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A new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohn's disease

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Abstract

Introduction: Fecal calprotectin (FC), as determined by the enzyme-linked immunoassay (ELISA) test, has been proposed as a promising biomarker of endoscopic activity in Crohn's disease (CD). However data on its accuracy in predicting endoscopic remission according to location and postoperative recurrence (POR) is scarce.

Our objective was to evaluate the ability of FC determined by a new quantitative point-of-care test (FC-QPOCT) to predict endoscopic remission and POR in CD patients.

Methods: FC was determined simultaneously by an enzyme-linked immunoassay test (FC-ELISA) and a FC-QPOCT in CD patients undergoing colonoscopy. Clinical disease activity was assessed according to the Crohn's Disease Activity Index (CDAI). Endoscopic results were assessed according to the Crohn's Disease Endoscopic Activity Index of Severity (CDEIS) and postoperative recurrence according to the Rutgeerts' score.

Results: A total of 115 ileocolonoscopies were performed (29 on patients with ileocolonic resection). FC levels correlated more closely with the CDEIS than leucocytes, platelets or CRP. The prediction of "endoscopic remission" (CDEIS < 3), using FC-QPOCT (cut-off 272 $\mu\text{g/g}$) and FC-ELISA (cut-off 274 $\mu\text{g/g}$) presented an AUC of 0.933 and 0.935 respectively. FC-QPOCT results correlated better with endoscopic activity in the ileocolonic location (Pearson's correlation, $r=0.879$; $P<0.001$), than the colonic ($r=0.725$; $P<0.001$) or the ileal location ($r=0.437$; $P=0.016$). Median FC-QPOCT levels discriminated Rutgeerts' score i0–i1 from i2–i4 (98 (range 30–306) $\mu\text{g/g}$ vs. 234.5 (range 100–612) $\mu\text{g/g}$ respectively, $P=0.012$).

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Conclusions: FC determined by rapid quantitative test predicts “endoscopic remission” and endoscopic postoperative recurrence in CD patients.

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1. Introduction

The healing of the mucosa has been associated with an improved disease outcome in Crohn's disease (CD) patients^{1–4} and is increasingly accepted as an endpoint of the treatment. Endoscopic assessment is still the gold standard for evaluation of the mucosa but is an expensive and invasive technique. Moreover, in CD patients, the performance of an endoscopy has the additional limitation that it does not always reach the affected segment of the intestine. A non-invasive assessment of disease activity is therefore relevant in CD and includes clinical assessment, image techniques and biomarkers.

Clinical activity has shown a poor correlation with endoscopic activity.^{5,6} Although image techniques such as computed tomography or magnetic resonance imaging, have a good accuracy in the detection of the active disease, they are expensive and not always available in many centers. Ultrasonography is less expensive and more accessible, but less accurate.^{7–9}

Among the various biomarkers, CRP has demonstrated a good response in CD, better than in UC.^{10,11} However, its accuracy in the prediction of endoscopic activity is modest.¹²

Fecal biomarkers have been proposed as a more accurate tool to evaluate the disease activity in IBD and among them fecal calprotectin (FC) has been the most studied. Calprotectin represents 60% of cytosolic proteins in granulocytes and its presence in feces is therefore proportional to neutrophil migration to the gastrointestinal tract and to the degree of inflammation. FC has shown a better correlation with endoscopic activity in IBD patients than CRP and the other biomarkers.¹³

However, we still have scarce and conflicting data on some aspects such as the FC levels according to the different locations of the disease or the value of FC as a surrogate marker of postoperative recurrence in CD patients with ileal or ileocecal resection.

There are two other relevant issues with reported inconsistent results that affect the use of FC in clinical practice: the optimal FC threshold for the prediction of endoscopic activity,^{14,21–24} and the reliability of the rapid point-of-care (POCT) tests compared to the enzyme-linked immunoassay (ELISA) test in IBD patients.

There is still no agreement on which FC cut-off point to use for predicting endoscopic activity. Different threshold concentrations ranging from 50 $\mu\text{g/g}$,^{14,21} 100 $\mu\text{g/g}$ ²² to 250 $\mu\text{g/g}$ ^{23,24} have been proposed.

The ELISA test is the most widely used test to measure FC. However, this test has some limitations: it is time-consuming (up to 4 h) and requires a laboratory and trained personnel to perform the test, and the collection of multiple samples in order to make the running test more cost effective. In this context, two kinds of POCT tests have been developed: semi-quantitative ones that provide a range of FC levels and a rapid quantitative one (Q-POCT) which is as fast as the

semi-quantitative tests but provides an exact number of FC, like the ELISA test. Previous studies^{25–28} using Q-POCT observed a good correlation with the ELISA test but none of them explored its ability to predict endoscopic activity in CD patients. In a previous study,²⁹ we assessed the accuracy of FC in the prediction of endoscopic activity in UC patients by using a Q-POCT and the ELISA test simultaneously, and the results demonstrated a good correlation between both techniques.

The objective of this study was to evaluate the accuracy of FC determined by a quantitative rapid test for the prediction of endoscopic activity and postoperative recurrence in CD patients. We have also studied the behavior of FC according to disease location.

2. Materials and methods

2.1. Participants

One hundred and fifteen adult patients with previously known CD, referred for colonoscopy to the Department of Gastroenterology of Bellvitge University Hospital (Hospitalet de Llobregat, Barcelona) between April 2011 and June 2012, were prospectively and consecutively included in the study.

The criteria for inclusion were: appropriate diagnosis for CD (with endoscopy, biopsies and/or image) within at least the 3 months previous to the study and an age range between 18 and 85 years. Exclusion criteria included: pregnancy, non-steroidal anti-inflammatory drug intake, concomitant gastrointestinal infection, surgery within the previous three months, colorectal cancer, predominant perianal symptoms and UC or indeterminate colitis.

2.2. Study design

Patients were visited in our IBD unit prior to their colonoscopy in order to invite them to participate in the study. We provided them with a collection kit, performed a clinical interview and carried out blood tests. Patients were told to collect stool samples the day before bowel preparation (preferably from the first stool in the morning) and then keep it in the fridge for 24–48 h before bringing it to the hospital. If diarrhea was present in the sample, we performed a microbiological study of the feces consisting of conventional culture, parasites and *Clostridium difficile* detection, in order to exclude gastrointestinal infection. At our center, the diagnosis of *C. difficile* consists of two techniques: toxin detection in fecal samples using cell cytotoxicity neutralization assay and anaerobic culture followed by toxin detection (i.e. toxigenic culture). Samples for microbiological analysis were processed in the Microbiology Department and fecal samples for FC were collected in the Endoscopy Department and stored at $-20\text{ }^{\circ}\text{C}$ until analysis.

2.3. Clinical activity

Clinical activity was assessed according to the clinical criteria of the Crohn's Disease Activity Index (CDAI). Symptomatic remission was defined as a CDAI level greater than 150.

2.4. Serological biomarkers

Blood tests included leukocytes (lower range 3.9 g/L), platelets (lower range 150.0 g/L), erythrocyte sedimentation rate (ESR) and CRP (upper limit of normal < 5 mg/l).

2.5. Endoscopic disease activity

One hundred and fifteen colonoscopies were performed, of which 89 were complete ileocolonoscopies. Two trained endoscopists (FRM, JGC) from our unit, who were blind to the other variables of the study, carried out the colonoscopies. Endoscopic activity was assessed according to CDEIS.^{30–32} "Endoscopic remission" was defined as CDEIS < 3; "mild endoscopic activity" was defined as CDEIS 3–6; "moderate endoscopic activity" as CDEIS 7–15 and "severe endoscopic activity" as CDEIS > 15. "Endoscopic activity" was globally defined as CDEIS ≥ 3 and "any grade of endoscopic activity" as CDEIS > 0. We also assessed endoscopic activity according to the "presence or absence" of ulcers. Endoscopic disease location was classified according to endoscopic findings in the ileal, colonic or ileocolonic locations. When no signs of endoscopic activity were found, the location was classified according to previously known disease location (Montreal classification). Postoperative disease activity of the neoterminal ileum was scored according to the Rutgeerts' score. Postoperative recurrence was defined as Rutgeerts' score ≥ 2.³³ All the colonoscopies were included for the analysis involving the presence or absence of ulcers (n = 115). In this context, uncompleted colonoscopies were included only when an ulcer was found (in this situation the fact of completing the colonoscopy doesn't change the classification of the patient in the group known as "presence of ulcers"). For the rest of the analysis involving the disease location or the calculation of the CDEIS and Rutgeerts' score, only the completed ones were considered (n = 89).

2.6. Fecal calprotectin determination

Stool sample collection was performed 1 to 3 days before the endoscopy and bowel preparation. All samples were stored at –20 °C, thawed and analyzed by an enzyme-linked immunoassay test (Calprotectin Bühlmann ELISA, Bühlmann, Schönenbuch, Switzerland) and by a quantitative point-of-care test (Quantum Blue, Bühlmann, Schönenbuch, Switzerland). The quantitative point-of-care test (FC-QPOCT) relies on lateral flow assay technology including an easy to use reader system, which gives a quantitative read out. There are two kinds of FC-QPOCT: the lower range Quantum Blue (LF-CAL) and the high range Quantum Blue (LF-CHR). Both provide quantitative results within minutes, ranging from 30–300 µg/g or 100–1800 µg/g FC respectively. The range of the enzyme-linked immunoassay test (FC-ELISA) is 10–1800 µg/g. The median time analysis required for FC-ELISA is 2 h for 88 samples and 12–15 min for each sample with FC-QPOCT depending on the kit (12 min for

LF-CAL and 15 min for LF-CHR). The sensitivity of both tests is < 10 µg/g. After the necessary training, two gastroenterologists and the IBD nurse (TLO, ALG) took the FC measurements. They were blind to endoscopic activity and the other variables. We started the study using the LFCAL kit and changed to the LF-CHR kit when it was available. All samples that scored > 300 µg/g with LF-CAL were tested again with the LF-CHR kit in order to obtain the exact value.

2.7. Statistical analysis

The statistical analysis was carried out using the SPSS version 19.0 statistical package (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed with median and interquartile range (IQR) or range, except when specified. Mann Whitney-U test was used to compare medians. Qualitative results were based on the Chi-square test. The correlation analysis between FC, endoscopic activity and other biomarkers was based on Pearson's correlation (r). Multivariate analysis consisted of multiple logistic regressions. Accuracy analysis included receiver operator characteristic (ROC) curve analyses with 95% confidence intervals, as well as test characteristics such as sensitivity, specificity, positive and negative predictive values (SENS, SPEC, PPV, and NPV), and overall accuracy. Statistical significance was accepted for $P < 0.05$. Correlation between FC-ELISA and FC-QPOCT was analyzed by Pearson's correlation and the interclass correlation index (ICI).

2.8. Ethical considerations

The ethics committee of Bellvitge University Hospital approved this study and all patients gave their informed written consent for participation.

3. Results

A total of 115 Crohn's disease patients were included between April 2011 and June 2012. Epidemiological, clinical, biological and endoscopic characteristics of patients are shown in Table 1.

3.1. Correlation between FC-QPOCT and FC-ELISA

Pearson's correlation between FC-QPOCT and FC-ELISA was 0.879 ($P < 0.001$). The interclass correlation index (ICI) was 0.878 (CI 95%: 0.827–0.914; $P < 0.001$). Results are shown in Fig. 1.

3.2. Relation of FC levels and the different grades of endoscopic activity

FC levels determined by QPOCT and ELISA presented a correlation with endoscopic activity of 0.769 and 0.722 respectively (Pearson's correlation; $P < 0.001$ in both cases).

FC-QPOCT discriminated between "endoscopic activity" defined as CDEIS ≥ 3 and "endoscopic remission" defined as CDEIS < 3 (median levels of 788.5 µg/g (range 115–1800) vs. 100 µg/g (range 30–1647) respectively; $P < 0.001$). FC-ELISA showed similar results (median levels of 101.8 µg/g (range 30–1620.9) and 1211.9 µg/g (range 122.4–1800) respectively; $P < 0.001$).

Table 1 Patient characteristics.

Patient characteristics	Number	Percent
Number of patients	115	–
Men	50	44
Age (years): median (IQR)	40 (32–58)	–
Disease duration (years): median (IQR)	8 (5–21)	–
Montreal classification		
Age (years) at diagnosis: A1 (≤ 16)/A2 (17–40)/A3 (>40)	3/102/8	3/90/7
Locations: L1/L2/L3 ^b	26/45/42	23/40/37
Behaviors: B1/B2/B3 ^b	85/12/16	75/11/14
Not known ^a	2	2
Medication at endoscopy ^c		
Oral/topical 5-ASA	32/1	29/1
CC (beclometasone/budesonide/systemic)	1/8/2	1/7/2
IMM (AZA/6MP/MTX)	46/6/8	39/5/7
TNF inhibitor (IFX/ADA)	7/11	6/10
Leucocytes (g/L): median (IQR)	6200 (4900–7925)	–
Platelets (g/L): median (IQR)	271 (205–320)	–
CRP (mg/dL): median (IQR)	2.5 (1–8.2)	–
ESR (mm): median (IQR)	7.5 (3–18.5)	–
Crohn's Disease Activity Index (CDAI)	50.8 (0–120)	–
Asymptomatic patients (CDAI < 150)	23	20
FC-ELISA ($\mu\text{g/g}$): median (range)	301.9 (30–1800)	–
FC-QPOCT ($\mu\text{g/g}$): median (range)	294.5 (30–1800)	–
CDEIS ^d : median \pm interquartile range	2 (0–32.5)	–
Reason for colonoscopy ^d		
Symptoms	28	24
Check mucosal healing	3	0
Dysplasia surveillance	29	25
Postoperative recurrence	50	44
To know extension	5	4

IQR, interquartile range; ASA, aminosalicylic acid; CC, corticosteroids; IMM, immunomodulators; AZA azathioprine; 6MP, 6 mercaptopurine; MTX, methotrexate; TNF, tumor necrosis factor; IFX, infliximab; ADA, adalimumab; FC-QPOCT, FC determined by the quantitative-point-of-care test; FC-ELISA, FC determined by ELISA; CDEIS, Crohn's Disease Endoscopic Index of Severity.

^a No previous reports regarding the extension of the disease and not completed colonoscopy in the study.

^b Behaviors: B1, non-stricturing, non-penetrating; B2, stricturing; and B3, penetrating. Locations: L1, ileal; L2, colonic; and L3, ileocolonic.

^c As therapy regimens overlapped, the total is 106.3%.

^d 115 colonoscopies and 89 complete ones. Please see "Material and methods" for further clarifications.

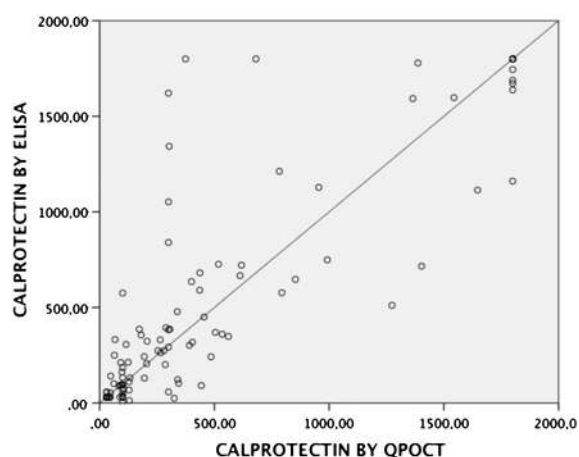


Figure 1 Correlation between FC-QPOCT and FC-ELISA. Scatterplot demonstrating the correlation between FC-ELISA and FC-QPOCT. Pearson's correlation coefficient $r = 0.879$, $P < 0.001$.

FC-QPOCT was able to discriminate between patients with and without ulcers (median levels of 128 $\mu\text{g/g}$ (range 30–1800) vs. 618 $\mu\text{g/g}$ (range 100–1800) and; $P < 0.001$). Similar results were observed with FC-ELISA (data not shown).

FC-QPOCT was also able to discriminate between the different grades of endoscopic activity. Median levels of FC-ELISA and FC-QPOCT for each endoscopic CDEIS grade are shown in Fig. 2.

Similar results were observed with FC-ELISA for CDEIS.

3.3. Accuracy of FC for the prediction of CDEIS < 3

The prediction of "endoscopic remission" defined as CDEIS < 3 with FC-QPOCT and FC-ELISA presented an area under the curve (AUC) of 0.933 and 0.935 respectively (Fig. 3). A 274 $\mu\text{g/g}$ cut-off value of FC-ELISA gave a sensitivity of 77%, a specificity of 97%, a NPV of 75%, and a PPV of 98% (global accuracy 85%). A 272 $\mu\text{g/g}$ cut-off value FC-QPOCT gave a sensitivity of 79%, a specificity of 97%, a NPV of 76%, and a PPV of 98% (global accuracy 86%).

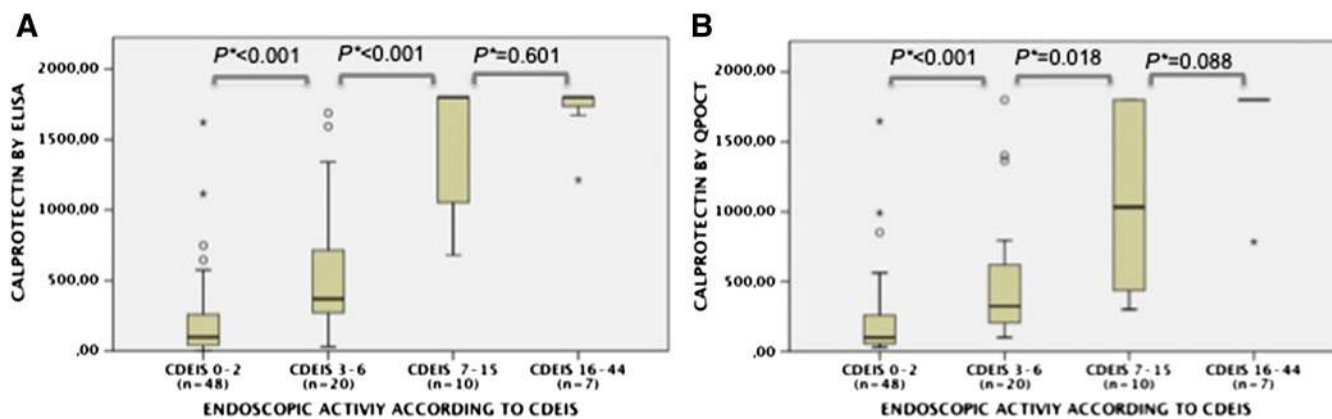


Figure 2 Median FC levels according to CDEIS. Fecal calprotectin concentrations in different groups of endoscopic activity are illustrated by boxplots. The box represents the lower and upper quartiles and the horizontal line in the middle of the box is the median. The 95% confidence interval is represented by whiskers and individual outliers are represented by values outside the whiskers. **A. FC-ELISA.** Median FC levels were 101.8 $\mu\text{g/g}$ for inactive disease (CDEIS 0–2); 371.65 $\mu\text{g/g}$ for mild disease (CDEIS 3–6); 1800 $\mu\text{g/g}$ for moderate disease (CDEIS 7–15) and 1800 $\mu\text{g/g}$ for severe disease (CDEIS 16–44) respectively (*U Mann Whitney). **B. FC-QPOCT.** Median FC levels were 100 $\mu\text{g/g}$ for inactive disease (CDEIS 0–2); 323 $\mu\text{g/g}$ for mild disease (CDEIS 3–6); 1034 $\mu\text{g/g}$ for moderate disease (CDEIS 7–15) and 1800 $\mu\text{g/g}$ for severe disease (CDEIS 16–44) respectively (*U Mann Whitney).

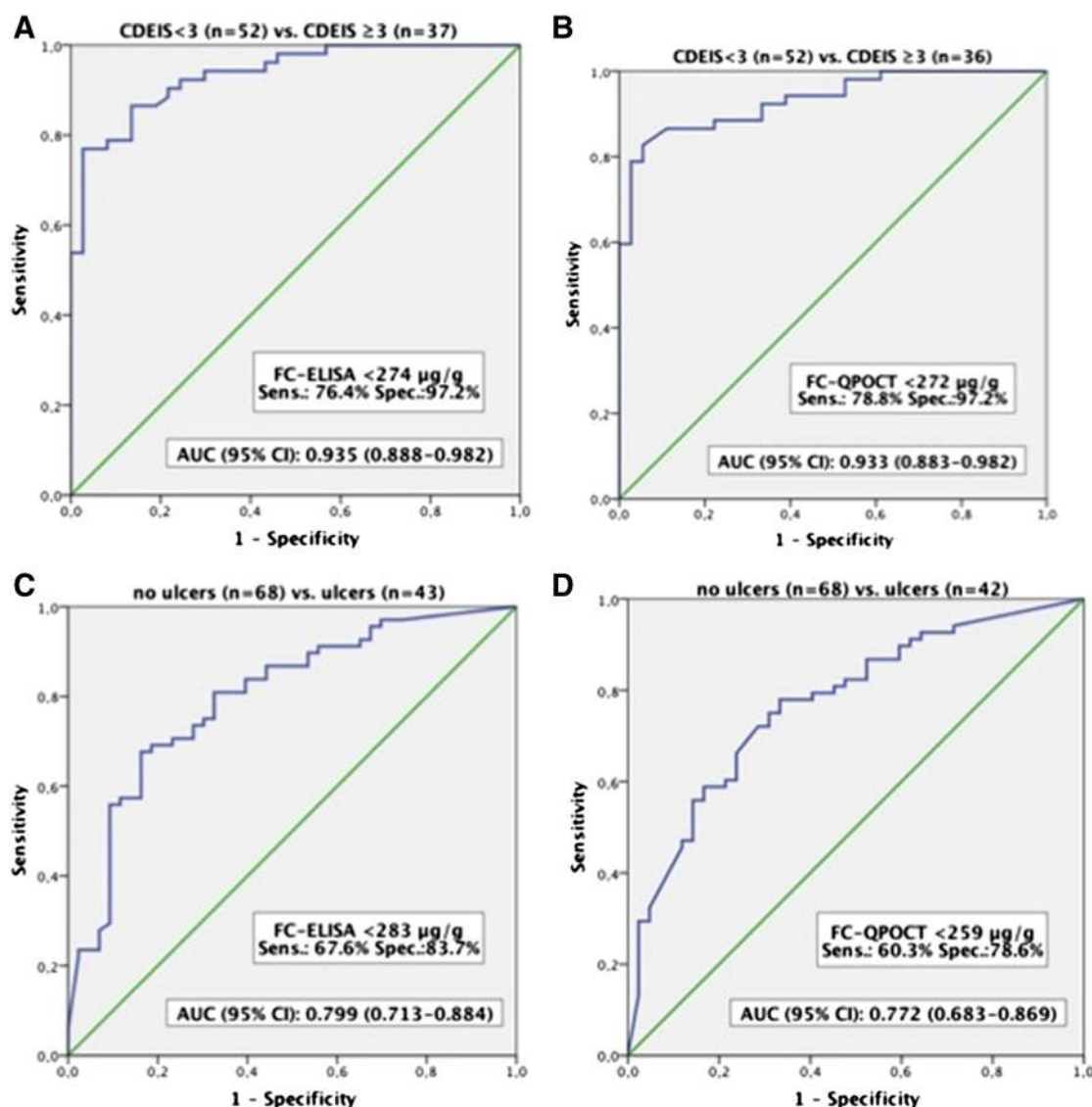


Figure 3 ROC curves for FC-ELISA: CDEIS 0–2 versus CDEIS 3–44 (A), absence vs presence of ulcers (C), ROC curves for FC-QPOCT: CDEIS 0–2 versus CDEIS 3–44 (B), and absence vs presence of ulcers (D). Clarifications on different sample sizes for each subgroup in the “Materials and methods” section.

Global accuracy in the prediction of “endoscopic remission” was better for FC with any of the techniques when compared to the global accuracy of CRP and clinical activity with CDAI. Accuracy in predicting “endoscopic remission” did not improve when combining FC with clinical activity or CRP (data not shown). Results are shown in Table 2.

3.4. Accuracy of FC for the prediction of CDEIS = 0

The prediction of “complete endoscopic remission” defined as CDEIS = 0 with FC-QPOCT and FC-ELISA presented an area under the curve (AUC) of 0.831 and 0.801 respectively. A 200 µg/g cut-off value of FC-QPOCT had a sensitivity of 75% and a specificity of 77%. A 261.8 µg/g cut-off value of FC-ELISA had a sensitivity of 75% and a specificity of 76%.

3.5. Accuracy of FC for the prediction of “absence of ulcers”

All patients with ulcers (n = 68) had a FC level of >250 µg/g with both techniques. And 13 out of 42 patients with no ulcers had a FC level of >250 µg/g. The prediction of “absence of ulcers” with FC-QPOCT and FC-ELISA presented an area under the curve (AUC) of 0.772 and 0.799 respectively (Fig. 3).

3.6. Relation between FC levels and location of endoscopic activity

When considering FC-QPOCT levels according to the location of endoscopic activity (ileum, colon or ileocolonic activity),

Table 2 Sensitivity (SENS), specificity (SPEC), positive predictive value (PPV), negative predictive value (NPV) and accuracy of fecal calprotectin, clinical activity and CRP in predicting "endoscopic remission" (CDEIS < 3).

	SENS (%)	SPEC (%)	PPV (%)	NPV (%)	Accuracy (%)	P ^a
FC-QPOCT < 272 µg/g	78.8	97.2	97.6	76.1	86.36	<0.001
FC-ELISA < 274 µg/g	76.4	97.2	97.6	74.5	85.23	<0.001
CRP < 5 mg/L	80.9	55.9	71.7	67.9	70.37	0.001
CDAI ^b < 150	83.3	27.8	60.6	42.9	59.52	0.285

CRP, C-reactive protein; FC-QPOCT, FC determined by the quantitative point of care test; FC-ELISA, FC determined by ELISA; CDAI: Crohn's Disease Activity Index.

^a P: Chi-square test.

the correlation between FC levels and CDEIS was better in the ileocolonic location (Pearson's correlation, $r = 0.879$; $P < 0.001$), followed by the colonic ($r = 0.725$; $P < 0.001$) and then the ileal location ($r = 0.437$; $P = 0.016$). Accuracy of FC-QPOCT in predicting "endoscopic remission" defined as CDEIS < 3 was also better in patients with endoscopic activity in the ileocolonic location (AUC 0.974), followed by the colon location (AUC 0.952) and the ileal location (AUC 0.940).

Out of the patients with "endoscopic activity" (CDEIS ≥ 3), median FC-QPOCT levels were higher in the ileocolonic location (1800 µg/g, range 300–1800), followed by the colonic location (1297 µg/g, range 518–1800) and the ileal location (420.5 µg/g, range 115–1800), and these differences were statistically significant ($P = 0.013$). However, in patients with "endoscopic remission", no significant differences were found between median FC-QPOCT levels in the ileocolonic location (100 µg/g, range 30–853), colonic location (100 µg/g, range 30–1647) and ileal location (100 µg/g, range 30–344, $P = 0.99$).

Median levels of FC-QPOCT for each endoscopic CDEIS grade according to disease location are shown in Fig. 4.

Cut-off values for predicting "endoscopic remission" were 293 µg/g for colonic disease (sensitivity of 79% and specificity of 100%) and 107.5 µg/g for ileal disease (sensitivity of 63%

and specificity of 100%). Similar results were observed with FC-ELISA (data not shown).

3.7. Relation of FC with endoscopic activity, clinical activity and serological markers

The CDEIS correlated more closely with FC-QPOCT (Pearson's correlation $r = 0.722$; $P < 0.001$) followed by CRP ($r = 0.362$; $P < 0.001$), leucocytes ($r = 0.327$; $P = 0.003$) and clinical activity (0.169; $P = 0.129$). The correlations between the CDEIS, FC (FC-ELISA and FC-QPOCT), clinical activity and serological variables are shown in Table 3.

In the multivariate analysis, after adjusting for clinical activity, CRP and FC-QPOCT only FC-QPOCT > 272 µg/g proved to be an independent predictor of endoscopic activity (OR 142.98, 12.187–16677.465 95% CI; $P < 0.001$). Results shown in Table 4.

3.8. Analysis of patients in clinical remission

In this study we found a poor correlation between clinical activity and endoscopic activity using the CDEIS (Table 3).

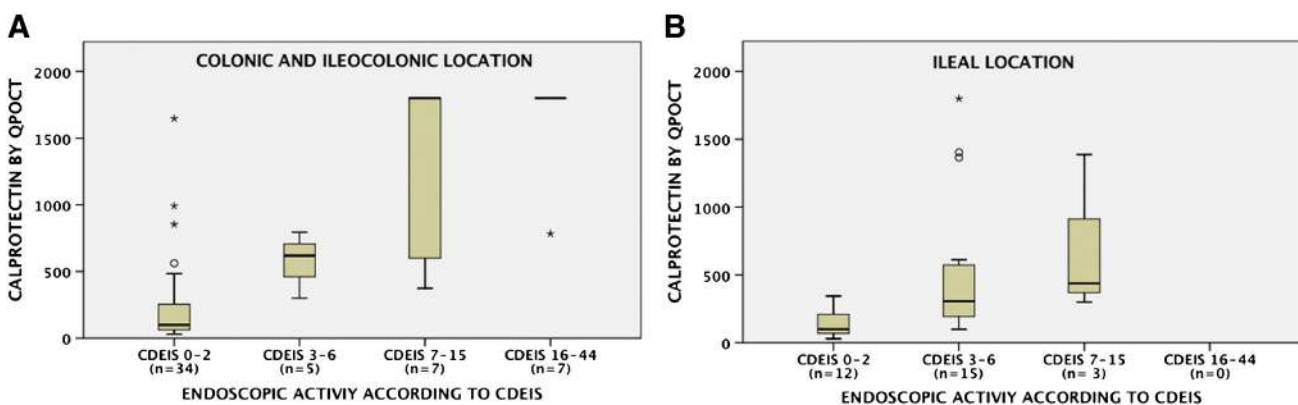


Figure 4 Median FC-QPOCT levels according to CDEIS and disease location. Fecal calprotectin concentrations in different groups of endoscopic activity are illustrated by boxplots. The box represents the lower and upper quartiles and the horizontal line in the middle of the box is the median. The 95% confidence interval is represented by whiskers and individual outliers are represented by values outside the whiskers. *No statistical analysis was performed because of the small size of final subgroups. **A. Colonic and ileocolonic location.** Median FC levels were 100 µg/g for inactive disease (CDEIS 0–2); 618 µg/g for mild disease (CDEIS 3–6); 1800 µg/g for moderate disease (CDEIS 7–15) and 1800 µg/g for severe disease (CDEIS 16–44) respectively. **B. Ileal location.** Median FC levels were 100 µg/g for inactive disease (CDEIS 0–2); 306 µg/g for mild disease (CDEIS 3–6) and 437 µg/g for moderate disease (CDEIS 7–15). There were no patients with severe disease (CDEIS 16–44) and ileal location.

Table 3 Pearson's correlation between FC-ELISA, FC-QPOCT, clinical activity, serum biomarkers and endoscopic activity.

	Leucocytes	FC-ELISA	FC-QPOCT	Platelets	Clinical activity ^a	CDEIS ^b
CRP	0.186 (<i>P</i> = 0.103)	0.439 (<i>P</i> < 0.001)	0.0358 (<i>P</i> < 0.001)	0.009 (<i>P</i> = 0.942)	0.076 (<i>P</i> = 0.512)	0.362 (<i>P</i> < 0.001)
Leucocytes		0.228 (<i>P</i> = 0.043)	0.250 (<i>P</i> = 0.023)	0.348 (<i>P</i> = 0.002)	0.232 (<i>P</i> = 0.41)	0.327 (<i>P</i> = 0.003)
FC-ELISA			0.867 (<i>P</i> < 0.001)	0.156 (<i>P</i> = 0.171)	0.134 (<i>P</i> = 0.235)	0.784 (<i>P</i> < 0.001)
FC-QPOCT				0.089 (<i>P</i> = 0.434)	0.072 (<i>P</i> = 0.525)	0.722 (<i>P</i> < 0.001)
Platelets					0.233 (<i>P</i> = 0.040)	0.024 (<i>P</i> = 0.835)
Clinical activity ^a						0.169 (<i>P</i> = 0.129)

CRP, C-reactive protein; FC-ELISA, FC determined by the ELISA test; FC-QPOCT, FC determined by the quantitative point-of-care test.

^a Clinical activity according to CDAI.

^b Crohn's Disease Endoscopic Index of Severity.

When analyzing the patients in clinical remission (*n* = 65) defined as CDAI < 150, we observed that 25 of them (39%) had "endoscopic activity" (defined by CDEIS ≥ 3) and all of them had a FC-QPOCT level > 272 µg/g and would therefore have been correctly classified by performing a FC test. In contrast, 9 out of 40 patients (23%) presenting "endoscopic remission" also had a FC-QPOCT level > 272 µg/g. Similar results were observed with FC-ELISA. Among the 25 patients in clinical remission and with "endoscopic activity", only 14 (56%) had a CRP > 5 mg/dL. In contrast, only 7 out of 37 patients (19%) with "endoscopic remission" had a CRP > 5 mg/dL.

3.9. Analysis of patients with normal CRP

When analyzing patients with normal CRP (*n* = 53) we observed that 15 of them (28%) had "endoscopic activity" and all had a FC-QPOCT > 272 µg/g and would therefore have been correctly classified by FC.

3.10. Patients with ileocecal resection

We included 29 patients with ileocecal resection who underwent a complete ileocolonoscopy. Median FC-QPOCT levels were able to discriminate between patients with no POR defined as Rutgeerts' score i0–i1 and patients with POR defined as a Rutgeerts' score i2–i4: 98 µg/g (range 30–306) vs. 234.5 µg/g (range 100–612) respectively, (*P* = 0.012). When considering median FC levels for each grade of Rutgeerts' score, we observed no significant differences between the different degrees. Median FC levels were 100, 43, 234.5 and 363.5 for Rutgeerts' score i0, i1, i2 and i3 respectively. The accuracy of FC-QPOCT in predicting POR defined as Rutgeerts' score i2–i4 presented an AUC of 71.53. A 283 µg/g FC-QPOCT cut-off value had a sensitivity of 67% and a specificity of 72%. With FC-ELISA, the AUC was 70.14 and a 203.34 cut-off value had a sensitivity of 75% and a specificity of 72%.

Neither clinical activity nor the other biomarkers had a significant correlation with POR, and none of them were able to discriminate between Rutgeerts' score i0–i1 and Rutgeerts' score i2–i4 (data not shown).

4. Discussion

This study demonstrated the accuracy of FC in predicting "endoscopic remission" through a rapid quantitative test, and its ability to discriminate between different degrees of endoscopic activity. We found a higher correlation between FC levels and endoscopic activity for colonic than for ileal disease. We also observed that FC was able to predict endoscopic postoperative recurrence in patients with an ileocecal resection. The study demonstrated a good correlation between the ELISA test and a rapid quantitative test.

CD patients presenting mucosal healing have a better prognosis, with a reduced number of surgeries, relapses and complications.^{1–4} Therefore, it would be advisable to monitor the disease according to endoscopic activity. To avoid the performance of repeated endoscopies, which is an invasive and expensive tool, there is an increased interest in surrogate and objective markers of endoscopic activity. Among them, fecal markers, and FC in particular, have been proposed as one of the most promising ones.

Multiple studies have assessed the accuracy of FC in predicting endoscopic activity. However, we still have conflicting and inconsistent data regarding a number of aspects, such as FC cut-off values, FC levels according to disease location and FC utility as a predictor of endoscopic postoperative recurrence. In addition, none of the previous studies have been carried out together with a rapid quantitative test.

Previous studies observed a good correlation between FC and endoscopic activity,^{6,14–20,34–37} but only some of the studies^{6,14,15,37} analyzed the capacity of FC to differentiate between different degrees of endoscopic activity. We observed that FC, measured both with the ELISA and the rapid quantitative test, was able to discriminate between the different levels of endoscopic activity, as well as to detect the presence or absence of ulcers.

Different cut-off values (varying from 70 to 250 µg/g) for predicting "endoscopic activity" or "endoscopic remission" have been described in the literature.^{14,15,24} Cut-off values vary depending on the FC determination technique, endoscopic score and the desired sensitivity and specificity according to the definition of endoscopic activity or MH. Schoepfer et al.¹⁴ observed an accuracy of 87% for FC in

Table 4 Multivariate analysis (logistic regression) for the prediction of "endoscopic remission" (CDEIS < 3).

Variable	Coefficient (β)	Standard error	Exp (B)	95% CI		P
FC-QPOCT < 272 $\mu\text{g/g}$	4.963	1.256	142.98	12.187	-16677.465	<0.001
CRP < 5 mg/dL	0.549	0.722	1.732	0.421	-7.132	0.447
Symptomatic remission ^a	1.713	1.159	5.544	0.572	-53.765	0.140

CRP, C-reactive protein; FC-QPOCT, FC determined by the quantitative-point-of-care test.

^a Symptomatic remission: CDAI < 150.

predicting active endoscopic disease defined as SES > 3 with a 70 $\mu\text{g/g}$ cut-off value. Sipponen et al.¹⁵ illustrated that, with a 200 $\mu\text{g/g}$ cut-off value, FC predicted CDEIS \geq 3 with a sensitivity of 70% and a specificity of 92%. D'Haens et al.,²⁴ in a study involving 87 CD patients, found a cut-off value of 250 $\mu\text{g/g}$ to be the most accurate for predicting the presence of large ulcers in CD (with a specificity of 100% and a sensitivity of 71%). In our study, a 272 $\mu\text{g/g}$ cut-off value FC-QPOCT had a sensitivity of 79%, a specificity of 97% and a global accuracy of 86%. Differences in the studied population and the FC determination technique may account for the divergence in these results. We used a technique based on monoclonal antibodies, which is more accurate than the previously used polyclonal techniques, both for ELISA and the rapid test.

As was also shown in previous studies,^{5,6} we observed a weak correlation between clinical activity and endoscopic activity. In our study, among the patients in clinical remission, 38% had endoscopic activity and all of them had FC-QPOCT > 272 $\mu\text{g/g}$, and would therefore have been correctly classified by using a FC rapid test. Similarly, among patients with a normal CRP (<5 mg/dL), 28% had endoscopic activity and all of them had a FC-QPOCT > 272 $\mu\text{g/g}$. Neither clinical activity, nor the usual biomarkers (CRP, leucocytes and platelets) nor a combination of these markers, achieved greater accuracy in predicting "endoscopic remission".

There is conflicting data in the literature on the levels of FC levels in the different locations of the disease. Some studies found no relation between FC levels and disease location.¹⁶⁻²⁰ In contrast, Schoepfer et al.,¹⁴ found a higher correlation between SES-CD and FC levels in ileocolonic than in colonic or ileal disease. We also found a greater correlation between endoscopic activity and FC in patients with ileocolonic disease, followed by those patients with colonic disease and ileal disease. In line with Sipponen et al.,¹⁵ we also observed significantly higher FC levels in the colon than in the ileum in patients with "endoscopic activity" (CDEIS > 2), but no differences in patients in "endoscopic remission" (CDEIS < 3). In our study, cut-off values for predicting "endoscopic remission" were higher in the colonic than in the ileal location. This highlights the importance of disease location when interpreting the results of FC levels in CD patients and of FC as a better marker of colonic than of ileal inflammation. Further studies need to be carried out in order to confirm whether it is necessary to consider different cut-off levels according to the location of the disease.

There is a scarcity of data on the relationship between different FC levels and the risk of postoperative recurrence. Lamb et al.³⁸ observed different FC levels in patients with and without endoscopic lesions, but there is no definition given for postoperative recurrence (POR). Conversely,

Scarpa et al.³⁹ showed no difference in FC levels between patients with and without endoscopic recurrence. However, in their study, they did not use Rutgeerts' score to define POR. A further limitation of this study is that the collection of samples was not carried out at the time of the colonoscopy. In our group of patients with ileocecal resection, we observed that FC was able to predict POR defined as Rutgeerts' score \geq 2-4 with both techniques. However, accuracy was lower than in non-resected patients, which limits its use in clinical practice.

As shown in previous studies,²⁵⁻²⁹ we observed a good relationship between the ELISA test and the FC-QPOCT.

Our study has several limitations. Firstly, we only assessed disease activity by ileocolonoscopy. In patients with Crohn's disease activity may exist in the upper gastrointestinal tract or in other segments of the small ileum which cannot be reached by performing an endoscopy. Secondly, the group of patients with ileocecal resection was small and therefore we may need to validate the cut-off value with a bigger sample. Lastly, this is a cross-sectional study and there is a lack of follow-up on the FC levels in our CD patients. FC would be most useful for example in evaluating response to treatment in a longitudinal study.

In conclusion, we observed that FC is a more accurate and better surrogate marker of endoscopic activity and postoperative recurrence than clinical activity and the other biomarkers. FC accuracy and therefore its cut-off levels vary significantly according to disease location. This accuracy was observed both with the ELISA and the rapid quantitative test, which allows us to utilize FC more easily in our clinical practice.

Specific author contributions

Study design, data collection, calprotectin measurements, statistical analysis, interpretation and manuscript writing: Triana Lobatón; data collection and calprotectin measurements: Alicia López-García; study design, colonoscopies, statistical analysis, interpretation and manuscript writing: Francisco Rodríguez-Moranta; data collection: Alexandra Ruiz; data collection: Lorena Rodríguez; study design, colonoscopies, statistical analysis and manuscript writing: Jordi Guardiola Capon.

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Conflict of interest

None.

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