Measurement of Fecal Calprotectin Improves Monitoring and Detection of Recurrence of Crohn's Disease Following Surgery

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Short title: Calprotectin in Post-Operative Crohn’s Disease

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ABSTRACT

Background & Aims: Crohn’s disease (CD) usually recurs after intestinal resection; post-operative endoscopic monitoring and tailored treatment can reduce chance of recurrence. We investigated whether monitoring levels of fecal calprotectin (FC) can substitute for endoscopic analysis of the mucosa.

Methods: We analyzed data collected from 135 participants in a prospective, randomized, controlled trial, performed at 17 hospitals in Australia and 1 in New Zealand, that assessed the ability of endoscopic evaluations and step-up treatment to prevent CD recurrence after surgery. Levels of FC, serum levels of c-reactive protein (CRP), and Crohn’s disease activity index (CDAI) scores were measured before surgery and then 6, 12, and 18 months after resection of all macroscopic Crohn’s disease. Ileo-colonoscopies were performed at 6 months after surgery in 90 patients and 18 months after surgery in all patients.

Results: Levels of FC were measured in 319 samples from 135 patients. The median FC decreased from 1347 µg/g before surgery to 166 µg/g at 6 months after surgery, but was higher in patients with disease recurrence (based on endoscopic analysis; Rutgeerts score ≥i2) than patients in remission (275 µg/g vs 72 µg/g; P<.001). Combined 6- and 18-month levels of FC correlated with the presence (r=0.42; P<.001) and severity (r=0.44; P<.001) of CD recurrence, but level of CRP and CDAI score did not. Levels of FC >100 µg/g indicated endoscopic recurrence with 89% sensitivity and 58% specificity, and a negative predictive value (NPV) of 91%; this means that colonoscopy could have been avoided for 47% of patients. Six months after surgery, levels of FC<51 µg/g in patients in endoscopic remission predicted maintenance of remission (NPV, 79%). In patients with endoscopic recurrence at
6 months who stepped-up treatment, levels of FC decreased from 324 µg/g at 6 months to 180 µg/g at 12 months and 109 µg/g at 18 months.

**Conclusion:** In an analysis of data from a prospective clinical trial, measurement of FC has sufficient sensitivity and NPV values to monitor for CD recurrence after intestinal resection. Its predictive value might be used to identify patients most likely to relapse. After treatment for recurrence, FC level can be used to monitor response to treatment. It predicts which patients will have disease recurrence with greater accuracy than level of CRP or CDAI score.

**KEYWORDS:** Inflammatory Bowel Disease; Fecal Biomarkers; Prognostic Factor; Prognosis
INTRODUCTION

Approximately seventy percent of patients with Crohn’s disease will require intestinal resection at some time in their life and, of these, up to 70 percent require a second operation. Endoscopically-identified post-operative disease recurrence occurs early and its severity predicts the subsequent clinical course. Within weeks of resection, new aphthous ulceration in the neo-terminal ileum can be identified, with disease recurrence identifiable endoscopically in 70 percent of patients at one year.

Monitoring of Crohn’s disease activity is often based on a combination of clinical assessment and biological markers of inflammation. However, there is often insufficient correlation between the clinical state and biological markers to engender confidence in their use as the sole basis for monitoring or treatment decision-making. Endoscopy is the gold standard for detecting and quantifying bowel inflammation, but is expensive, labor intensive, inconvenient for the patient and carries some risk. The correlation between clinical scoring systems, such as the Crohn’s Disease Activity Index (CDAI) and the Harvey Bradshaw Index (HBI), and endoscopic findings in Crohn’s disease is poor. The correlation between serum biochemical markers of inflammation, such as C-reactive protein (CRP), and endoscopic findings in Crohn’s disease is also inconsistent.

We have recently undertaken a controlled clinical trial examining different strategies for managing patients after Crohn’s disease resection of all macroscopic disease. The Post-Operative Crohn’s Endoscopic Recurrence (POCER) study has demonstrated that initial post-operative therapy according to clinical risk of recurrence, with colonoscopy performed six months after intestinal resection and treatment step-up for recurrence, is significantly
superior to standard drug therapy alone, in preventing post-operative Crohn’s disease recurrence. This study utilized ileo-colonoscopy as the main means for disease monitoring. However the limitations of colonoscopy create a need for a non-invasive measure to identify disease recurrence after surgery. As yet no simple diagnostic test has been validated against endoscopy in large populations to monitor for disease recurrence post-operatively.

Calprotectin is a member of the S100 family of calcium-binding proteins and is abundant in all body fluids in proportion to the degree of inflammation present. It can be readily quantified in feces using enzyme-linked immunosorbent assay (ELISA) or an immunoassay.

Fecal calprotectin (FC) has been shown to reflect endoscopic disease activity in Crohn’s disease. FC is more sensitive than CDAI or CRP at detecting endoscopic inflammation, and is a reliable surrogate marker of mucosal healing in patients with Crohn’s disease. Increased FC concentrations are associated with an increased risk of clinical relapse.

Its value in the post-operative setting, however, is uncertain.

Only small studies have evaluated the role of fecal biomarkers post-operatively, few have correlated measurements with endoscopic findings, and results have been inconsistent. FC concentrations have been shown to fall after intestinal resection for Crohn’s disease. Lobaton et al studied 29 patients post-operatively, and demonstrated that the FC concentration was significantly lower among those in remission (i0 and i1) than those with recurrent disease (98 vs. 235µg/g, p=0.012). Lasson and colleagues studied 30 patients for one year post-operatively performing monthly FC and ileo-colonoscopy at one year. One year after surgery the median values of FC were not significantly different between the patients in endoscopic remission (n=17) and the patients with an endoscopic recurrence.
(n=13) 189 vs. 227µg/g; p=0.25. However, most patients with low values were in remission and all patients with high (>600µg/g) FC values had recurrent disease.

These data suggest an emerging role for FC in monitoring patients in the post-operative setting. However prospective, longitudinal evaluation of fecal biomarkers in a large post-operative population with colonoscopy performed early is required to fully determine the role of this test in this setting. Whether FC can replace colonoscopy in monitoring for Crohn’s disease recurrence post-operatively needs to be determined.

The aim of this study was to examine whether FC, CRP or CDAI can be used as surrogate markers of recurrent mucosal lesions in the neo-terminal ileum and at the anastomosis. This study aimed to determine the accuracy of these biomarkers in reflecting the presence and severity of recurrent disease, and predicting future recurrence, following intestinal resection of all macroscopic disease.

**MATERIALS AND METHODS**

**The Clinical Post-Operative Recurrence Study**

The POCER study was a prospective, randomized, controlled trial which aimed to assess the value of post-operative endoscopic assessment and treatment step-up for early mucosal recurrence. Patients were stratified according to risk of recurrence. Smokers, patients with perforating disease, or patients with ≥1 previous resections were classified as “high-risk”; all others were “low-risk”. All patients underwent resection of all macroscopic disease.
Patients may have had previous upper gut disease (Table 1), 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 NSAID therapy and were instructed to avoid these during the study.

Immediately post-operatively all patients received 3 months of metronidazole. High-risk patients also received daily azathioprine (2mg/kg/day) or 6-mercaptopurine (1.5mg/kg/day). High risk patients intolerant of thiopurine received adalimumab induction (160mg/80mg) and then 40mg two-weekly. Low-risk patients received no further medication.

Patients were randomized to colonoscopy at 6 months (active care) or no colonoscopy (standard care). For endoscopic recurrence (Rutgeerts score ≥i2) at 6 months patients stepped-up to thiopurine, fortnightly adalimumab with thiopurine, or weekly adalimumab. The primary end-point was endoscopic recurrence at 18 months. Endoscopic remission was defined as Rutgeert’s score i0 or i1 (i0 = no lesions, i1 = mild small superficial anastomotic lesions), and recurrence defined as i2, i3 or i4 (moderate to severe lesions).

One hundred and seventy four patients were included at 17 hospitals in Australia and one in New Zealand. One hundred and one of 122 patients randomized to endoscopic intervention (6 month colonoscopy) were high-risk, compared to 44 of 56 in the standard care arm (Figure 1).

As part of the study protocol stool samples were taken pre-operatively (baseline), and at 6,
12 and 18 months post-operatively for calprotectin measurement. Also at these time points CDAI was calculated and serum CRP measured.

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

Endoscopic Visual Assessment

At ileo-colonoscopy mucosal recurrence at the anastomosis and neo-terminal ileum was assessed according to the Rutgeerts score\textsuperscript{3} by the endoscopist, who was not blinded to patient treatment. Photographs of the anastomosis and neo-terminal ileum were, however, scored again by two senior investigators (PDC and MAK) blinded to the endoscopist’s score and the patient’s identity and treatment. A final consensus score was determined by the two blinded assessors.

For the 6 and 18 month colonoscopies endoscopic remission was defined as Rutgeerts score i0 (no lesions) or i1 (\(\leq 5\) 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)\textsuperscript{3}. Two secondary measures of endoscopic disease activity were also calculated: the Crohn’s Disease Endoscopic Index of Severity (CDEIS)\textsuperscript{28} and the Simple Endoscopic Score for Crohn’s Disease (SES-CD),\textsuperscript{29} to ensure robustness of the Rutgeerts score.

Stool collection and storage
Stool samples were collected pre-operatively when a patient joined the study before surgery and collected at 6, 12 and 18 months after surgery. Patients were instructed to collect stool samples no more than three days prior to the study visit, or if colonoscopy was to be performed, three days prior to colonoscopy before commencing bowel preparation. Samples were stored at -20 degrees Celsius in patients’ home freezer, transported on ice, stored at -80 degrees Celsius at study centres until conclusion of the clinical study. All samples were then analyzed simultaneously in a central laboratory.

**Fecal Biomarker Assays**

FC was measured by a quantitative enzyme immunoassay (Smart-Prep, Bühlmann, Schonenbuch, Switzerland) as per manufacturer’s instructions, without knowledge of patient data. Concentrations were expressed as µg/g of stool.

The upper limit of the normal range of FC in patients without gut inflammation is well defined as <50µg/g.\(^{30}\)

**Statistical analysis**

For the clinical POCER study\(^{10}\) 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.\(^{31,32}\) Allowing for a 31% drop-out of patients 170 patients (113 active and 57 standard care arms) were needed. The sample size for the study was based on the clinical study design comparing
two management strategies to prevent disease recurrence. It was not based on the calprotectin component of the study which was not separately powered.

Data were analyzed using STATA12 (StataCort, Texas, USA). Associations between categorical data were assessed using either Chi-square or Fisher’s exact test. Associations between endoscopic disease and FC, CDAI, and CRP were assessed by logistic regression analysis for binary outcomes and by the determination of Spearman’s rank correlation coefficient (rho) for non-parametric correlations. The optimal cut-off values for FC concentration for assessment and prediction of endoscopic recurrence were determined using logistic regression in combination with the senspec command in STATA12 and Youden Index.\textsuperscript{33, 34}

There were three cohorts used for analysis:

\textit{Cross Sectional Analysis}

This analysis allowed for median FC concentrations at all time-points (pre-operative, 6, 12 and 18 months) to be calculated.

\textit{Endoscopic Validation Analysis}

The patients included in this analysis are shown in Table 1. This analysis included FC measurements taken at 6 or 18 months in which an endoscopic assessment was performed at the same time point. FC, CRP and CDAI data from 6 and 18 month time-points were correlated to endoscopic recurrence (Rutgeerts scores i0 or i1) and scored endoscopic
severity (i0 - i4).

To determine if FC can be used to predict future endoscopic recurrence FC from all patients in the standard care arm at six months (who did not undergo any treatment change between 6 and 18 months) were considered in relation to their endoscopic findings at 18 months. FC results from patients in the active care arm in endoscopic remission at 6 months (who also did not change treatment between 6 and 18 months) were considered in relation to their endoscopic findings at 18 months.

*Longitudinal Analysis*

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

**RESULTS**

Figure 1 shows the study patient disposition. Demographic details of patients who provided stool samples are shown in Table 1.

Baseline patient demographics were similar for the three 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 Calprotectin Concentration in Relation to Surgery, Mucosal Recurrence and Remission

Three hundred and nineteen fecal samples from 135 patients (44% male, median age 38 years (range 28 - 40)) were studied (Table 1). At 6 months 91 patients underwent colonoscopy (active care arm) and of these 31 (32%) had endoscopic recurrence. At 18 months 108 patients underwent colonoscopy, of whom 45 (33%) had endoscopic recurrence.

FC concentrations were elevated pre-operatively (median 1402 µg/g, IQR: 426 µg/g-2825 µg/g). At 6 months FC concentration fell (all patients median 166 µg/g, IQR: 56 µg/g-424 µg/g), but was higher in those with recurrent endoscopic disease than endoscopic remission (275 µg/g, IQR: 163 µg/g-540 µg/g vs. 72 µg/g, IQR: 32 µg/g-190 µg/g, p<0.001) (Figure 2A). At 18 months FC concentration was higher in recurrent endoscopic disease than endoscopic remission (FC: 331 µg/g, IQR: 159 µg/g-550 µg/g vs. 75 µg/g, IQR: 37 µg/g-258 µg/g, p<0.002) (Figure 2B). When 6 and 18 month observations were combined median FC in those with recurrent endoscopic disease was 330 µg/g, IQR: 163 - 540 versus 75 µg/g, IQR: 37 – 258, for those in endoscopic remission (p<0.001) (Figure 2C).

The cut-off values for FC and corresponding sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the receiver operator characteristic (AUROC) curve analysis results in detecting endoscopic recurrence are shown in Table 3. The calculated best cut-off for FC as a marker of endoscopic recurrence, from combined 6
and 18 month endoscopic observations, was 135µg/g. However for clinical utility and in order to optimize specificity, whilst maintaining optimal sensitivity and NPV, a best-fit cut-off of 100µg/g was selected. FC >100µg/g identified endoscopic recurrence (≥2) with a sensitivity of 0.89, specificity of 0.58, PPV 53%, NPV 91%, and AUROC 0.763. AUROC for CRP and CDAI were significantly lower at 0.568 and 0.541 respectively when 6 and 18 month observations were combined.

Logistic regression showed that patients with FC >100µg/g at 18 months were significantly more likely to have endoscopic recurrence compared to those with FC ≤100µg/g (OR 10.5, 95% CI 2.7-40.4, p=0.001).

For the SES-CD a value of ≥4 is regarded as reflecting at least mildly active endoscopic Crohn’s disease.35 In the current study a FC of >139 was sensitive and modestly specific for a SES-CD ≥4 with a sensitivity of 0.79, specificity 0.63, PPV 56%, NPV 83% and AUROC 0.720. For the CDEIS a value of ≥6 reflects endoscopic Crohn’s disease activity, with an FC of >92 found to have a sensitivity of 0.73, specificity of 0.72, PPV of 90%, NPV 44% and AUROC of 0.730.36

**Relationship Between Fecal Calprotectin Concentration and Endoscopic Findings**

One hundred and thirty six patients had matched endoscopic, FC, CRP and CDAI results available which were included in a correlation analysis. FC correlated with both the presence of endoscopic recurrence (r=0.42, p<0.001) and scored endoscopic severity (Rutgeert’s score) (r=0.44, p<0.001) when all 6 and 18 month endoscopic observations were considered. FC correlated with both the presence of endoscopic recurrence and
scored endoscopic severity when 6 (endoscopic recurrence r=0.49, p < 0.001; endoscopic severity r=0.56, p<0.001) and 18 month (endoscopic recurrence r=0.38, p=0.002; endoscopic severity r=0.35, p = 0.004) outcomes were considered independently.

CRP and CDAI did not correlate with FC, endoscopic recurrence or scored endoscopic severity. FC also correlated with SES-CD (r=0.49, p<0.001) and CDEIS (r=0.47, p<0.001). (Table 4).

Nineteen patients had colonic recurrence, separate to the anastomosis, at 6 months, 18 months or both. No patient with both colonic recurrence and anastomotic recurrence had a FC ≤100µg/g. Seven patients with colonic recurrence had no anastomotic recurrence; their FC ranged from 10-3040µg/g, with 5 patients >100µg/g. The two patients with colonic recurrence only and low FC had superficial colonic inflammation only. Twelve patients with colonic recurrence and anastomotic recurrence had a FC of 467- 4421µg/g.

Extreme FC values were not completely reliable in excluding or confirming endoscopic recurrence. Five patients with FC ≤100µg/g had a Rutgeerts score indicating recurrence (Figure 2D). Of these five, two had a FC <50µg/g. Therefore even a low FC did not absolutely preclude disease recurrence, although it was unlikely. Conversely a high concentration, for example FC >1000µg/g, was sometimes associated with no colonoscopic recurrence (data not shown), most likely due to either microscopic disease or upper gut disease.

In our cohort, if colonoscopy was performed at 6 months post-operatively only in those with a FC >100µg/g, 47% of patients without endoscopic disease recurrence would have
avoided a colonoscopy. If colonoscopy was performed at 6 or 18 months post-operatively only in those with a FC >100µg/g colonoscopy would have been avoided in 58% of patients without endoscopic disease recurrence. Figure 2D shows all FC concentrations <300µg/g from the endoscopic validation cohort (combined 6 and 18 month observations). The dotted line illustrates the proposed cut-off of 100µg/g.

When 6 and 18 month post-operative endoscopic assessments were considered five patients had endoscopic disease recurrence (four with i2 and one with i4) but a FC ≤100µg/g (FC 20, 36, 50, 54 and 93 µg/g). Of these five patients, four had a paired CRP result available - only one (with i2 recurrence) had an elevated CRP (30mg/L).

In patients who did not have an endoscopy at 6 months FC >100µg/g at 6 months predicted endoscopic recurrence (Rutgeerts ≥i2) at 18 months with a sensitivity 0.75, specificity 0.45, PPV 46%, NPV 74%, and AUROC 0.889. The optimal AUROC-derived cut-off in this group was determined as 252µg/g giving a sensitivity of 0.63, specificity 0.76, PPV 63%, and NPV 76%.

FC >100µg/g at 6 months in patients in endoscopic remission (i0 or i1) predicted subsequent recurrence at 18 months with a sensitivity 0.50, specificity 0.43, PPV 25%, NPV 71%, and AUROC 0.477. The AUROC-derived optimal cut-off in this group was determined as 51µg/g giving a sensitivity 0.50, specificity 0.68, PPV 36% and NPV 79%.

**Fecal Calprotectin Concentration in Response to Treatment**

Fecal calprotectin decreased significantly in response to intensification of drug therapy
(Figure 3). In patients in the active study arm in endoscopic remission at 6 months who did not step-up medical therapy, median FC concentration rose from 129µg/g to 153µg/g at 12 months (p=0.194) and 178µg/g at 18 months (p=0.245). In patients with endoscopic recurrence at 6 months who stepped-up treatment the median FC concentration at 6 months fell from 324µg/g to 180µg/g at 12 months (p=0.005) and 109µg/g at 18 months (p=0.004).

**DISCUSSION**

The majority of patients with Crohn’s disease require a resection at some time, and most of these will come to a further operation. The POCER study has recently demonstrated that post-operative endoscopic monitoring, together with treatment intensification for early recurrence, is superior to standard drug therapy alone, in preventing disease recurrence, at least in the short term.\(^\text{10}\) However such endoscopic monitoring is invasive, expensive, cannot be repeated frequently, and, in some patients will yield a normal result. In two thirds of abdominal Crohn’s operations all macroscopically involved intestine is resected.\(^\text{37}\) Such a situation, of surgically-induced and verified remission, is an ideal starting-point for the use of a non-invasive marker to monitor for recurrent inflammation.

In our study we have demonstrated that FC is markedly elevated prior to surgery and falls substantially after resection of all macroscopic disease at six months, consistent with findings from Lamb et al.\(^\text{25}\)
The present study has demonstrated that FC is sufficiently sensitive to monitor recurrence of Crohn’s disease, and has a high enough negative predictive value to be confident that few patients with recurrence have been missed.

The Rutgeerts endoscopic scoring system used in this study has not been formally validated. However the severity of the endoscopic findings one year post-operatively, based on this scoring system, has been shown previously to predict subsequent symptomatic recurrence. We used a Rutgeerts score of i2 to define recurrence, as in previous studies. This scoring system has also been used in other randomized, controlled trials of drug therapy in the post-operative setting, with good discrimination between treatment arms. The outcomes observed using other endoscopic scoring systems provide support for the primary measure used in this study (Rutgeert’s score). Although the Crohn’s Disease Endoscopic Index of Severity (CDEIS) and the Simple Endoscopic Score for Crohn’s Disease (SES-CD) are not specific for the post-operative setting, they were used in the context of removal of all macroscopic disease.

Selecting the most appropriate cut-off value for fecal calprotectin measurement is critical to its performance as a screening test. Such a value should have a high negative predictive value so that few patients with active disease are missed for subsequent colonoscopy. Whilst a cut-off of 50 µg/g has more commonly been used as a cut of to diagnose inflammatory bowel disease in patients presenting with gastrointestinal symptoms this value is inappropriate for evaluating patients with established Crohn’s disease. In patients with Crohn’s disease there is likely to be microscopic inflammation even in the setting of macroscopic normality which will marginally increase calprotectin but this is unlikely to be relevant clinically. At each time point in our study a cut-off of 100µg/g had an NPV of ≥90%
with the best combination of sensitivity and specificity. Reducing the cut-off to 50µg/g in this study would have resulted in a marked reduction in test specificity to 0.38 with a reduction in PPV to < 50% rendering the test clinically irrelevant.

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

Fecal calprotectin measurement would appear to be of modest value in predicting future endoscopic recurrence in the post-operative setting. A low fecal calprotectin in patients in endoscopic remission at six months had a limited predictive value for the development of endoscopic recurrence one year later. We therefore recommend serial measurement of fecal calprotectin at regular intervals in the post-operative period in preference to relying on a single FC measurement to predict future endoscopic behavior. It may be expected that repeated testing would improve test sensitivity.

Our findings illustrate the potential value of fecal calprotectin testing routinely in the post-operative setting as part of a management algorithm in asymptomatic patients. These results confirm the accuracy, utility and superiority of fecal calprotectin over CRP or CDAI as a monitoring tool and screening test for endoscopic recurrence of Crohn’s disease in the post-operative population. These data suggest that FC may have an important role in monitoring Crohn’s disease post-operatively, with colonoscopy reserved for those with an elevated calprotectin or those with a clinical indication.
It would appear that fecal calprotectin concentration measurement reflects the severity of endoscopic recurrence post-operatively (Table 4). Fecal calprotectin also appears to reflect the response to intensified drug therapy. Fecal calprotectin measurement may therefore have a further role in monitoring the response to treatment, with colonoscopy reserved for patients who fail to show a fall in calprotectin to within the normal range.

In this study single calprotectin measurements were taken at specific time points. Although intra-individual variation may occur with repeat testing, it is most important whether there is substantial variation within the range of values that discriminates active from inactive disease. In this regard fecal calprotectin has been shown to have low day-to-day variability in Crohn’s disease patients. Nonetheless every test needs to be considered in the clinical context, including the patient’s history, risk of recurrence, and the presence of symptoms.

Our data suggest that fecal calprotectin can be used to monitor for recurrence and to follow patients’ response to treatment. These data do not suggest that such measurements replace the need for colonoscopy, but rather serve as a complementary investigation. Calprotectin could be measured frequently, to identify early and relatively-easily treated recurrence, with colonoscopy reserved for certain intervals. In patients who have had frequent or severe previous recurrences endoscopy may still be the preferred first-line investigation.

To date small studies evaluating the utility of fecal calprotectin measurement to diagnosis post-operative recurrence have provided conflicting results. The strengths of this study
lie in the large sample size, prospective measurements, endoscopic validation, and longitudinal intra-individual assessment of fecal calprotectin.

The small number of patients with severe recurrence (Rutgeerts score i3 - 5 patients and i4 - 7 patients) limits conclusions regarding the role of fecal calprotectin in the diagnosis of severe post-operative recurrence.

Interpretation of calprotectin measurement as a marker of anastomotic Crohn’s recurrence is limited by the possible effect of upper gastrointestinal ulceration or proximal small bowel Crohn’s disease on calprotectin concentration. Upper gut imaging was not performed in this study. To be included in the study patients had to have had all macroscopic disease removed at the time of surgery. However we cannot exclude 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 fecal calprotectin without endoscopic anastomotic recurrence. Similarly, a small number of patients had colonic recurrence without anastomotic recurrence, some of whom had an elevated calprotectin concentration.

In conclusion, fecal calprotectin measurement has a potentially valuable role to play in monitoring Crohn’s disease patients after intestinal resection. In the setting of detecting early recurrence and monitoring the response to treatment it is superior to the serum marker of inflammation, CRP, and the clinical index CDAI. Fecal calprotectin testing can be performed non-invasively, frequently, cheaply and easily. Calprotectin testing can be integrated into the type of post-operative management algorithm demonstrated in the POCER study to decrease post-operative recurrence.
FIGURE LEGENDS

Figure 1. Consort Diagram: The POCER Study

Figure 2. Fecal calprotectin (FC) in endoscopic remission vs. recurrence at (A) 6 months, (B) 18 months; and (C) combined 6 and 18 months. Median FC in remission vs recurrence was 72 vs 275 at 6 months (p<0.001), 77 vs 331 at 18 months (p<0.002) and 75 vs 330 when 6 and 18 month observations were combined (p<0.001). Figure 2D shows all matched endoscopy and FC assessments where FC is <300µg/g. The dashed line represents the 100µg/g cut-off point. The vast majority of patients with a FC ≤100µg/g in this study were in endoscopic remission (Rutgeerts i0 or i1).

Figure 3. Fecal calprotectin (FC) in relation to 6 month endoscopic findings and in response to treatment step-up or no change in treatment. Treatment step-up at 6 months was associated with a significant reduction in FC at 12 (p=0.005) and 18 months (p=0.004). FC trended to increase at both 12 and 18 months in patients in endoscopic remission at 6 months who did not step up treatment but these changes were not statistically significant.
REFERENCES


**Table 1.** Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>All (n=135)</th>
<th>Endoscopic Validation Cohort (n=99)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n =</td>
<td>%</td>
</tr>
<tr>
<td><strong>Sex (Male)</strong></td>
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</tr>
<tr>
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<td>(26-47)</td>
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<tr>
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<td>17.0</td>
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<td>High Risk</td>
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<tr>
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<td>Active Care</td>
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<td><strong>Resection Type</strong></td>
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<td></td>
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<tr>
<td>Ileocaecal</td>
<td>104</td>
<td>77.0</td>
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<td>Isolated ileal</td>
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<td>Subtotal colectomy</td>
<td>6</td>
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<tr>
<td>Ileocaecal and sigmoid</td>
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<td>3.7</td>
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<tr>
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<tr>
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<td><strong>Pre-Operative CDAI, median (IQR)</strong></td>
<td>222 (136.5 - 315.5)</td>
<td>218 (138 - 311)</td>
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<tr>
<td><strong>Pre-Operative CRP, median (IQR)</strong></td>
<td>10.5 (5 - 48)</td>
<td>9 (5 - 48.5)</td>
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**Table 2** Analysis cohorts and endoscopic recurrence
<table>
<thead>
<tr>
<th>Analysis Cohort</th>
<th>Patients (n=)</th>
<th>Samples (n=)</th>
<th>Patients with Recurrence at 6 months</th>
<th>Patients with Recurrence at 18 months</th>
</tr>
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<tbody>
<tr>
<td>Cross Sectional</td>
<td>135</td>
<td>319</td>
<td>31 (36%)</td>
<td>45 (45%)</td>
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<tr>
<td>Endoscopic Validation</td>
<td>99</td>
<td>137</td>
<td>23 (34%)</td>
<td>24 (35%)</td>
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<tr>
<td>Longitudinal</td>
<td>80</td>
<td>203</td>
<td>20 (28%)</td>
<td>22 (34%)</td>
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**Table 3.** Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under the receiver operator characteristic (AUROC) of fecal calprotectin (FC) in identifying endoscopic recurrence (Rutgeerts ≥12) at 6 months, 18 months, and combined 6 and 18 months. The calculated best cut-off for FC is shown first in each table.

<table>
<thead>
<tr>
<th>FC (µg/g)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>AUROC</th>
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<tr>
<td><strong>6 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=68</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calculated best cut-off of 135</td>
<td>0.91</td>
<td>0.62</td>
<td>55</td>
<td>93</td>
<td>0.799</td>
</tr>
<tr>
<td>50</td>
<td>0.96</td>
<td>0.38</td>
<td>44</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.91</td>
<td>0.56</td>
<td>51</td>
<td>93</td>
<td></td>
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<tr>
<td>150</td>
<td>0.78</td>
<td>0.67</td>
<td>55</td>
<td>86</td>
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<tr>
<td>200</td>
<td>0.74</td>
<td>0.77</td>
<td>61</td>
<td>85</td>
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</tr>
<tr>
<td>1000</td>
<td>0.22</td>
<td>0.93</td>
<td>63</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td><strong>18 months</strong></td>
<td></td>
<td></td>
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<td>n=69</td>
<td></td>
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<td>0.79</td>
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<tr>
<td>200</td>
<td>0.71</td>
<td>0.71</td>
<td>57</td>
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<td>0.17</td>
<td>0.89</td>
<td>44</td>
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<tr>
<td><strong>Combined 6 and 18 months</strong></td>
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<td>n=139</td>
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<tr>
<td>Calculated best cut-off of 135</td>
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<td>0.66</td>
<td>56</td>
<td>91</td>
<td>0.763</td>
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<tr>
<td>50</td>
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<tr>
<td>100</td>
<td>0.89</td>
<td>0.58</td>
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<td>150</td>
<td>0.77</td>
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<tr>
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<td>0.19</td>
<td>0.90</td>
<td>50</td>
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Table 4. Correlation between fecal calprotectin (FC) and endoscopic recurrence (Rutgeerts score i2 - i4), scored endoscopic severity (score i0 - i4), Simple Endoscopic Score for Crohn’s Disease (SES-CD), Crohn’s Disease Endoscopic Index of Severity (CDEIS), C-reactive protein (CRP) and Crohn’s Disease Activity Index (CDAI).

<table>
<thead>
<tr>
<th></th>
<th>Endoscopic Recurrence (Rutgeerts score i2, i3 or i4)</th>
<th>Scored Endoscopic Severity (i0-i4)</th>
<th>SES-CD</th>
<th>CDEIS</th>
<th>CRP</th>
<th>CDAI</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>0.491</td>
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<td>CDAI</td>
<td>-0.153</td>
<td>1.000</td>
<td>-0.174</td>
<td>0.978</td>
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<td>X</td>
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<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>
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</table>
Patients Screened and Consented
n = 212

Surgery

STRATIFICATION
n = 184

HIGH RISK
n = 153
One or more of:
- Previous surgery
- Penetrating Disease
- Smoker

LOW RISK
n = 31
No risk factors

RANDOMIZATION
n = 184

Did not receive allocated treatment
n = 4

ACTIVE CARE
n = 120

HIGH RISK
n = 101

LOW RISK
n = 21

Did not receive allocated treatment
n = 4

STANDARD CARE
n = 30

HIGH RISK
n = 44

LOW RISK
n = 8

Withdraw during or 6 Months
n = 18
- 10 administrative reasons
- 6 symptom recurrence
- 2 comorbidity

Colonoscopy at 6 months
n = 104

Rutgeerts score ≥12 or ≥13
No change in therapy

Rutgeerts score ≥12 - ≤14
Step-up therapy

Withdraw between 6 and 18 months
n = 18
- 8 administrative reasons
- 7 symptom recurrence
- 4 comorbidity

PRIMARY ENDPOINT
Colonoscopy at 18 months
n = 118
P < 0.002

Calprotectin (μg/g)

Remission
n = 45

Recurrence
n = 24
A box plot showing the distribution of calprotectin levels in stool samples from patients in remission and recurrence. The plot indicates a statistically significant difference between the two groups, with a p-value of less than 0.001. The number of samples in the remission group is 90, and in the recurrence group, it is 47.
A cloud chart showing the distribution of Calprotectin (μg/g) across different Rutgeerts Score categories (i0 to i4). The x-axis represents the Rutgeerts Score categories, while the y-axis shows the Calprotectin levels. Data points are scattered across the chart, with a horizontal dashed line indicating a threshold level of 100 μg/g.
The graph shows the change in Caproetin (µg/g) over time (6 months, 12 months, 18 months) for different conditions:

- **Red line** represents endoscopic remission at 6 months without treatment step-up. The y-axis ranges from 0 to 350. The P-values are 0.005 and 0.004 at 6 and 12 months, respectively.

- **Gray line** represents endoscopic recurrence at 6 months with treatment step-up. The P-values are 0.194 and 0.245 at 6 and 12 months, respectively.