Accepted Manuscript

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

Emily K. Wright, Gastroenterology Fellow, Michael A. Kamm, Professor of Gastroenterology, Peter De Cruz, Gastroenterologist, Amy L. Hamilton, Scientist, Kathryn J. Ritchie, Research Nurse, Efrosinia O. Krejany, Clinical Scientist, Steven Leach, Post-Doctoral Scientist, Alexandra Gorelik, Statistician, Danny Liew, Professor of Clinical Epidemiology, Lani Prideaux, Gastroenterologist, Ian C. Lawrance, Professor of Gastroenterology, Jane M. Andrews, Professor of Gastroenterology, Peter A. Bampton, A/Prof of Gastroenterology, Simon L. Jakobovits, Gastroenterologist, Timothy H. Florin, Professor of Gastroenterology, Peter R. Gibson, Professor of Gastroenterology, Henry Debinski, A/Prof of Gastroenterology, Finlay A. Macrae, Professor of Gastroenterology, Douglas Samuel, Gastroenterologist, Ian Kronborg, Gastroenterologist, Graeme Radford-Smith, A/Prof of Gastroenterology, Warwick Selby, A/Prof of Gastroenterology, Michael J. Johnston, Colorectal Surgeon, Rodney Woods, Colorectal Surgeon, P Ross Elliott, Gastroenterologist, Sally J. Bell, Gastroenterologist, Steven J. Brown, Gastroenterologist, William R. Connell, Gastroenterologist, Andrew S. Day, Professor of Pediatric Gastroenterology, Paul V. Desmond, A/Prof of Gastroenterology, Richard B. Gearry, A/Prof of Gastroenterology

PII: S0016-5085(15)00111-0
DOI: 10.1053/j.gastro.2015.01.026
Reference: YGAST 59577

To appear in: *Gastroenterology*
Accepted Date: 17 January 2015

Please cite this article as: Wright EK, Kamm MA, De Cruz P, Hamilton AL, Ritchie KJ, Krejany EO, Leach S, Gorelik A, Liew D, Prideaux L, Lawrance IC, Andrews JM, Bampton PA, Jakobovits SL, Florin TH, Gibson PR, Debinski H, Macrae FA, Samuel D, Kronborg I, Radford-Smith G, Selby W, Johnston MJ, Woods R, Elliott PR, Bell SJ, Brown SJ, Connell WR, Day AS, Desmond PV, Gearry RB, Measurement of Fecal Calprotectin Improves Monitoring and Detection of Recurrence of Crohn's Disease Following Surgery, *Gastroenterology* (2015), doi: 10.1053/j.gastro.2015.01.026.

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

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

Emily K Wright
Gastroenterology Fellow

Michael A Kamm
Professor of Gastroenterology

Peter De Cruz
Gastroenterologist

Amy L Hamilton
Scientist

Kathryn J Ritchie
Research Nurse

Efrosinia O Krejany
Clinical Scientist

Steven Leach
Post-Doctoral Scientist

Alexandra Gorelik
Statistician

Danny Liew
Professor of Clinical Epidemiology

Lani Prideaux
Gastroenterologist

Ian C Lawrance
Professor of Gastroenterology

Jane M Andrews
Professor of Gastroenterology

Peter A Bampton
A/Prof of Gastroenterology

Simon L Jakobovits
Gastroenterologist

Timothy H Florin

Professor of Gastroenterology

Peter R Gibson
Professor of Gastroenterology

Henry Debinski
A/Prof of Gastroenterology

Finlay A Macrae
Professor of Gastroenterology

Douglas Samuel
Gastroenterologist

Ian Kronborg
Gastroenterologist

Graeme Radford-Smith
A/Prof of Gastroenterology

Warwick Selby
A/Prof of Gastroenterology

Michael J Johnston
Colorectal Surgeon

Rodney Woods
Colorectal Surgeon

P Ross Elliott
Gastroenterologist

Sally J Bell
Gastroenterologist

Steven J Brown
Gastroenterologist

William R Connell
Gastroenterologist

Andrew S Day
Professor of Pediatric Gastroenterology

Paul V Desmond
A/Prof of Gastroenterology

Richard B Gearry
A/Prof of Gastroenterology

Department of Gastroenterology, St Vincent's Hospital, Melbourne, Australia
Department of Medicine, University of Melbourne, Melbourne, Australia
Christchurch Hospital, Christchurch, New Zealand
University of Otago, Christchurch, New Zealand

AFFILIATIONS: Department of Gastroenterology, St Vincent's Hospital and University of Melbourne, Melbourne, Australia (E.K.W., P.D.C., M.A.K., A.L.H., K.J.R., E.O.K., L.P., M.J.J., R.W., P.R.E., S.J.B., S.J.B., W.R.C., and P.V.D.); School of Women's and Children's Health, University of NSW, Sydney, Australia (S.L.); Melbourne EpiCentre, University of Melbourne and Melbourne Health, Melbourne. Australia (A.G., D.L.); Centre for Inflammatory Bowel Diseases, Fremantle Hospital and The University of Western Australia, Fremantle, Australia (I.C.L.); Department of Gastroenterology and Hepatology, Royal Adelaide Hospital and University of Adelaide, Adelaide, Australia (J.M.A.); Department of Gastroenterology and Hepatology, Flinders Medical Centre and Flinders University, Adelaide, Australia (P.A.B.); Department of Gastroenterology, Alfred Health (S.L.J.); Department of Gastroenterology, Mater Health Services, University of Queensland Brisbane, Australia (T.H.F.); Department of Gastroenterology, Alfred Health and Monash University, Melbourne, Australia (P.R.G.); Melbourne Gastrointestinal Investigation Unit, Cabrini Hospital, Melbourne, Australia (H.D.); Department of Colorectal Medicine and Genetics, and Department of Medicine, Royal Melbourne Hospital and University of Melbourne, Melbourne, Australia (F.A.M); Department of Gastroenterology, Bankstown Hospital (D.S.); Department of Gastroenterology, Western Hospital, Melbourne, Australia (I.K.); Department of Gastroenterology, Royal Brisbane and Womens Hospital, Brisbane; IBD Group Queensland Institute of Medical Research; University of Queensland (G.R.S.); AW Morrow Gastroenterology and Liver Centre, Royal Prince Alfred Hospital, Sydney, Australia (W.S.); Department of Paediatrics, University of Otago, Christchurch, New Zealand (A.S.D.); Department of Medicine, University of Otago, Christchurch, New Zealand (R.B.G.).

DISCLOSURES: EK Wright, KJ Ritchie, EO Krejany, S Leach, A Gorelik, L Prideaux, SL Jakobovits, TH Florin, H Debinski, D Samuel, I Kronborg, G Radford-Smith, MJ Johnston, R Woods, PR Elliott and PV Desmond have no conflicts of interest to declare. P De Cruz has received travel grant support from Abbott and Schering-Plough. MA Kamm has acted as an advisor to Abbott and Janssen, has received research support from Abbott, and has acted as a speaker at symposiums sponsored by Abbott and Janssen. A Hamilton has received an educational grant from Abbott. D Liew has served on advisory boards and received research grants from Abbott. IC Lawrance has been on an advisory board for Abbott and Janssen, a speaker for Abbott and Janssen, and has held research and travel grants from Abbott and Janssen. JM Andrews has been an advisory board member for both Janssen and Abbott, spoken for both Abbott and Janssen, received research funds from both Abbott and Janssen, and received travel grants from both Abbott and Janssen. PA Bampton has been on advisory boards for Janssen and Abbott, has received research funding from Abbott, and travel sponsorship from both Abbott and Janssen. PR Gibson has received consulting fees from Abbott, Janssen, and Schering-Plough; research support from Abbott; and payments for lectures from Abbott and Janssen. FA Macrae has been on an advisory board to Janssen, has received travel grants from Abbott, and has received clinical research support from Janssen, Abbott and MSD. W Selby has been on an advisory board for Abbott. SJ Bell has received travel assistance from Abbott. SJ Brown has received travel support and speaker fees from both Abbott and Janssen. WR Connell has been on advisory board for Janssen and a speaker for Abbott and Janssen. AS Day has been an

advisor to Janssen. RB Gearry has been on an advisory board for Abbott and Janssen, a speaker for Abbott and Janssen, and held research, educational and travel grants from Abbott and Janssen.

ACKNOWLEDGEMENTS: JI Keenan, L Burgess and A Ashcroft at The University of Otago (Christchurch, New Zealand) Gastroenterology laboratory who were responsible for the processing and testing of specimens and provided supervision and laboratory support to EKW. St Vincent's Research Endowment Fund provided research support. Bülhmann provided Smart-Prep testing kits. The National Health and Medical Research Council (NHMRC) supported EKW, PDC and MAK. We are grateful to colleagues and centres for their participation.

AUTHOR CONTRIBUTIONS: EKW, PDC and MAK – 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; obtained funding. ALH – acquisition of data; analysis and interpretation of data; drafting of the manuscript. KJR and EOK – acquisition and monitoring of data. ASD, RBG, SL – laboratory support; acquisition of data; critical revision of the manuscript for important intellectual content; obtained funding. AG and DL – analysis and interpretation of data; drafting of the manuscript; statistical analysis. LP, ICL, JMA, PAB, SLJ, THF, PRG, HD, FAM, DS, IK, GRS, WS, MJJ, RW, RRE, SJB, SJB, WRC, PVD – acquisition of data and critical review of manuscript.

Author for Correspondence:

Professor Michael Kamm
St Vincent's Hospital
Victoria Parade
Fitzroy 3065
Melbourne
Australia
Tel: + 61 3 9417 5064
Fax: + 61 3 9416 2485
E: mkamm@unimelb.edu.au

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.[1] 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,[2] with disease recurrence identifiable endoscopically in 70 percent of patients at one year.[3]

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.[4, 5] Endoscopy is the gold standard for detecting and quantifying bowel inflammation, but is expensive, labor intensive, inconvenient for the patient and carries some risk.[6] 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.[7, 8] The correlation between serum biochemical markers of inflammation, such as C-reactive protein (CRP), and endoscopic findings in Crohn's disease is also inconsistent.[7, 9]

We have recently undertaken a controlled clinical trial examining different strategies for managing patients after Crohn's disease resection of all macroscopic disease.[10] 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.[11] 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[12] and is abundant in all body fluids in proportion to the degree of inflammation present.[13] It can be readily quantified in feces using enzyme-linked immunosorbent assay (ELISA) or an immunoassay.

Fecal calpotectin (FC) has been shown to reflect endoscopic disease activity in Crohn's disease.[8, 9] FC is more sensitive than CDAI or CRP at detecting endoscopic inflammation,[9, 14] and is a reliable surrogate marker of mucosal healing in patients with Crohn's disease.[8, 15] Increased FC concentrations are associated with an increased risk of clinical relapse.[16-21] 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.[22-27] FC concentrations have been shown to fall after intestinal resection for Crohn's disease.[25] 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).[26] Lasson and colleagues[22] 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.[10] 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[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 (≤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)[3]. Two secondary measures of endoscopic disease activity were also calculated: the Crohn's Disease Endoscopic Index of Severity (CDEIS)[28] and the Simple Endoscopic Score for Crohn's Disease (SES-CD),[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.[33, 34]

There were three cohorts used for analysis:

Cross Sectional Analysis

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

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 ≥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 1402µg/g, IQR: 426µg/g-2825µg/g). At 6 months FC concentration fell (all patients median 166µg/g, IQR: 56µg/g-424µg/g), but was higher in those with recurrent endoscopic disease than endoscopic remission (275µg/g, IQR: 163µg/g-540µg/g vs. 72µg/g, IQR: 32µg/g-190µg/g, $p<0.001$) (Figure 2A). At 18 months FC concentration was higher in recurrent endoscopic disease than endoscopic remission (FC: 331µg/g, IQR: 159µg/g-550µg/g vs. 75µg/g, IQR: 37µg/g-258µg/g, $p<0.002$) (Figure 2B). When 6 and 18 month observations were combined median FC in those with recurrent endoscopic disease was 330µg/g, IQR: 163 - 540 versus 75µg/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 (≥i2) 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.[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.[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.[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.[2] 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.[32] 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)[28] and the Simple Endoscopic Score for Crohn's Disease (SES-CD)[29] 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[38] 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.[39, 40] 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.[22-26] 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.[41] 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

1. Caprilli R, Gassull MA, Escher JC, et al. European evidence based consensus on the diagnosis and management of Crohn's disease: special situations. Gut 2006;55 Suppl 1:i36-58.
2. Rutgeerts P, Geboes K, Vantrappen G, et al. Predictability of the postoperative course of Crohn's disease. Gastroenterology 1990;99:956-63.
3. Rutgeerts P, Geboes K, Vantrappen G, et al. Natural history of recurrent Crohn's disease at the ileocolonic anastomosis after curative surgery. Gut 1984;25:665-72.
4. Best WR, Becktel JM, Singleton JW, et al. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology 1976;70:439-44.
5. Crama-Bohbouth G, Pena AS, Biemond I, et al. Are activity indices helpful in assessing active intestinal inflammation in Crohn's disease? Gut 1989;30:1236-40.
6. Gisbert JP, McNicholl AG, Gomollon F. Questions and answers on the role of fecal lactoferrin as a biological marker in inflammatory bowel disease. Inflamm Bowel Dis 2009;15:1746-54.
7. Jones J, Loftus EV, Jr., Panaccione R, et al. Relationships between disease activity and serum and fecal biomarkers in patients with Crohn's disease. Clinic Gastroenterol Hepatol 2008;6:1218-1224.
8. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012;18:2218-24.
9. Sipponen T, Savilahti E, Kolho KL, et al. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. Inflamm Bowel Dis 2008;14:40-6.
10. De Cruz P, Kamm MA, Hamilton AL, et al. Crohn's disease management after intestinal resection: a randomised trial. Lancet 2014.
11. Biancone L, Onali S, Calabrese E, et al. Non-invasive techniques for assessing postoperative recurrence in Crohn's disease. Dig Liver Dis 2008;40 Suppl 2:S265-70.
12. Steinbakk M, Naess-Andresen CF, Lingaas E, et al. Antimicrobial actions of calcium binding leucocyte L1 protein, calprotectin. Lancet 1990;336:763-5.
13. Roseth AG, Fagerhol MK, Aadland E, et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol 1992;27:793-8.
14. Vieira A, Fang CB, Rolim EG, et al. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. BMC Res Notes 2009;2:221.
15. Sipponen T, Savilahti E, Karkkainen P, et al. Fecal calprotectin, lactoferrin, and endoscopic disease activity in monitoring anti-TNF-alpha therapy for Crohn's disease. Inflamm Bowel Dis 2008;14:1392-8.
16. Tibble JA, Sigthorsson G, Bridger S, et al. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology 2000;119:15-22.
17. D'Inca R, Dal Pont E, Di Leo V, et al. Can calprotectin predict relapse risk in inflammatory bowel disease? Am J Gastroenterol 2008;103:2007-14.
18. Gisbert JP, Bermejo F, Perez-Calle JL, et al. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. Inflamm Bowel Dis 2009;15:1190-8.
19. Sipponen T, Kolho KL. Faecal calprotectin in children with clinically quiescent inflammatory bowel disease. Scand J Gastroenterol 2010;45:872-7.

20. de Suray N, Salleron J, Vernier-Massouille G, et al. Close monitoring of CRP and fecal calprotectin is able to predict clinical relapse in patients with Crohn's disease in remission after infliximab withdrawal. A sub-analysis of the STORI study. Gastroenterology 2012;142:S-149.
21. Louis E, Mary J-Y, Vernier-Massouille G, et al. Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. Gastroenterology 2012;142:quiz e31.
22. Lasson A, Strid H, Ohman L, et al. Fecal calprotectin one year after ileocaecal resection for Crohn's disease - A comparison with findings at ileocolonoscopy. J Crohns Colitis 2014.
23. Orlando A, Modesto I, Castiglione F, et al. The role of calprotectin in predicting endoscopic post-surgical recurrence in asymptomatic Crohn's disease: a comparison with ultrasound. Eur Rev Med Pharmacol Sci 2006;10:17-22.
24. Scarpa M, D'Inca R, Basso D, et al. Fecal lactoferrin and calprotectin after ileocolonic resection for Crohn's disease. Dis Colon Rectum 2007;50:861-9.
25. Lamb CA, Mohiuddin MK, Gicquel J, et al. Faecal calprotectin or lactoferrin can identify postoperative recurrence in Crohn's disease. Br J Surg 2009;96:663-74.
26. Lobaton T, Lopez-Garcia A, Rodriguez-Moranta F, et al. A new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohn's disease. J Crohns Colitis 2013;7:e641-51.
27. Yamamoto T, Kotze PG. Is fecal calprotectin useful for monitoring endoscopic disease activity in patients with postoperative Crohn's disease? J Crohns Colitis 2013.
28. Mary JY, Modigliani R. Development and validation of an endoscopic index of the severity for Crohn's disease: a prospective multicentre study. Groupe d'Etudes Therapeutiques des Affections Inflammatoires du Tube Digestif (GETAID). Gut 1989;30:983-9.
29. Daperno M, D'Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc 2004;60:505-12.
30. von Roon AC, Karamountzos L, Purkayastha S, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. Am J Gastroenterol 2007;102:803-813.
31. D'Haens GR, Vermeire S, Van Assche G, et al. Therapy of metronidazole with azathioprine to prevent postoperative recurrence of Crohn's disease: a controlled randomized trial. Gastroenterology 2008;135:1123-9.
32. Regueiro M, Schraut W, Baidoo L, et al. Infliximab prevents Crohn's disease recurrence after ileal resection. Gastroenterology 2009;136:441-50 e1; quiz 716.
33. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32-5.
34. Ruopp MD, Perkins NJ, Whitcomb BW, et al. Youden Index and optimal cut-point estimated from observations affected by a lower limit of detection. Biom J 2008;50:419-30.
35. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol 2010;105:162-9.
36. Mary JY, Lemann M, Colombel J-F. Endosocpic remission and response in Crohn's disease: an objective definition using the CDEIS. Gut 2006;37 (Suppl 1):A55.
37. Ng SC, Lied GA, Arebi N, et al. Clinical and surgical recurrence of Crohn's disease after ileocolonic resection in a specialist unit. Eur J Gastroenterol Hepatol 2009;21:551-7.

38. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ 2010;341:c3369.
39. Naismith GD, Smith LA, Barry SJ, et al. A prospective single-centre evaluation of the intra-individual variability of faecal calprotectin in quiescent Crohn's disease. Aliment Pharmacol Ther 2013;37:613-21.
40. Moum B, Jahnsen J, Bernklev T. Fecal calprotectin variability in Crohn's disease. Inflamm Bowel Dis 2010;16:1091-2.
41. Manz M, Burri E, Rothen C, et al. Value of fecal calprotectin in the evaluation of patients with abdominal discomfort: an observational study. BMC Gastroenterol 2012;12:5.

Table 1. Patient demographics

	All (n=135)		Endoscopic Validation Cohort (n=99)	
	n =	**%**	**n =**	**%**
Sex (Male)	59	43.7	46	46.5
Age (Median)	36 (26-47)		38 (29-47)	
Risk Stratification				
Low Risk	23	17.0	18	18.2
High Risk	112	83.0	81	81.8
Randomisation				
Standard Care	39	28.9	21	21.2
Active Care	96	71.1	78	78.8
Resection Type				
Ileocaecal	104	77.0	78	78.8
Isolated Ileal	9	6.7	6	6.1
Subtotal colectomy	6	4.4	6	6.1
Ileocaecal and sigmoid	5	3.7	3	3.0
Ileocaecal and small bowel	11	8.1	6	6.1
Disease Location at Surgery				
Ileum only (L1)	71	52.6	57	57.6
Colon only (L2)	10	7.4	7	7.1
Ileum and colon (L3)	54	40.0	35	35.4
Upper gastrointestinal disease (L4)	6	4.4	5	5.1
Disease Phenotype at Surgery				
Inflammatory (B1)	12	8.9	9	9.1
Stricturing (B2)	49	36.3	37	37.4
Penetrating (B3)	74	54.8	53	53.5
Perianal disease (P)	14	10.4	10	10.1
Smoking at Study Entry				
Never	60	44.4	43	43.4
Current	39	28.9	27	27.3
Past	36	26.7	29	29.3
Immediate Post-Operative Drug Therapy from Study Commencement				
Metronidazole only (Low Risk Patients)	23	17.0	18	18.2
Thiopurine (High Risk Patients)	76	53.6	55	55.6
Adalimumab (High Risk Thiopurine Intolerant Patients)	36	26.7	26	26.3
Pre-Operative CDAI, median (IQR)	222 (136.5 - 315.5)		218 (138 - 311)	
Pre-Operative CRP, median (IQR)	10.5 (5 - 48)		9 (5 - 48.5)	

Table 2 Analysis cohorts and endoscopic recurrence

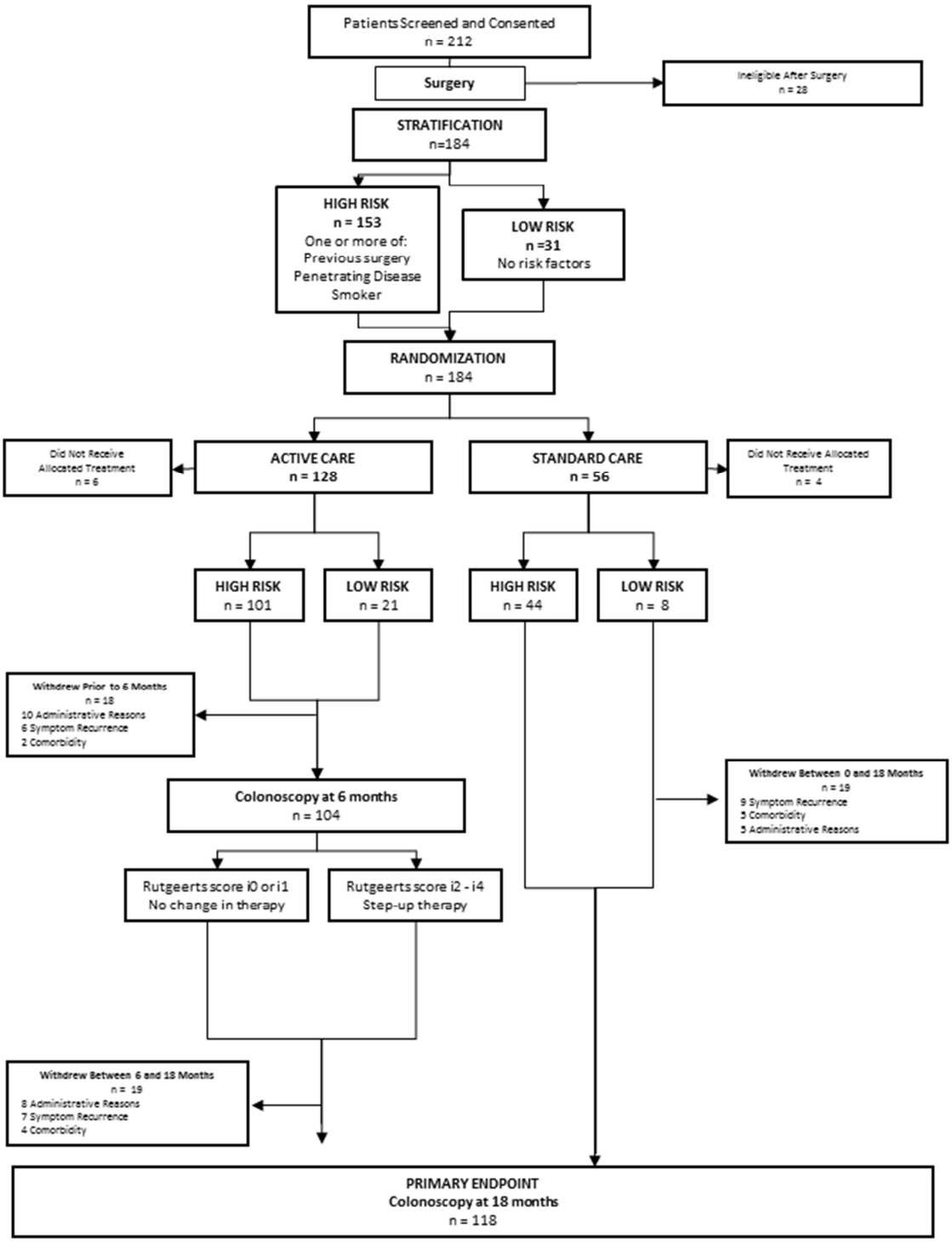
Analysis Cohort	Patients (n=)	Samples (n=)	Patients with Recurrence at 6 months	Patients with Recurrence at 18 months
Cross Sectional	**135**	**319**	**31 (36%)**	**45 (45%)**
Endoscopic Validation	**99**	**137**	**23 (34%)**	**24 (35%)**
Longitudinal	**80**	**203**	**20 (28%)**	**22 (34%)**

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 ≥i2) at 6 months, 18 months, and combined 6 and 18 months. The calculated best cut-off for FC is shown first in each table.

	FC (µg/g)	Sensitivity	Specificity	PPV (%)	NPV (%)	AUROC
6 months n=68	Calculated best cut-off of 135	0.91	0.62	55	93	0.799
	50	0.96	0.38	44	94	
	100	0.91	0.56	51	93	
	150	0.78	0.67	55	86	
	200	0.74	0.77	61	85	
	1000	0.22	0.93	63	70	
18 months n=69	Calculated best cut-off of 127	0.88	0.67	58	91	0.727
	50	0.96	0.36	44	94	
	100	0.88	0.58	53	90	
	150	0.79	0.69	58	86	
	200	0.71	0.71	57	82	
	1000	0.17	0.89	44	67	
Combined 6 and 18 months n=139	Calculated best cut-off of 135	0.87	0.66	56	91	0.763
	50	0.96	0.38	45	94	
	100	0.89	0.58	53	91	
	150	0.77	0.68	55	85	
	200	0.71	0.74	59	83	
	1000	0.19	0.90	50	68	

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).

n=136	Endoscopic Recurrence (Rutgeerts score i2, i3 or i4)		Scored Endoscopic Severity (i0-i4)		SES-CD		CDEIS		CRP		CDAI	
	r	***P***	***R***	***P***	***r***	***P***	***r***	***P***	***r***	***P***	***r***	***P***
FC	0.419	<0.001	0.439	<0.001	0.491	<0.001	0.467	<0.001	0.240	0.121	0.115	1.000
CDAI	-0.153	1.000	-0.174	0.978	X	X	X	X	-0.167	1.000	X	X
CRP	0.091	1.000	0.166	1.000	X	X	X	X	X	X	X	X


Patients Screened and Consented
n = 212
Surgery
Ineligible After Surgery
n = 28
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 = 6
ACTIVE CARE
n = 128
STANDARD CARE
n = 56
Did Not Receive Allocated
Treatment
n = 4
HIGH RISK
n = 101
LOW RISK
n = 21
HIGH RISK
n = 44
LOW RISK
n = 8
Withdrew Prior to 6 Months
n = 18
10 Administrative Reasons
6 Symptom Recurrence
2 Comorbidity
Withdrew Between 0 and 18 Months
n = 19
9 Symptom Recurrence
5 Comorbidity
5 Administrative Reasons
Colonoscopy at 6 months
n = 104
Rutgeerts score i0 or i1
No change in therapy
Rutgeerts score i2 - i4
Step-up therapy
Withdrew Between 6 and 18 Months
n = 19
8 Administrative Reasons
7 Symptom Recurrence
4 Comorbidity
PRIMARY ENDPOINT
Colonoscopy at 18 months
n = 118