Fecal calprotectin is not superior to serum C-reactive protein or the Harvey–Bradshaw index in predicting postoperative endoscopic recurrence in Crohn's disease

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Background Fecal calprotectin (FC) is a widely used noninvasive marker of gut inflammation that is associated with endoscopic severity in Crohn's disease (CD). However, FC has been inconsistent in predicting postoperative recurrence of CD, and its utility in the postoperative setting remains unclear.

Materials and methods Blood and fecal samples were collected in consecutively recruited patients with CD who had undergone ileocolonic resection and required a colonoscopy to assess postoperative recurrence, as defined by the Rutgeerts score (RS).

Results A total of 86 patients were prospectively recruited at five centers. Overall, 49 (57%) had CD recurrence (RS ≥ i2). FC concentrations trended to increase with RS severity; FC median (interquartile range) was significantly higher in patients with endoscopic recurrence than those in endoscopic remission [172.5 (75–375) vs. 75 (36.5–180.5) μg/g, respectively]. The same occurred for C-reactive protein (CRP) [0.5 (0.1–0.95) vs. 0.1 (0.02–0.27)] mg/dl and the Harvey–Bradshaw index (HBI) [4 (2–7) vs. 1 (0–3.5)]. The three variables significantly correlated. The area under the curve to discriminate between patients in endoscopic remission and recurrence was 0.698 for FC, with 62 μg/g being the optimal cut-off point. This indicated FC would have 85.7% sensitivity and 45.9% specificity in detecting any recurrence, having positive predictive value and negative predictive value of 67.7 and 70.8%, respectively. Area under the curve for CRP and HBI were both 0.710. The combination of CRP and HBI provided a positive predictive value 95.7 and a diagnostic odds ratio of 30.8.

Conclusion FC is not better than CRP combined with HBI to predict endoscopic postoperative recurrence of CD. Eur J Gastroenterol Hepatol 30:1521–1527

Introduction

Crohn's disease (CD) is an inflammatory bowel disease (IBD) with a chronic and relapsing clinical course. The appearances of complications of CD, including abscesses, fistula, perforations, bleeding, and failure of response to medication, are common and may lead to surgery [1]. Resection of the affected bowel segment will be required in half of the patients with CD within 10 years after diagnosis, but this figure may increase up to 80% during their lives, the most common of which being an ileocolic resection [2]. However, surgery is not a cure, and recurrence of mucosal inflammation is observed endoscopically in 70–90% of adult patients with CD within a year of surgery, although only one-third of them will present with symptoms [3,4].

Postoperatively, patients with CD require regular monitoring to check for endoscopic recurrence and disease progression after ileocolonic resection. Endoscopic indexes constitute the gold standard in defining IBD relapse owing to their high sensitivity and specificity [5,6]. However, colonoscopy is an invasive, expensive, and not always well-tolerated technique, so finding alternative monitoring methods has been actively pursued. Clinical symptoms [7] and some unspecific noninvasive markers [8] are far from optimal to predict postoperative inflammatory activity in endoscopy, especially for mild flares, and have limited utility in other concomitant processes of IBD [9].

Fecal markers such as calprotectin are potentially more specific than blood markers because they are unaffected by extraintestinal processes. Calprotectin constitutes the main cytosolic protein of neutrophils and macrophages, exudated to the lumen from the inflammation of the intestinal mucosa [10]. Fecal calprotectin (FC) levels correlate with endoscopic disease activity in CD [11] and were proposed

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as a simple, adequate and noninvasive method in evaluating recurrence in postoperative patients with CD [12]. More recent studies, however, have recommended against using calprotectin as a substitute for colonoscopy for CD monitoring and treatment adjustment [13–15]. Data regarding the usefulness of FC levels to predict relapse in patients with CD receiving biological therapies are limited and conflicting [16,17]. The prognostic value of FC in patients undergoing different treatments and with varying degrees of severity of the disease requires further evaluation.

The aim of the study was to evaluate the predictive value of a rapid test of FC for the presence and severity of postoperative endoscopic recurrence in patients with CD, compared with CRP and the clinical evaluation of disease activity. In addition, we sought to identify whether treatment modalities modify the predictive capacity of such markers.

Materials and methods

Study design

A cross-sectional, observational, multicenter cohort study was performed within the Ciudad Real province IBD working group, which represents all adult IBD units in this region of Spain [18]. Consecutive recruitment was designed and conducted prospectively from April 2016 to September 2017 on patients who required assessment of postoperative recurrence of CD. Inclusion criteria consisted of adult patients with a previous diagnosis of CD based on clinical, endoscopic, radiologic, and histologic criteria [19], who underwent ileocolic resection for active CD at any point after the diagnosis of the disease, and accepted undertaking a colonoscopy and FC determination. All patients received standard postoperative care, including metronidazole for 3 months. Therapy to maintain disease remission was being used for at least 6 months before at stable doses. Exclusion criteria were patients aged under 18 years, those with incomplete colonoscopy or lack of FC examination, pregnant women, and patients with IBD unclassified.

Assessment of clinical activity

Inclusion criteria were determined at baseline by the IBD physicians in charge at the point of recruitment. Clinical activity was assessed by using the Harvey–Bradshaw index (HBI) [20], wherein general welfare, abdominal pain, stool frequency and consistency, abdominal mass, erythema nodosum and uveitis were recorded. Clinical remission was considered as an index less than or equal to 5 [21]. Blood and stool analyses were requested to be performed before endoscopy, including C-reactive protein (CRP) serum concentration and FC. An appointment for an endoscopy to be performed in less than a month from inclusion was offered to all patients. Epidemiological and clinical data obtained at the time of diagnosis included patient age, sex, type and location of the disease according to the Montreal classification system [22], and date of the surgical resection/s.

Stool tests and laboratory analyses

Stool samples were collected from baseline and 3 days before endoscopy and routinely processed in clinical laboratories of the participating centers. All used the same type of test to determine FC, the Quantum Blue Calprotectin kit (Bühlmann Laboratories, Schönenbuch, Switzerland). The values provided by the laboratories ranged from less than 30 to more than 1000 μg/g. Normal values for serum CPR ranged at 0.1–1 mg/dl.

Endoscopic assessment

A complete ileocolonoscopy was performed under sedation by trained endoscopists of the IBD unit at each participating hospital. From the ileocolonoscopy, mucosal recurrence at the anastomosis and neoterminal ileum was assessed according to the Rutgeerts score (RS) [23] by the endoscopist, who was not blinded to patient treatment. Endoscopic remission was defined as a RS of i0 (no lesions) or i1 (five aphthous lesions), and recurrence was defined as a RS of i2 (>5 aphthous lesions or larger lesions confined to anastomosis), i3 (diffuse ileitis), or i4 (diffuse inflammation with large ulcers and/or narrowing).

Statistical analysis

Results for continuous variables are expressed as the mean and SD or as the median and interquartile range (IQR); qualitative variables are presented as absolute and relative frequencies. The χ^2 (Fisher's exact test, where appropriate) and Mann–Whitney *U*-test were used to compare qualitative and quantitative variables, respectively. Multivariate logistic regression models for the variables with statistical significance or clinical relevance in the bivariate analysis were obtained. Odds ratios (OR) with 95% CIs were calculated for significant variables.

A diagnostic study was then conducted calculating for each variable its cut-off point with the best diagnostic value (best balance between sensitivity and specificity) according to the Youden method [24]. The sensitivity, specificity, predictive values, likelihood ratios (LH+), and diagnostic odds ratios were calculated by each cut-off. Receiver operating characteristic (ROC) curves and area under curve (AUC) were performed.

A significance level of 0.05 was used throughout. Analyses and summaries were carried out with the PASW statistical program (version 18.0; SPSS Inc., Chicago, Illinois, USA) and Epidat v3.1 (General Directorate of Public Health of the Galician Health Service, Spain).

Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki principles and following the rules for Good Clinical Practice. The registries supporting this study were approved by the local ethics or research committees at every participating center, and all patients gave their informed consent before inclusion.

Results

Patients characteristics

A total of 89 patients diagnosed with CD and ileocolonic resection underwent a colonoscopy to assess endoscopic recurrence; 86 of them (including 45 males and 41 women) who had matched complete colonoscopy with ileal intubation, FC, CRP results and HBI constituted the

study cohort. Two of the patients who were initially recruited did not provide stool samples for FC and a third did not have serum CRP results around the time of the colonoscopy; hence, they were removed from the analyses. The median age at CD diagnosis was 30.8 years (IQR: 22.7–45.7). At the point of inclusion, the median age was 46.2 (IQR: 35.3–53.2) years old. Active smoking at the point of inclusion was present in 21 (34.4%) patients whereas 12 (19.7%) patients were former smokers and 28 (45.9%) had never smoked.

A total of 36 (41.9%) patients were being treated with immunosuppressant drugs at the point of inclusion and 23 (26.7%) were receiving antitumor necrosis factor-α therapy. Combination therapy was being used in 21 (24.4%) patients and six (6.9%) patients were receiving neither of these options. Table 1 shows the characteristics of the study cohort.

Colonoscopic assessment, blood and fecal biomarkers

Among the 86 patients enrolled, 37 (43%) were in endoscopic remission (i.e. RS i0 or i1), whereas endoscopic recurrence (RS ≥ i2) was evidenced in 49 (57%) patients. Median (IQR) FC concentration was 172.5 (75–375) μg/g in patients with a relapse of CD and 75 (36.5–180.5) μg/g in those maintaining endoscopic remission ($P = 0.003$). The same occurred with median (IQR) CRP [0.5 (0.1–0.95) vs. 0.1 (0.02–0.27) mg/dl; $P = 0.001$] and the HBI [4 (2–7) vs. 1 (0–3.5); $P = 0.001$] (Fig. 1).

FC concentrations trended to increase gradually in accordance with the severity of postoperative endoscopic recurrence (Table 2): RS correlated significantly with FC values ($\rho = 0.327$, $P = 0.002$), and also with CRP ($\rho = 0.360$; $P = 0.001$) and HBI ($\rho = 0.366$; $P = 0.001$). In addition, FC concentrations were associated with those of CRP ($\rho = 0.354$; $P = 0.001$) and HBI scores ($\rho = 0.370$; $P = 0.001$).

Table 1. Baseline clinical and demographic characteristics of patients with Crohn's disease at the time of endoscopy

	Overall cohort ($N = 86$)
Sex (male/female)	45 (52.3)/41 (47.7)
Age at diagnosis [median (IQR)] (years)	30.8 (22.7–45.7)
Disease duration [median (IQR)] (years)	7.9 (3.9–16.5)
Age at colonoscopy [median (IQR)] (years)	46.2 (35.3–53.2)
Time from last ileocecal resection [median (IQR)] (years)	4.5 (2–9.7)
Smoking habits at study inclusion [*N* (%)]	
Never smoked	28 (45.9)
Former smoker	12 (19.7)
Current smoker	21 (34.4)
Disease behavior at surgery [*N* (%)]	
B1	11 (12.8)
B2	49 (57)
B3	26 (30.2)
Disease location al surgery [*N* (%)]	
L1	38 (44.2)
L2	3 (3.5)
L3	35 (40.7)
L1 + L4	3 (3.5)
L2 + L4	1 (1.2)
L3 + L4	6 (7)
Number of intestinal surgery [median (rank)]	1 (1–3)
Drug therapy for CD at study inclusion [*N* (%)]	
Immunosuppressants	36 (41.9)
Antitumor necrosis factor agents	23 (26.7)
Combined immunosuppressants + antitumor necrosis factor agents	21 (24.4)
Mesalazine	6 (6.9)
Harvey–Bradshaw index [median (IQR)]	2.5 (0–6)
FC [median (IQR)] (μg/g)	110.5 (60–289.3)
CPR [median (IQR)] (mg/dl)	0.2 (0.09–0.62)
Rutgeerts score [*N* (%)]	
i0	24 (27.9)
i1	13 (15.1)
i2	24 (27.9)
i3	10 (11.6)
i4	15 (17.4)

CD, Crohn's disease; CRP, C-reactive protein; FC, fecal calprotectin; IQR, interquartile range.

Diagnostic accuracy of fecal calprotectin and C-reactive protein in discriminating postoperative endoscopic remission from recurrence in postoperative Crohn's disease

For FC and CRP concentrations, and for the HBI score, ROC curves were made, keeping all the continuous data in order to find the optimal cut-off point. For FC, an AUC of 0.698 (95% CI: 0.58–0.81), and an optimal cut-off of 62 μg/g using the Youden test were obtained. This indicated that calprotectin would have 85.7% sensitivity and 45.9% specificity in detecting any recurrence. Positive predictive value (PPV) was 67.7% and the negative predictive value was 70.8%, thus showing that FC would not detect 29.2% of patients with CD with postoperative recurrence.

An AUC of 0.710 (95% CI: 0.60–0.82) was calculated for CRP, with 0.32 being the optimal cut-off value according to the Younden test. HBI score values also provided an AUC of 0.710 (95% CI: 0.6–0.82); the optimal cut-off value to discriminate endoscopic remission from recurrence was 2, according to the Youden test (Table 3 and Fig. 2). Figure 3 reflects a graph with curves for the three variables and their corresponding AUC. In addition, the sensitivity and specificity values for other cutoff points were calculated (Table 3). Subgroup analyses revealed that the time elapsed since surgery did not significantly modify the predictive performance of the three markers (Supplementary Table 1, Supplemental digital content 1, *http://links.lww.com/EJGH/A353*).

Owing to the limitations in the individual predictive capacity of each of these variables, we next looked to see if particular combinations could increase their diagnostic performance. The combination of a CRP concentration over 0.32 mg/dl and an HBI index over two provided a PPV of 95.7, a positive LH+ of 16.85 and a diagnostic OR of 30.8. When these two variables were joined to a FC concentration more than 62 μg/g, the PPV resulted in 94.1, LH+ was 12.26 and the diagnostic OR was 18.06. Supplementary Table 2 (Supplemental digital content 2, *http://links.lww.com/EJGH/A354*), shows the diagnostic performance of other combinations with their 95% CI.

Effect of Crohn's disease therapy in the diagnostic performance of fecal calprotectin and C-reactive protein

Since previous research has documented a reasonable predictive ability for FC in patients with IBD receiving antitumor necrosis factor-α therapy [17], we next aimed to analyze the potential effect of CD therapy on the diagnostic performance of FC, CRP and HBI in predicting postoperative recurrence through subgroup analysis, according to CD therapy at the point of endoscopy and RS assessment. No significant differences were found in the predictive capacities of AUC for FC concentration, serum

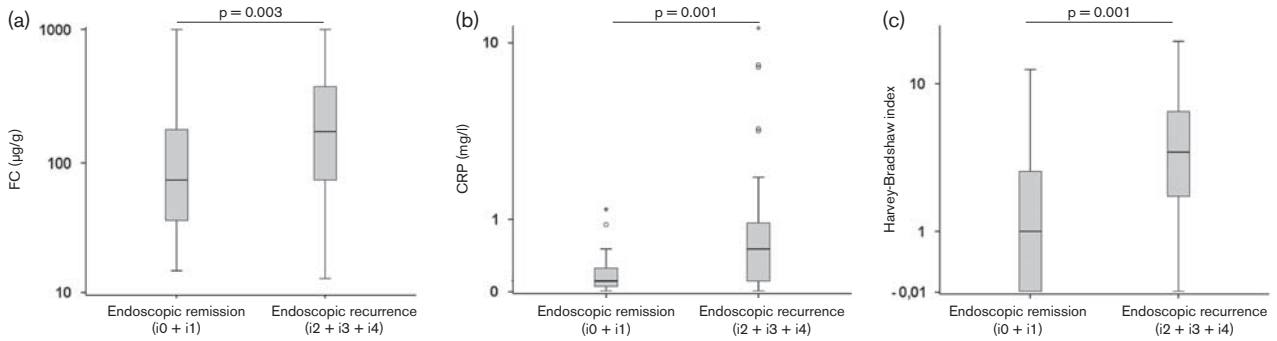

Fig. 1. Concentration of fecal calprotectin (a), C-reactive protein (b) and Harvey–Bradshaw index (HBI) (c) on logarithmic scales in patients with Crohn's disease according to explanatory variable recurrence/nonrecurrence after colonoscopy, where nonrecurrence is Rutgeerts score i0 to i2a. The boxplots show median, upper, and lower quartiles of the data; the whiskers indicate the 95% confidence interval of the values.

Table 2. Mean concentrations of fecal calprotectin, C-reactive protein and Harvey–Bradshaw index according to the Rutgeerts score for postoperative recurrence of Crohn's disease

Rutgeerts score (number of patients)	FC [median (IQR)] (µg/g)	CPR [median (IQR)] (mg/l)	Harvey–Bradshaw index [median (IQR)]
i0 ($n = 27$)	67.5 (26.3–154.5)	0.1 (0.03–0.26)	1 (0–4)
i1 ($n = 13$)	100 (46.5–210.5)	0.1 (0.02–0.3)	1 (0–3)
i2 ($n = 24$)	166.5 (64.8–316.3)	0.45 (0.11–0.62)	4 (1–6)
i3 ($n = 10$)	98.5 (63–625)	0.1 (0.05–0.73)	6 (3–8)
i4 ($n = 15$)	259 (131–543)	0.93 (0.15–3.8)	5 (2–9)

CRP, C-reactive protein; FC, fecal calprotectin; IQR, interquartile range.

CRP and HBI score between therapies (Table 4). Finally, no significant effect was demonstrated for the smoking status on the diagnostic performance of FC for endoscopic postoperative recurrence of CD. AUC of FC in those who never smoked, current smokers and former smokers were 0.654, 0.741 and 0.625, respectively ($P = 0.536$).

Discussion

The present study explores the relationship between FC concentrations and the magnitude of postoperative endoscopic recurrence in a cross-sectional cohort of patients with CD, recruited from several IBD facilities in Central Spain, and who received ileocolonic resection. According to our results, the predictive ability of FC in this clinical scenario is more limited than previously shown in nonoperated patients with CD [25,26], and not superior to standard evaluation with clinical symptoms and serum CRP in identifying patients with postoperative recurrence of CD.

Evidence on the cost-effectiveness of FC with regard to screening patients of all ages with a suspicion of IBD, has been provided [27], and resulting in good sensitivity and specificity values, generally higher in adults than in children [28]. However, FC concentration values are ineffective in distinguishing between CD and ulcerative colitis [29]. Once a diagnosis of IBD has been achieved, FC has been used repeatedly to monitor disease activity. According to studies recruiting patients with IBD in remission, FC could be also considered as an appropriate tool for predicting relapse of the disease [30–33], with a meta-analysis that provided a pooled AUC of 0.79 and 0.78 in patients with CD and UC, respectively [34], thus allowing for early changes in treatment.

The capacity of FC to predict endoscopic postoperative recurrence of CD in patients who underwent colonoscopy as a reference test was later assessed in several studies, subsequently pooled in a meta-analysis [12]. The AUC values provided for endoscopic recurrence ranged from 0.86 to 0.74 [13,37]. It should be noted that widely variable cut-off values for FC and standards for relapse were used in font studies, and the sensitivity and specificity of FC greatly depended on the cut-off value considered, with no commonly accepted threshold provided [35]. Additional reasons for this variability include that the accuracy of the different tests for assessing IBD varies from one to the other [12,36], and differences in inclusion criteria among the studies (some of them considering exclusively asymptomatic patients [37–39]). The prognostic value of FC probably differs in patients receiving different treatments and in those suffering from different severities of the disease. It has already been shown that FC is a very accurate marker in preventing relapse within the following 2 months after administration of infliximab in nonoperated patients with IBD (who potentially would have a severe disease). Low levels of FC just before infliximab administration prevented relapse with 100% accuracy, up to the next dose 8 weeks later [17]. Although these results have not been universally reproduced [16], it was necessary to check these differences in the predictive precision of FC in patients with CD with ileocecal resection receiving different treatment modalities.

We found that FC in this scenario provided an overall AUC of 0.698 which ranged from 0.60 to 0.805 when patients were subgrouped according to CD therapies. A nonsignificant increase in the diagnostic performance of FC from patients treated with immunomodulators to those under a combination therapy was found (Table 4), with no differences demonstrated for CRP or HBI. Time since surgery did not influence the diagnostic performance of any of the three markers assessed.

By using the Younden test, we calculated that the optimal cut-off concentration of FC was 62 µg/g, with 85.7% sensitivity and 45.9% specificity, for identifying any CD recurrence. Additional studies also showed that relatively low FC concentration performed well in terms of providing acceptable-to-good negative predictive values. Optimal cut-off of 100 µg/g FC postoperatively was calculated by Boschetti *et al.* [37] in France and by Wright *et al.* [38] in Australia. In Spain, Lobaton *et al.* [40]

Table 3. Diagnostic accuracy of fecal calprotectin, C-reactive protein and Harvey–Bradshaw index to predict endoscopic postoperative recurrence of Crohn's disease in patients who had undergone ileocecal resection

	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	PPV (95% CI) (%)	NPV (95% CI) (%)	LR+ (95% CI)	LR− (95% CI)	OR diagnostic (95% CI)	AUROC
FC cut-off (µg/g)								0.698 (0.58–0.81)
50	89.8 (78.2–95.6)	40.5 (26.3–56.5)	66.7 (54.7–76.8)	75 (53.1–88.8)	1.51 (1.14–2)	0.25 (0.10–0.61)	6 (1.93–18.65)	
62 (Youden test)	85.7 (73.3–92.9)	45.9 (31–61.6)	67.7 (55.4–78)	70.8 (50.8–85.1)	1.59 (1.15–2.18)	0.31 (0.15–0.65)	5.1 (1.82–14.27)	
100	67.3 (53.4–78.8)	56.8 (40.9–71.3)	67.3 (53.4–78.8)	56.8 (40.9–71.3)	1.56 (1.03–2.36)	0.58 (0.36–0.92)	2.71 (1.12–6.55)	
200	46.9 (33.7–60.6)	78.4 (62.8–88.6)	74.2 (56.8–86.3)	52.7 (39.8–65.3)	2.17 (1.10–4.29)	0.68 (0.49–0.93)	3.21 (1.22–8.4)	
250	42.9 (30–56.7)	88.6 (72–94.1)	80.8 (62.1–91.5)	53.3 (40.9–65.4)	3.17 (1.32–7.62)	0.66 (0.5–0.88)	4.8 (1.6–14.4)	
CRP cut-off (mg/l)								0.710 (0.6–0.82)
0.32 (Youden test)	57.1 (43.3–70)	85.7 (70.6–93.7)	84.8 (69.1–93.3)	58.8 (45.2–71.2)	4 (1.71–9.33)	0.5 (0.35–0.71)	8 (2.66–24.1)	
0.5	40.8 (28.2–54.8)	91.4 (77.6–97)	87 (67.9–95.5)	52.5 (40.2–64.5)	4.76 (1.53–14.79)	0.65 (0.5–0.84)	7.36 (1.98–27.36)	
0.75	30.6 (19.5–44.5)	91.4 (77.6–97)	83.3 (60.8–94.2)	48.5 (36.8–60.3)	3.57 (1.12–11.41)	0.76 (0.6–0.96)	4.71 (1.24–17.8)	
1	22.4 (13–35.9)	94.3 (81.4–98.4)	84.6 (57.8–95.7)	46.5 (35.4–58)	3.93 (0.93–16.63)	0.82 (0.67–1.01)	4.78 (0.99–23.12)	
1.5	14.3 (7.1–26.7)	100 (90.1–100)	100 (64.6–100)	45.5 (34.8–56.5)	–	0.86 (0.76–0.96)	–	
Harvey–Bradshaw index cut-off								0.710 (0.6–0.82)
1	83 (69.9–91.1)	40.5 (26.3–56.5)	63.9 (51.4–74.8)	65.2 (44.9–81.2)	1.4 (1.04–1.88)	0.42 (0.21–0.85)	3.32 (1.22–9.08)	
2 (Youden test)	78.7 (65.1–88)	56.8 (40.9–71.3)	69.8 (56.5–80.5)	67.7 (50.1–81.4)	1.82 (1.22–2.71)	0.37 (0.21–0.68)	4.86 (1.87–12.61)	
3	61.7 (47.4–74.2)	64.9 (48.8–78.2)	69 (54–80.9)	57.1 (42.2–70.9)	1.76 (1.07–2.87)	0.59 (0.39–0.90)	2.97 (1.22–7.28)	
4	57.4 (43.3–70.5)	75.7 (59.9–86.6)	75 (58.9–86.2)	58.3 (44.3–71.2)	2.36 (1.27–4.39)	0.56 (0.38–0.82)	4.20 (1.63–10.84)	

AUROC, area under the receiver operating characteristic curve; CI, confidence interval; CRP, C-reactive protein; FC, fecal calprotectin; LR, likelihood ratio; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value.

Fig. 2. Concentrations of fecal calprotectin (FC) (expressed in µg/g of stool) on a logarithmic scale among Crohn's disease patients according to the endoscopic score of severity (Rutgeerts score) after ileocolonic resection. The boxplots show median, upper, and lower quartiles of the data; the whiskers indicate the 95% confidence interval of the values.

Fig. 3. Comparison of receiver operating characteristic (ROC) curves for fecal calprotectin (FC), C-reactive protein (CRP) and Harvey–Bradshaw index (HBI) as predictors of endoscopic recurrence, and area under curve (AUC) of each.

determined 107.5 µg/g as the optimal cut-off point for ileal recurrence of CD whereas Herranz-Bachiller *et al.* [13] found that 60 µg/g was the point with the highest discriminative ability. A meta-analysis of 10 prospective trials aimed at assessing the value of using FC as a surrogate marker of postoperative recurrence of CD and found pooled sensitivity and specificity of 0.82 (95% CI: 0.73–0.89) and 0.61 (95% CI: 0.51–0.71), respectively, with a diagnostic OR of 7.19. These pooled results are very close to our own (0.86, 0.46 and 5.1, respectively) and support evidence as to the limitations of FC as a surrogate marker of endoscopic recurrence in postoperative CD.

Overall, and contrary to some previous research [37], our results show that FC alone was not superior to serum

Table 4. Comparison of receiver operating characteristic curves for fecal calprotectin, C-reactive protein and Harvey–Bradshaw index as predictors of endoscopic recurrence, according to type of therapy in a prospectively recruited series of patients with Crohn's disease who had undergone ileocecal resection

	Maintenance therapy for Crohn's disease			
	Immunomodulators	Antitumor necrosis factor α inhibitors	Combination therapy	*P* value
Fecal calprotectin	0.600 (0.410–0.790)	0.644 (0.330–0.958)	0.805 (0.601–1)	0.147
C-reactive protein	0.739 (0.569–0.909)	0.583 (0.248–0.919)	0.691 (0.456–0.926)	0.461
Harvey–Bradshaw index	0.640 (0.457–0.823)	0.789 (0.507–1)	0.755 (0.536–0.974)	0.249

CRP or HBI in predicting postoperative endoscopic recurrence of CD. Differences in inclusion criteria (by recruiting exclusively asymptomatic patients [37,38] or also those with a clinically active disease [13,14] as in our case) might contribute to these differences. However, combinations of these variables markedly improved the diagnostic performance: increased values of CRP together with an HBI over two points provided a high PPV of 95.7, a positive LH+ of 16.85 and a diagnostic OR of 30.8. Notably, adding FC values to the combination did not increase the likelihood of diagnosing postoperative CD recurrence.

Our study has strength in that it included a relatively large number of patients with CD, prospectively recruited in several IBD units and who were assessed for postoperative recurrence by the RS (the standard score routinely used in clinical practice and most research). FC concentration was determined using the same method in each clinical laboratory facility in all recruiting centers, thus avoiding the variances reported with different tests [36]. FC levels, as evaluated by the Quantum Blue rapid test, have been shown to correlate well with the standard ELISA test [41]. As for CRP, biomarkers were determined very close to endoscopic evaluation. Owing to the inclusion criteria, and in agreement with previous research [38,40], recruited patients presented with variable degrees of clinical activity, thus improving the representativeness of our sample as the clinical spectrum of real patients with CD who attend IBD clinics.

However, we should also acknowledge some limitations of our research. First, disease recurrence and activity was exclusively assessed by ileocolonoscopy, even when a proportion of patients in our cohort (8.1%) had CD involving upper gastrointestinal tract (L4) which could not be assessed by performing ileocolonoscopies. Second, the cross-sectional inclusion of patients with differing timescales since their last surgery and having a variety of therapies precluded the identification of a potential clinical profile for an optimal FC performance. Third, clinical remission of CD was defined exclusively based on the HBI, without its utility having been validated in the postoperative setting [42]. Fourth, potential differences in outcomes according to the surgical technique after ileocolic resection (i.e. side-to-side or end-to-end anastomosis) were not evaluated. Lastly, the gastroenterologist undertaking the evaluation of the gold-standard RS was not blinded to the treatment and clinical course of recruited patients, thus risking potential bias in the postoperative assessment.

Conclusion

The capacity of FC to predict endoscopic postoperative recurrence in patients with CD who have undergone surgery was not superior to the combination of CRP with HBI. FC exhibited a low negative predictive value of around 70% despite establishing a very low optimal cut-off point of 60 μg/g. As a result, FC does not constitute a substitute for colonoscopy in monitoring operated on patients with CD, as 1 out of three will present endoscopic recurrence despite negative FC values. A plethora of substitute and/or complementary markers to calprotectin are currently being evaluated [43]; it is expected that some of them will have an optimal diagnostic performance to obviate the currently still needed colonoscopy in the evaluation of the postoperative recurrence of CD disease.

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Conflicts of interest

There are no conflicts of interest.

References

1 Peyrin-Biroulet L, Loftus EV, Colombel JF, Sandborn WJ. The natural history of adult Crohn's disease in population-based cohorts. *Am J Gastroenterol* 2010; 105:289–297.
2 Connelly TM, Messaris E. Predictors of recurrence of Crohn's disease after ileocolectomy: a review. *World J Gastroenterol* 2014; 20:14393–14406.
3 Buisson A, Chevaux JB, Allen PB, Bommelaer G, Peyrin-Biroulet L. Review article: the natural history of postoperative Crohn's disease recurrence. *Aliment Pharmacol Ther* 2012; 35:625–633.
4 De Cruz P, Kamm MA, Hamilton AL, Ritchie KJ, Krejany EO, Gorelik A, *et al*. Crohn's disease management after intestinal resection: a randomised trial. *Lancet* 2015; 385:1406–1417.
5 Baert F, Moortgat L, van Assche G, Caenepeel P, Vergauwe P, de Vos M, *et al*. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. *Gastroenterology* 2010; 138:463–468.
6 Colombel JF, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, *et al*. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011; 141:1194–1201.
7 Viscido A, Corrao G, Taddei G, Caprilli R. Crohn's disease activity index is inaccurate to detect the post-operative recurrence in Crohn's disease. A GISC study. Gruppo Italiano per lo Studio del Colon e del Retto. *Ital J Gastroenterol Hepatol* 1999; 31:274–279.
8 Papamichael K, Karatzas P, Mantzaris GJ. Faecal calprotectin but not C-reactive protein (CRP) or Crohn's Disease Activity Index (CDAI) may

predict post-operative endoscopic recurrence of Crohn's disease. *J Crohns Colitis* 2013; 7:e700–e701.
9 Tibble JA, Sigthorsson G, Foster R, Forgacs I, Bjarnason I. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterology* 2002; 123:450–460.
10 Johne B, Fagerhol MK, Lyberg T, Prydz H, Brandtzaeg P, Naess-Andresen CF, *et al*. Functional and clinical aspects of the myelomonocyte protein calprotectin. *Mol Pathol* 1997; 50:113–123.
11 D'Haens G, Ferrante M, Vermeire S, Baert F, Noman M, Moortgat L, *et al*. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. *Inflamm Bowel Dis* 2012; 18:2218–2224.
12 Qiu Y, Mao R, Chen B, He Y, Zeng Z, Xue L, *et al*. Fecal calprotectin for evaluating postoperative recurrence of Crohn's disease: a meta-analysis of prospective studies. *Inflamm Bowel Dis* 2015; 21:315–322.
13 Herranz Bachiller MT, Barrio Andres J, Fernandez Salazar L, Ruiz-Zorrilla R, Sancho Del Val L, Atienza Sanchez R. The utility of faecal calprotectin to predict post-operative recurrence in Crohn's disease. *Scand J Gastroenterol* 2016; 51:720–726.
14 Orlando A, Modesto I, Castiglione F, Scala L, Scimeca D, Rispo A, *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.
15 Hukkinen M, Pakarinen MP, Merras-Salmio L, Koivusalo A, Rintala R, Kolho KL. Fecal calprotectin in the prediction of postoperative recurrence of Crohn's disease in children and adolescents. *J Pediatr Surg* 2016; 51:1467–1472.
16 Laharie D, Mesli S, El Hajbi F, Chabrun E, Chanteloup E, Capdepont M, *et al*. Prediction of Crohn's disease relapse with faecal calprotectin in infliximab responders: a prospective study. *Aliment Pharmacol Ther* 2011; 34:462–469.
17 Ferreiro-Iglesias R, Barreiro-de Acosta M, Otero Santiago M, Lorenzo Gonzalez A, Alonso de la Peña C, Benitez Estevez AJ, *et al*. Fecal calprotectin as predictor of relapse in patients with inflammatory bowel disease under maintenance infliximab therapy. *J Clin Gastroenterol* 2016; 50:147–151.
18 Lucendo AJ, Hervías D, Roncero O, Lorente R, Bouhmidi A, Angueira T, *et al*. Epidemiology and temporal trends (2000–2012) of inflammatory bowel disease in adult patients in a central region of Spain. *Eur J Gastroenterol Hepatol* 2014; 26:1399–1407.
19 Van Assche G, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, *et al*. The second European evidence-based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *J Crohns Colitis* 2010; 4:7–27.
20 Harvey RF, Bradshaw MJ. Measuring Crohn's disease activity. *Lancet* 1980; 1:1134–1135.
21 Vermeire S, Schreiber S, Sandborn WJ, Dubois C, Rutgeerts P. Correlation between the Crohn's disease activity and Harvey–Bradshaw indices in assessing Crohn's disease severity. *Clin Gastroenterol Hepatol* 2010; 8:357–363.
22 Silverberg MS, Satsangi J, Ahmad T, Arnott IDR, Bernstein CN, Brant SR, *et al*. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; 19 (Suppl A):5A–36A.
23 Rutgeerts P, Geboes K, Vantrappen G, Kerremans R, Coenegrachts JL, Coremans G. Natural history of recurrent Crohn's disease at the ileocolonic anastomosis after curative surgery. *Gut* 1984; 25:665–672.
24 Ruopp MD, Perkins NJ, Whitcomb BW, Schisterman EF. Youden Index and optimal cut-point estimated from observations affected by a lower limit of detection. *Biom J* 2008; 50:419–430.
25 Schoepfer AM, Beglinger C, Straumann A, Trummler M, Vavricka SR, Bruegger LE, *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; 105:162–169.
26 Mosli MH, Zou G, Garg SK, Feagan SG, MacDonald JK, Chande N, *et al*. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol* 2015; 110:802–819.
27 Yang Z, Clark N, Park KT. Effectiveness and cost-effectiveness of measuring fecal calprotectin in diagnosis of inflammatory bowel disease in adults and children. *Clin Gastroenterol Hepatol* 2014; 12:253–262.
28 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.
29 Wang S, Wang Z, Shi H, Heng L, Juan W, Yuan B, *et al*. Faecal calprotectin concentrations in gastrointestinal diseases. *J Int Med Res* 2013; 41:1357–1361.
30 Costa F, Mumolo MG, Ceccarelli L, Bellini M, Romano MR, Sterpi C, *et al*. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005; 54:364–368.
31 D'Inca R, Dal Pont E, Di Leo V, Benazzato L, Martinato M, Lamboglia F, *et al*. Can calprotectin predict relapse risk in inflammatory bowel disease? *Am J Gastroenterol* 2008; 103:2007–2014.
32 Walkiewicz D, Werlin SL, Fish D, Scanlon M, Hanaway P, Kugathasan S. Fecal calprotectin is useful in predicting disease relapse in pediatric inflammatory bowel disease. *Inflamm Bowel Dis* 2008; 14:669–673.
33 Gisbert JP, Bermejo F, Perez-Calle JL, Taxonera C, Vera I, McNicholl AG, *et al*. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease. *Inflamm Bowel Dis* 2009; 15:1190–1198.
34 Mao R, Xiao Y, Gao X, Chen B, He Y, Yang L, *et al*. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. *Inflamm Bowel Dis* 2012; 18:1894–1899.
35 Zittan E, Kelly OB, Kirsch R, Milgrom R, Burns J, Nguyen GC, *et al*. Low fecal calprotectin correlates with histological remission and mucosal healing in ulcerative colitis and colonic Crohn's disease. *Inflamm Bowel Dis* 2016; 22:623–630.
36 Jang HW, Kim HS, Park SJ, Hong SP, Kim TI, Kim WH, *et al*. Accuracy of three different fecal calprotectin tests in the diagnosis of inflammatory bowel disease. *Intest Res* 2016; 14:305–313.
37 Boschetti G, Laidet M, Moussata D, Stefanescu C, Roblin X, Phelip G, *et al*. Levels of fecal calprotectin are associated with the severity of postoperative endoscopic recurrence in asymptomatic patients with Crohn's disease. *Am J Gastroenterol* 2015; 110:865–872.
38 Wright EK, Kamm MA, De Cruz P, Hamilton AL, Ritchie KJ, Krejany EO, *et al*. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. *Gastroenterology* 2015; 148:938–947.
39 Lopes S, Andrade P, Afonso J, Rodrigues-Pinto E, Dias CC, Macedo G, *et al*. Correlation between calprotectin and modified Rutgeerts score. *Inflamm Bowel Dis* 2016; 22:2173–2181.
40 Lobaton T, Lopez-Garcia A, Rodriguez-Moranta F, Ruiz A, Rodriguez L, Guardiola J. A new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohn's disease. *J Crohns Colitis* 2013; 7:e641–e651.
41 Coorevits L, Baert FJ, Vanpoucke HJ. Faecal calprotectin: comparative study of the Quantum Blue rapid test and an established ELISA method. *Clin Chem Lab Med* 2013; 51:825–831.
42 Garcia-Planella E, Manosa M, Cabre E, Marin L, Gordillo J, Zabana Y, *et al*. Fecal calprotectin levels are closely correlated with the absence of relevant mucosal lesions in postoperative Crohn's disease. *Inflamm Bowel Dis* 2016; 22:2879–2885.
43 Di Ruscio M, Vernia F, Ciccone A, Frieri G, Latella G. Surrogate fecal biomarkers in inflammatory bowel disease: rivals or complementary tools of fecal calprotectin? *Inflamm Bowel Dis* 2017; 24:78–92.