>&lt;0.001</td>
<td>0.240</td>
<td>0.121</td>
</tr>
<tr>
<td>FL</td>
<td>0.306</td>
<td>0.008</td>
<td>0.348</td>
<td>&lt;0.001</td>
<td>0.274</td>
<td>0.032</td>
</tr>
<tr>
<td>FS</td>
<td>0.176</td>
<td>0.937</td>
<td>0.168</td>
<td>1.000</td>
<td>0.181</td>
<td>0.807</td>
</tr>
<tr>
<td>CDAI</td>
<td>−0.153</td>
<td>1.000</td>
<td>−0.174</td>
<td>0.978</td>
<td>−0.167</td>
<td>1.000</td>
</tr>
<tr>
<td>CRP</td>
<td>0.091</td>
<td>1.000</td>
<td>0.166</td>
<td>1.000</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
compared the accuracy of FC and FL for the diagnosis of CD, quantification of intestinal inflammation, prediction of treatment response and disease relapse, and for the diagnosis of postoperative recurrence.7,15,16,20,35,39,62,65 Most studies have found that FC and FL correlate well with each other without 1 biomarker consistently outperforming the other. FC and FS have been compared in one small study by Sipponen et al66 and performed similarly.

This is the first study, using endoscopic validation, to compare the 3 main biomarkers in CD. FC appeared to be the optimal marker for identification of endoscopic postoperative recurrence, with high sensitivity and NPV. FC measurement is sufficiently sensitive in the postoperative setting after resection of all macroscopic disease to monitor for CD recurrence. Although FS showed similar sensitivity (0.91) to FC (0.89) and FL (0.70), its specificity and PPV were poor at 0.12 and 35%. This combined with a modest NPV of 71% makes FS a poor screening test. In clinical practice, only one fecal test is likely to be used; combining fecal tests does not seem to be of greater diagnostic value than using one alone.57

FL correlated with both the endoscopic recurrence and endoscopic score; however, these correlations were weaker than those seen with FC. FL had lower specificity and PPV when compared with FC, making it an inferior screening test for endoscopic recurrence of CD in the postoperative population.

Our findings illustrate the potential value of routine fecal biomarker testing in the postoperative setting as part of a management algorithm in asymptomatic patients. They identify the value of these tests for screening for disease recurrence; however, such recurrence should then be confirmed. Sensitivity and NPV, particularly for FC, are high but PPV is poor. A proportion of patients even with mucosal normality at the anastomosis (Rutgeerts i0) had an elevated FC. This may be due to the possible effect of upper gastrointestinal ulceration or proximal small bowel CD on FC concentration.65 Upper gut imaging was not performed in this study. To be included in the study, patients had to have all macroscopic disease removed at the time of surgery. However, we cannot ignore the possibility that a small number of patients may have had upper gut microscopic disease or undetected proximal aphthous ulcers. Such minor disease may account for the small number of patients who had an elevated FC without endoscopic anastomotic recurrence. Similarly, a small number of patients had colonic recurrence without anastomotic recurrence, some of whom had an elevated FC.

Intraindividual variation is observed with repeat FC testing. However, it is most important whether there is substantial variation within the range of values that discriminates active from inactive disease. In this regard, FC has been shown to have low day-to-day variability in patients with CD.66,67 In the study by Moun et al,59 there was little intraindividual variability in patients with CD within the window which discriminated between a normal and abnormal result. Such analyses of variability have not been performed for FL or FS. These data for FC support the use of a single measurement.

Nineteen patients had colonic recurrence, separate to the anastomosis, at 6, 18 months, or both. All patients with both colonic recurrence and anastomotic recurrence had FC and FL results above the cutoffs defined in this study (FC 146–3540 μg/g, FL 7.8 to >100 μg/g), but for FS, results fell both above and below our defined optimal cutoff for endoscopic recurrence of 10.5 μg/g (1.2–126 μg/g). Seven patients with colonic recurrence had no anastomotic recurrence; their FC ranged from 100 to 3040 μg/g, FL from 4.1 to >100 μg/g, and FS <0.39 to 65.6 μg/g.

A small number of patients had endoscopically detectable disease recurrence but had normal fecal biomarker concentrations. CRP was not additionally helpful in identifying these patients.

Fecal calprotectin measurement has previously been shown to be of modest value in predicting future endoscopic recurrence in the postoperative setting.28 FL and FS were not analyzed specifically with respect to prediction of future endoscopic recurrence in this study.

Variation in the expression of these inflammatory proteins may explain their difference in performance. FS is expressed almost exclusively by granulocytes, whereas FC and FL have a broader pattern of expression in both granulocytes and monocytes, with FL being most broadly expressed.39,68

Our results confirm the accuracy, utility, and superiority of fecal biomarkers over CRP or CDAI as monitoring tools and screening tests for endoscopic recurrence of CD in the postoperative population. These data suggest that fecal biomarkers may have an important role in monitoring CD postoperatively, with colonoscopy reserved for those with an elevated biomarker result or a clinical indication. Alternatively, a strategy that combines regular fecal marker measurement, with additional infrequent colonoscopy, may offer the ideal means of disease monitoring, but this remains to be tested.

Fecal biomarkers can be used to indicate the presence and severity of CD recurrence after intestinal resection of all macroscopic disease and their measurement provides a guide to treatment response. This study shows that FC is the optimal marker for diagnosing and monitoring endoscopic postoperative recurrence. But every test must be considered in its clinical context, including the patient’s history, risk of recurrence, and the presence of symptoms. The results of this study, from a derivation cohort, should now be verified with findings in a validation cohort.

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Author contributions: Study concept and design, acquisition of data analysis, data interpretation, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis, and obtained funding, E. K. Wright, M. A. Kamm, and P. D. Cruz; acquisition of data, analysis and interpretation of data, and drafting of the manuscript, A. L.
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