A New Rapid Quantitative Test for Fecal Calprotectin Predicts Endoscopic Activity in Ulcerative Colitis

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Background: Fecal calprotectin (FC) determined by the enzyme-linked immunosorbent assay (ELISA) test has been proposed as a promising biomarker of endoscopic activity in ulcerative colitis (UC). However, data on its accuracy in predicting endoscopic activity is scarce. Besides, FC determined by the quantitative-point-of-care test (FC-QPOCT) that provides rapid and individual results could optimize its use in clinical practice. The aims of our study were to evaluate the ability of FC to predict endoscopic activity according to the Mayo score in patients with UC when determined by FC-QPOCT and to compare it with the ELISA test (FC-ELISA).

Methods: FC was determined simultaneously by FC-ELISA and FC-QPOCT in patients with UC undergoing colonoscopy. Clinical disease activity and endoscopy were assessed according to the Mayo score. Blood tests were taken to analyze serological biomarkers.

Results: A total of 146 colonoscopies were performed on 123 patients with UC. FC-QPOCT correlated more closely with the Mayo endoscopic subscore (Spearman's correlation coefficient rank $r = 0.727$, $P < 0.001$) than clinical activity ($r = 0.636$, $P < 0.001$), platelets ($r = 0.381$, $P < 0.001$), leucocytes ($r = 0.300$, $P < 0.001$), and C-reactive protein ($r = 0.291$, $P = 0.002$). The prediction of "endoscopic remission" (Mayo endoscopic subscore ≤ 1) with FC-QPOCT (280 μg/g) and FC-ELISA (250 μg/g) presented an area under the curve of 0.906 and 0.924, respectively. The interclass correlation index between both tests was 0.904 (95% confidence interval, 0.864–0.932; $P < 0.001$).

Conclusions: FC determined by QPOCT was an accurate surrogate marker of "endoscopic remission" in UC and presented a good correlation with the FC-ELISA test.

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Key Words: fecal calprotectin, mucosal healing, endoscopic activity, ulcerative colitis

The management of inflammatory bowel disease (IBD) is today increasingly based on the objective assessment of mucosal integrity and tissue inflammation.[1] Classically, clinical activity has guided the monitoring of patients with IBD. However, symptoms may underestimate endoscopic activity in up to 30% of patients with ulcerative colitis (UC).[2] Moreover, C-reactive protein (CRP) often has a negligible response on disease activity in UC, especially in mild and moderate cases.[3,4]

It has been demonstrated that the improvement of the mucosa indicates a better outcome of the disease,[5–7] both in UC and in Crohn's disease (CD). However, direct evaluation of the mucosa requires the performance of an endoscopy, which is invasive, time consuming, expensive, and does not always reach the affected segment of the intestine. Therefore, surrogate markers of intestinal inflammation would be of great value to the clinician for monitoring inflammatory disease activity and for measuring the effects of treatment. Fecal calprotectin (FC) represents 60% of cytosolic proteins in granulocytes. The presence of FC in feces is therefore proportional to neutrophil migration to the gastrointestinal tract and to the degree of inflammation.

The role of several biomarkers in assessing endoscopic activity in IBD has been recently reviewed by Lewis.[8] Among these biomarkers, FC showed the best correlation with endoscopic activity in both CD and UC.[9–19] In addition, 2 meta-analyses demonstrated the value of FC in discriminating IBD from non-IBD diagnoses.[20,21] However, only 2 studies have assessed the accuracy of FC in predicting mucosal healing (MH) in UC.[19,22] Moreover, there are still scarce data about 2 relevant points affecting the use of FC in clinical practice: the optimal thresholds of FC for the prediction of endoscopic activity and the reliability of the rapid point-of-care tests compared with the enzyme-linked immunosorbent assay (ELISA) test in patients with IBD.

Regarding the cutoff levels of FC, different threshold concentrations ranging from 50,[10,22] 100,[19] and to 250 μg/g[23] have been proposed in the literature. However, there is still no agreement on what cutoff level of FC should be considered for predicting endoscopic activity.

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The ELISA test has been the most widely used technique for measuring FC. This technique, though highly reliable, presents several drawbacks: the fact that it is time consuming (up to 4-hour duration), the need to collect multiple samples to make the running test more cost effective, and the availability of a laboratory and trained personnel to perform the test. To overcome these limitations, 2 kinds of point-of-care tests have been developed: semi-quantitative ones that provide a range of FC levels and a rapid quantitative (QPOCT), which is as fast as the semi-quantitative tests but provides an exact number as the ELISA test. Previous studies[24–27] using QPOCT observed a good correlation with the ELISA test but none of them explored its ability to predict endoscopic activity in IBD population.

The aims of our study were to evaluate the accuracy of FC and to determine the optimal cutoff levels for the prediction of endoscopic activity. We have also studied the reliability of the rapid quantitative test for the determination of FC compared with the previously used ELISA test.

MATERIALS AND METHODS

Participants

One hundred and twenty-three adult patients with previous known UC referred for colonoscopy to the Department of Gastroenterology of Bellvitge University Hospital (Hospitalet de Llobregat, Barcelona) between December 2010 and February 2012 were prospectively and consecutively included in the study. Nineteen of the 123 patients were referred twice for endoscopic study and 2 of them a third time during this period, so 146 colonoscopies and/or rectosigmoidoscopies were performed. Inclusion criteria included appropriated diagnosis for UC (with endoscopy, biopsies, and/or image) at least within the 3 previous months of the study and age between 18 and 85 years. Exclusion criteria included pregnancy, nonsteroidal anti-inflammatory drugs intake, concomitant gastrointestinal infection, surgery within the past 3 months, colorectal cancer, predominant perianal symptoms, and CD or indeterminate colitis diagnosis.

Study Design

Patients were visited in our IBD unit previous to their colonoscopy to invite them to participate in the study, provide them with the recipient for FC collection, perform a clinical interview, and carry out blood tests. If diarrhea was present, we also performed a microbiological study of feces consisting of conventional culture, parasites, and *Clostridium difficile* detection to exclude gastrointestinal infection. At our center, the diagnosis of *C. difficile* consists of 2 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°C until analysis.

Clinical Activity

Clinical activity was assessed according to the clinical criteria of the Mayo score. Symptomatic remission was based on the patient-rated Mayo subscores of stool frequency (0 points, normal number for the patient; 1 point, 1–2 stools more than normal; 2 points, 3–4 stools more than normal; and 3 points, ≥5 stools more than normal) and rectal bleeding (0 points, no blood seen; 1 point, streaks of blood with stool less than half the time; 2 points, obvious blood with stool most of the time; and 3 points, blood alone passes). Symptomatic remission was defined as a Mayo stool frequency subscore of 0 or 1 and a Mayo rectal bleeding subscore of 0.[7]

Serological Biomarkers

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

Endoscopic Disease Activity

One hundred and forty-six colonoscopies were performed of which 108 were complete to cecum and 38 were rectosigmoidoscopies. Rectosigmoidoscopies were performed instead of colonoscopies in a flare context to avoid iatrogenic damage or in cases of distal UC when total affected mucosa was evaluated and normal mucosa was reached. Two trained endoscopists (F.R.-M. and J.G.C.) from our unit, who were blind for the other variables from the study, carried out the colonoscopies. Endoscopic activity was assessed according to the Mayo endoscopic subscore. "Endoscopic remission" was defined as Mayo endoscopic subscore grade ≤1, and "no endoscopic activity" was defined as Mayo endoscopic subscore grade 0. "Endoscopic activity" was defined as Mayo endoscopic subscore grade ≥2, and "any degree of endoscopic activity" was defined as Mayo endoscopic subscore grade ≥1. Extension of disease activity was reported according to the Montreal classification (proctitis, left-sided colitis, or extensive colitis) when any degree of endoscopic activity was observed.

FC Determination

Stool sample collection was performed within 1 to 3 days before endoscopy and bowel preparation. All samples were stored at −20°C, thawed, and analyzed by an ELISA test (Calprotectin Bühlmann ELISA; Bühlmann, Schonenbuch, Switzerland) and by a QPOCT (Quantum Blue; Bühlmann). The quantitative point-of-care test (FC-QPOCT) relies on the lateral flow assay technology including an easy to use reader system allowing for a quantitative read out. There are 2 kinds of FC-QPOCT: the lower range Quantum Blue (LF-CAL) and the high range Quantum Blue (LF-CHR), which provide quantitative results within minutes, ranging from 30 to 300 μg/g or 100 to 1800 μg/g FC, respectively. The range of the ELISA test (FC-ELISA) is 10 to 1800 μg/g. The median time analysis required for FC-ELISA is 2 hours for 88 samples and 12 to 15 minutes for each sample with FC-QPOCT depending on the kit (12 minutes for LF-CAL and 15 minutes for LF-CHR). The sample amount needed is 50 to 150 μL and 80 μL

for FC-QPOCT. Sensitivity of both tests is <10 μg/g. FC measurements were taken by 3 gastroenterologists and the IBD nurse (T.L.O., A.L.G., L.R.-A., and E.S.P.) who were blind for endoscopic activity and other variables, following appropriate training. We started the study using the LF-CAL kit and changed to the LF-CHR kit when available. All samples scoring >300 μg/g with the LF-CAL were performed again with the LF-CHR kit to obtain their exact value.

Statistical Analysis

The statistical analysis was carried out using the SPSS version 19.0 statistical package (SPSS, Inc., Chicago, IL). Numerical results were given as the mean ± standard deviation and range, except when specified. Student's T test, Mann–Whitney U test, and analysis of variance factor were used to compare means. Qualitative results were based on the chi-square test. The correlation analysis between FC, endoscopic activity, and other biomarkers was based on Spearman's correlation coefficient rank (r). Multivariate analysis consisted of multiple logistic regression. Accuracy analysis included receiver operator characteristic curve analyses with its 95% confidence intervals and test characteristics, such as sensitivity, specificity, positive and negative predictive values, and overall accuracy. Statistical significance was accepted for $P < 0.05$. Correlation between FC-ELISA and FC-QPOCT was analyzed by Spearman's correlation coefficient rank and interclass correlation index.

Ethical Considerations

This study was approved by the ethics committee of Bellvitge University Hospital and all patients gave their informed written consent for participation.

RESULTS

A total of 146 endoscopies were performed on 123 patients with UC between December 2010 and February 2012. Epidemiological, clinical, biological, and endoscopic characteristics of patients are shown in Table 1.

Correlation Between Both Techniques

Spearman's correlation coefficient rank between FC-QPOCT and FC-ELISA was 0.911 ($P < 0.001$). The interclass correlation index was 0.904 (95% confidence interval, 0.864–0.932; $P < 0.001$). Results are shown in Figure 1.

Relation of FC with Endoscopic Activity, Clinical, and Serological Markers

FC determined by QPOCT and ELISA presented a correlation with endoscopic activity of 0.727 and 0.741, respectively (Spearman's correlation coefficient rank; $P < 0.001$ in both cases).

FC-QPOCT discriminated "endoscopic activity" defined as Mayo endoscopic subscore ≥2 from "endoscopic remission" defined as Mayo endoscopic subscore ≤1 (mean levels of 1368.39 ± 618.18 μg/g and 294.91 ± 456.36 μg/g, respectively, $P < 0.001$). FC-ELISA showed similar results (mean levels of 1299.49 ± 608.17 μg/g and 246.37 ± 357.73 μg/g, respectively, $P < 0.001$). Median levels of FC-ELISA and FC-QPOCT for each endoscopic Mayo grade are shown in Figure 2.

Correlations between Mayo endoscopic subscore, FC (FC-ELISA and FC-QPOCT), clinical activity, and the serological variables are shown in Table 2. The Mayo endoscopic subscore correlated more closely with FC-QPOCT (Spearman's correlation coefficient rank r = 0.727, $P < 0.001$) followed by clinical activity (r = 0.636, $P < 0.001$), platelets (r = 0.381, $P < 0.001$), leucocytes (r = 0.300, $P = 0.001$), and CRP (r = 0.291, $P = 0.002$). Similar results were obtained with FC-ELISA.

TABLE 1. Patient Characteristics

Patient Characteristics	Number	Percent
Number of patients	146	—
Men (%)	97	66.40
Age, mean ± SD, yr	47 ± 17	—
Disease duration, mean ± SD, yr	12.9 ± 10.1	—
UC extension (Montreal classification)		
Proctitis	18	12.30
Left-sided colitis	56	38.40
Extensive colitis	71	48.60
Not known[a]	1	0.70
Medication at endoscopy[b]		
Oral/topical 5-ASA	107/44	74.80/31.20
CC (beclomethasone/systemic/topical)	10/5/6	6.80/3.40/4.10
IMM (AZA/6MP/MTX/CyA/Tacrolimus)	18/2/3/1/1	12.30/1.40/2/0.70/0.70
TNF inhibitor	5	3.40
Leucocytes, mean ± SD, g/L	7.2 ± 2.1	—
Platelets, mean ± SD, g/L	285.7 ± 980.0	—
CRP, mean ± SD, mg/dL	14.74± 38.69	—
ESR, mean ± SD, mm	2.68 ± 1.89	—
Asymptomatic patients	85	58.22
FC-ELISA, mean ± SD, μg/g	796.7± 728.8	—
FC-QPOCT, mean ± SD, μg/g	862.7± 766.4	—
Mayo endoscopic subscore		
Mayo 0	35	24
Mayo 1	33	22.60
Mayo 2	71	48.60
Mayo 3	7	4.80

[a]No previous reports regarding the extension of the disease and not completed colonoscopy in the study.
[b]As therapy regimens overlapped, the total is 140.8%.
ASA, aminosalicylic acid; AZA, azathioprine; CC, corticosteroids; CyA, cyclosporine; IMM, immunomodulators; 6MP, 6-mercaptopurine; MTX, methotrexate; SD, standard deviation; TNF, tumor necrosis factor.

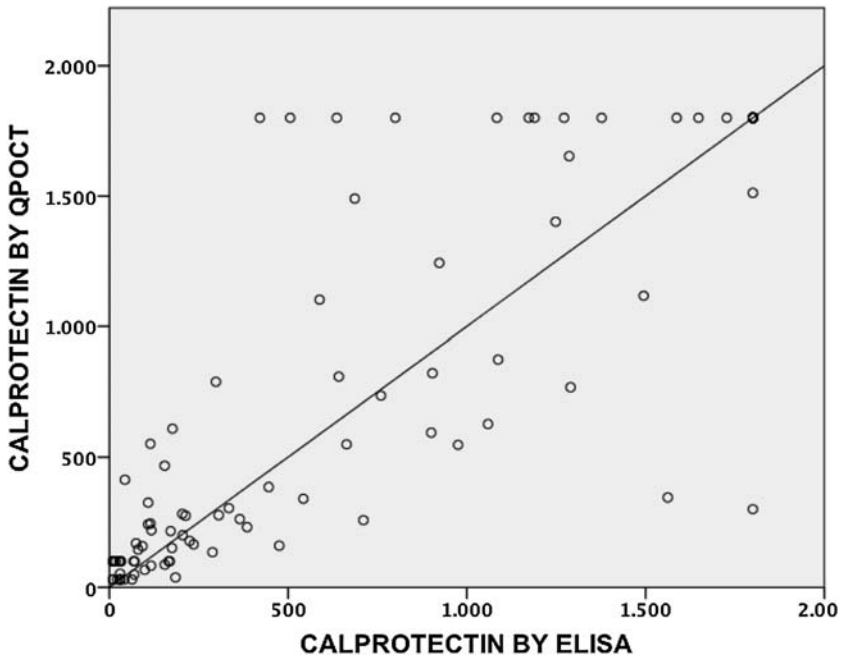

FIGURE 1. Correlation between FC-QPOCT and FC-ELISA. Scatterplot demonstrating the correlation between FC-ELISA and FC-QPOCT. Spearman' s rank correlation coefficient r = 0.911, $P < 0.001$.

In the multivariate analysis, FC-QPOCT and clinical assessment were both independent predictors of endoscopic activity (Table 3). Similar results were obtained with FC-ELISA (data not shown).

Accuracy of FC for the Prediction of Mayo Endoscopic Subscore ≤1

The prediction of "endoscopic remission" defined as Mayo endoscopic subscore ≤1 with FC-QPOCT and FC-ELISA

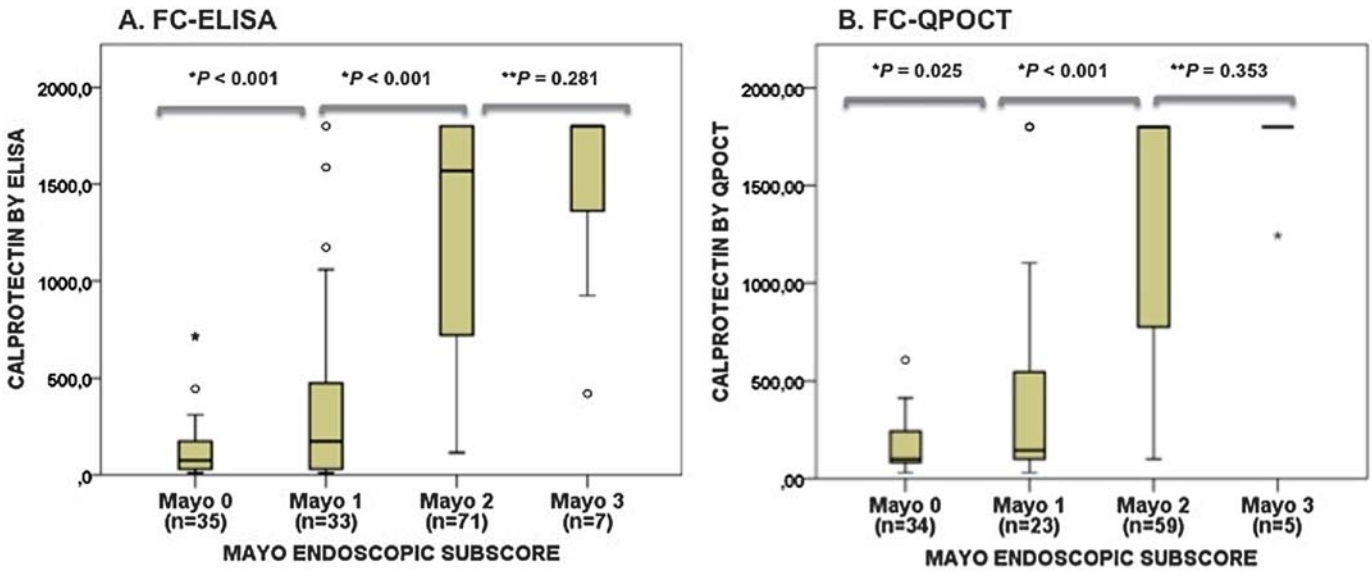

FIGURE 2. Median FC levels according to the Mayo endoscopic subscore. FC concentrations in different groups of endoscopic activity are illustrated by box plots. The box represents the lower and upper quartiles, and the horizontal line in the middle of the box is the median. The 95% confidence interval is represented by whiskers, and individual outliers are represented by values outside the whiskers. A, FC-ELISA. Median FC levels were 74.6 μg/g for Mayo 0; 173.65 μg/g for Mayo 1; 1569.28 μg/g for Mayo 2; and 1800 μg/g for Mayo 3. *Student's T test. **Mann–Whitney U test. B, FC-QPOCT. Median FC levels were 100 μg/g for Mayo 0; 145 μg/g for Mayo 1; 1800 μg/g for Mayo 2; and 1800 μg/g for Mayo 3. *Student's T test. **Mann–Whitney U test.

TABLE 2. Spearman's Correlation Coefficient Rank Between FC-ELISA, FC-QPOCT, Clinical Activity, Serum Biomarkers, and Endoscopic Activity

	Leucocytes	FC-ELISA	FC-QPOCT	Platelets	Clinical Activity[a]	Mayo Endoscopic Subscore
CRP	0.169 ($P = 0.056$)	0.316 ($P = 0.001$)	0.292 ($P = 0.002$)	0.250 ($P = 0.004$)	0.443 ($P < 0.001$)	0.307 ($P < 0.001$)
Leucocytes		0.227 ($P = 0.008$)	0.300 ($P < 0.001$)	0.305 ($P < 0.001$)	0.398 ($P < 0.001$)	0.262 ($P = 0.026$)
FC-ELISA			0.911 ($P < 0.001$)	0.317 ($P < 0.001$)	0.692 ($P < 0.001$)	0.741 ($P < 0.001$)
FC-QPOCT				0.381 ($P < 0.001$)	0.636 ($P < 0.001$)	0.727 ($P < 0.001$)
Platelets					0.303 ($P < 0.001$)	0.340 ($P < 0.001$)
Clinical activity[a]						0.629 ($P < 0.001$)

[a]Clinical activity according to Mayo score.
CRP, C-reactive protein; FC-ELISA, FC determined by the ELISA test; FC-QPOCT, FC determined by the quantitative point-of-care test.

presented an area under the curve of 0.906 and 0.924, respectively (Fig. 3). A 250 μg/g cutoff level of FC-ELISA (n = 146) had a sensitivity of 73.5%, a specificity of 89.7%, a negative predictive value of 79.5%, and a positive predictive value of 86.2% (global accuracy 82.2%). A 280 μg/g cutoff level FC-QPOCT (n = 121) had a sensitivity of 75.4%, a specificity of 89.1%, a negative predictive value of 80.3%, and a positive predictive value of 86% (global accuracy 82.7%). Results are shown in Table 4.

Accuracy of FC for the Prediction of Mayo Endoscopic Subscore 0

The prediction of "no endoscopic activity" defined as Mayo endoscopic subscore grade 0 with FC-QPOCT and FC-ELISA presented an area under the curve of 0.864 and 0.843, respectively (Fig. 3).

A 160 mg/g cutoff level had a sensitivity of 64.9% and a specificity of 83.9% with FC-QPOCT and a sensitivity of 66.7% and a specificity of 84.5% with FC-ELISA. Results shown in Table 5.

Among the asymptomatic patients (n = 72), 30.6% (n = 22) presented endoscopic activity. FC would have classified them correctly because 81.8% (18 out of 22) had an FC equal or superior to 280 mg/g.

Given that both FC and clinical activity are independently related to endoscopic activity, we created a score to predict "endoscopic remission" including both variables. When we combined clinical activity and FC-QPOCT with a 280 μg/g cutoff level, specificity improved to 93.8% (from 67.9% using only clinical activity), whereas sensitivity decreased to 70.2% (from 88.2% using only clinical activity). Similar results were observed with FC-ELISA. An asymptomatic patient with a FC-QPOCT <280 μg/g would have a 90.9% probability of having "endoscopic remission" (Table 4).

Relation of FC Levels to Extension of UC

When considering only patients who underwent complete colonoscopy (intubation until cecum; n = 108), we observed that FC-ELISA levels were related to the extension of endoscopic activity in our univariate analysis (F = 23.11, $P < 0.001$). However, in the multivariate analysis, FC was not an independent predictor for extension of the disease when adjusting for endoscopic activity. Similar results were observed with FC-QPOCT (data not shown).

Correlation with Presence of Pseudopolyps

We found no relation between the presence of pseudopolyps and FC levels.

DISCUSSION

This study shows a significant correlation between FC levels and endoscopic activity in UC. Our results demonstrate that FC, with a 250 μg/g cutoff level for FC-ELISA or a 280 μg/g

TABLE 3. Multivariated Analysis (Logistic Regression) for the Prediction of "Endoscopic Remission" (Mayo Endoscopic Subscore ≤1)

Variable	Coefficient (β)	Standard Error	Exp(B)	95% CI Exp(B)	*P*
CRP < 5 mg/dL	−0.166	0.680	0.847	0.223–3.210	0.807
Symptomatic remission[a]	2.137	0.624	8.476	2.496–28.786	0.001
FC-QPOCT < 280 μg/g	2.584	0.561	13.249	4.413–39.781	<0.001
Constant	−2.090	0.478	0.124		<0.001

[a]Symptomatic remission: Mayo stool frequency subscore of 0 or 1 and a Mayo rectal bleeding subscore of 0.
CRP, C-reactive protein; FC-QPOCT, FC determined by the quantitative point-of-care test.

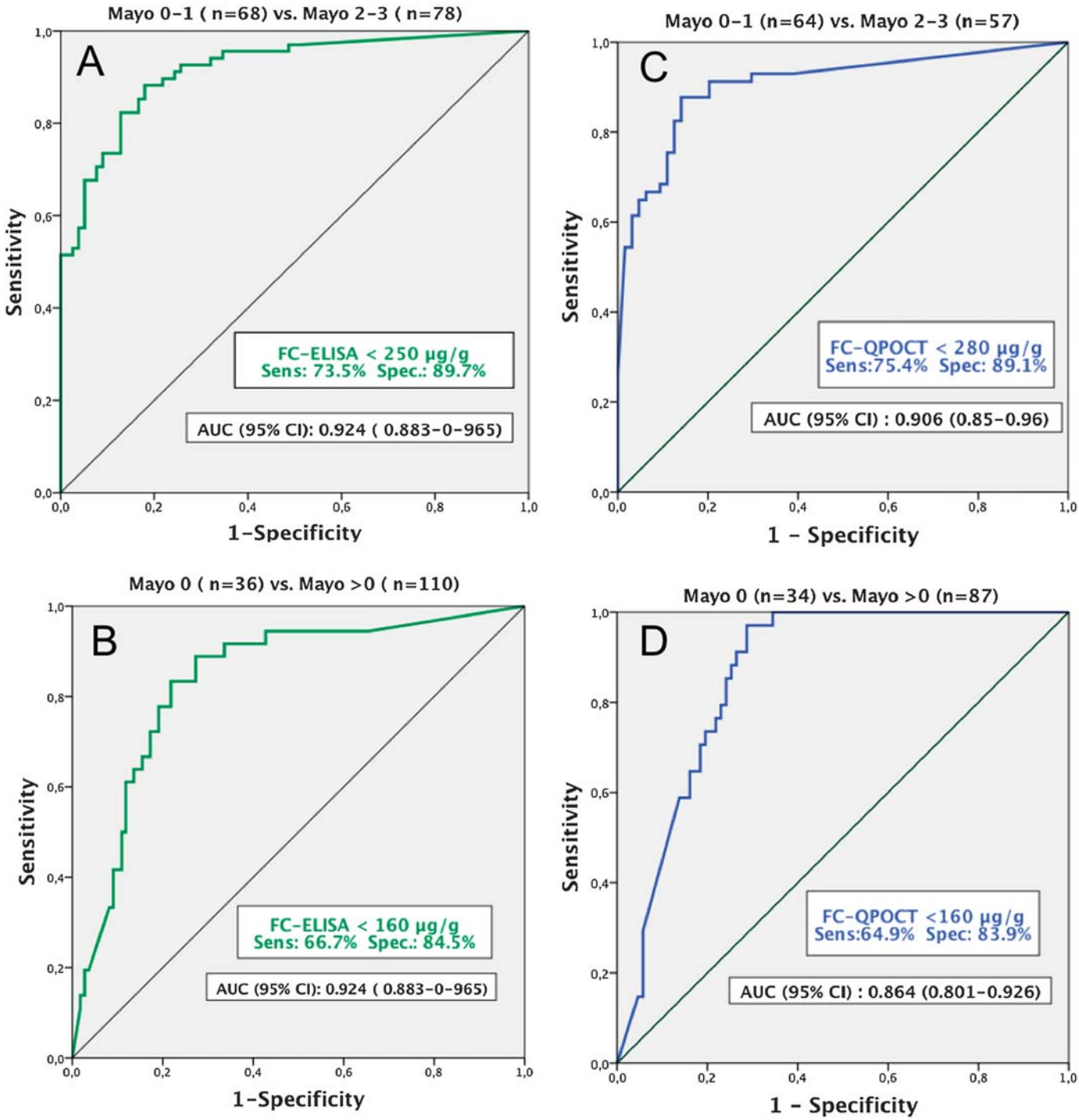

FIGURE 3. Receiver operator characteristic curves for FC-ELISA: Mayo 0 to 1 versus Mayo 2 to 3 (A), Mayo 0 versus Mayo >0 (B); FC-QPOCT: Mayo 0 to 1 versus Mayo 2 to 3 (C), Mayo 0 versus Mayo >0 (D).

cutoff level for FC-QPOCT, is a more accurate marker of endoscopic activity than clinical activity and other frequently used biomarkers such as CRP. In the multivariate analysis, FC and clinical activity were independently related to endoscopic activity. Thus, the combination of both variables may be useful to exclude endoscopic activity in clinical practice. We found FC to be especially useful in asymptomatic patients. In line with Regueiro et al,[2] we observed that clinical assessment underestimated endoscopic activity in one third of our patients and that the use of FC would have classified them correctly in most cases (Table 4). This

TABLE 4. Sensitivity (SENS), Specificity (SPEC), Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Accuracy of FC, Clinical Activity, and CRP in Predicting "Endoscopic Remission" (Mayo Endoscopic Subscore ≤1)

	SENS (%)	SPEC (%)	PPV (%)	NPV (%)	Accuracy (%)	P^a
FC-ELISA < 250 μg/g	73.5	89.7	86.2	79.5	82.2	<0.001
FC-QPOCT < 280 μg/g	75.4	89.1	86	80.3	82.7	<0.001
Symptomatic remission[b]	88.2	67.9	70.6	86.9	77.4	<0.001
CRP < 5 mg/L	83.9	37.8	50.5	75.7	57.7	0.010
Symptomatic remission[b] and FC-ELISA < 250 μg/g	69.1	96.2	94	78.1	83.6	<0.001
Symptomatic remission[b] or FC-ELISA < 250 μg/g	92.6	61.5	67.7	90.6	76	<0.001
Symptomatic remission[b] and FC-QPOCT < 280 μg/g	70.2	93.8	90.9	77.9	82.7	<0.001
Symptomatic remission[b] or FC-QPOCT < 280 μg/g	93	60.9	67.9	90.7	73.0	<0.001

[a]*P*: chi-square test.
[b]Symptomatic remission: Mayo stool frequency subscore of 0 or 1 and a Mayo rectal bleeding subscore of 0.
CRP, C-reactive protein; FC-QPOCT, FC determined by the quantitative point-of-care test; FC-ELISA, FC determined by ELISA.

study is the first to use ELISA and a quantitative rapid test simultaneously for FC determination in patients with UC, and the correlation between both techniques was excellent (Fig. 1). The use of the FC-QPOCT test provides a quantitative FC result within minutes, allowing fast and adequate decision-making.

Previous studies[5–7] have shown that MH is associated with a higher response to treatment, lower rates of relapse and hospitalization, and a reduced need for surgery. In addition, because chronic inflammation has been associated with an increased risk of colorectal cancer, MH would be protective in this context.[28] Reliable biomarkers of MH could avoid the need for endoscopic evaluation of the mucosa.

Among the biomarkers of endoscopic activity, FC has been put forward as one of the most promising for the management of patients with IBD. In patients with UC, FC has a particularly important role as a biomarker for endoscopic activity because of the negligible response of CRP and the underestimation of endoscopic activity by clinical assessment. However, previously published correlations between FC levels and endoscopic activity varied greatly depending on the study (correlations ranging from 0.51 to 0.83). This is probably because of the different endoscopic scores used in these studies: Mayo index,[7,9,12] Matt's index,[13] and Rachmilewitz index.[19] We decided to use the Mayo endoscopic subscore because it is the most common one, is easy to use, and has a demonstrated prognostic value.[7] In patients with UC, Mayo endoscopic subscore grade ≤1 has been used as a criterion for endoscopic remission.[7,29,30] In our study, FC proved to be an accurate biomarker of "endoscopic remission" (Mayo endoscopic subscore grade ≤1). FC also proved to be an accurate biomarker of "no endoscopic activity" (Mayo endoscopic subscore grade 0) to exclude any degree of endoscopic activity (Fig. 3).

Current studies also provide controversial data regarding the cutoff level of FC for the prediction of endoscopic activity in UC. Cutoff levels vary depending on the technique used, the endoscopic

TABLE 5. Sensitivity (SENS), Specificity (SPEC), Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Accuracy of FC, Clinical Activity and CRP in Predicting "No Endoscopic Activity" (Mayo Endoscopic Subscore 0)

	SENS (%)	SPEC (%)	PPV (%)	NPV (%)	Accuracy (%)	P^a
FC-ELISA < 160 μg/g	66.7	84.5	58.5	88.6	80.1	<0.001
FC-QPOCT < 160 μg/g	64.9	83.9	61.1	85.9	78.5	<0.001
Symptomatic remission[b]	91.7	52.7	38.8	95.1	75.2	<0.001
CRP ≥ 5 mg/L	81.8	32	29	83.8	44.6	0.18
Symptomatic remission[b] and FC-ELISA < 160 μg/g	61.1	86.4	59.5	87.2	80.1	<0.001
Symptomatic remission[b] or FC-ELISA < 160 μg/g	94.4	50	38.2	96.5	60.9	<0.001
Symptomatic remission[b] and FC-QPOCT < 160 μg/g	61.8	84.1	60	85.1	77.9	<0.001
Symptomatic remission[b] or FC-QPOCT < 160 μg/g	100	52.9	45.3	100	66.1	<0.001

[a]*P*: chi-square test.
[b]Symptomatic remission: Mayo stool frequency subscore of 0 or 1 and a Mayo rectal bleeding subscore of 0.
CRP, C-reactive protein; FC-QPOCT, FC determined by the quantitative point-of-care test; FC-ELISA, FC determined by ELISA.

score, and the desired sensitivity and the specificity according to the definition of endoscopic activity or MH. Previous studies indicated a cutoff level varying from 50 to 250 μg/g. Xiang et al[22] showed that FC with a 50 μg/g cutoff level had a specificity of 79.4% and a sensitivity of 91.9% in the discrimination of active and inactive disease according to the Sutherland score. Schoepfer et al[19] illustrated that with a 100 mg/L cutoff level, FC predicted endoscopic activity as defined by the Rachmilewitz index ≥4 with a specificity of 88% and a sensitivity of 86%. Recently, D'Haens et al,[23] in a study involving 39 patients with UC and 87 patients with CD, found a 250 μg/g cutoff level to be the most accurate for predicting the presence of any mucosal inflammation in patients with UC defined by the Mayo endoscopic subscore ≥1 (a specificity of 100% and a sensitivity of 71%) and the presence of large ulcers in CD. In line with these results, we found a 250 and 280 μg/g cutoff levels for FC-ELISA and FC-QPOCT, respectively, to be the most accurate for predicting endoscopic remission as defined by the Mayo endoscopic subscore ≤1 (Table 4). To maximize specificity for the diagnosis of endoscopic remission (Mayo endoscopic subscore ≤1), a 110 μg/g cutoff level would have a specificity of 100% and a sensitivity of 51.4%. In contrast, to maximize sensitivity, a cutoff level of 845 μg/g would have a sensitivity of 92.6% and a specificity of 74.4%. When using a more strict definition as "no endoscopic activity" (Mayo endoscopic subscore 0), the most accurate cutoff level was 160 μg/g for both techniques (Table 5). After the adjustment by endoscopic activity we found no relation between FC and the extension of the disease, which confirms the results of the study by Roseth et al.[9]

We also explored the reliability of a rapid QPOCT against the FC-ELISA test, which is the only test used in the previous studies. The FC-ELISA test is an accurate technique that requires the availability of a laboratory and the collection of multiple samples. In contrast, the FC-QPOCT test is an easy and rapid technique that also provides quantitative results but that is more easily available and that can be performed individually. Unlike other rapid tests, the FC-QPOCT test provides a quantitative result that allows us to choose among different cutoff levels according to the clinical endpoint being considered.

Lastly, our study shows that the combination of clinical data and FC levels (both with FC-QPOCT or FC-ELISA) optimizes the utility of FC in clinical practice. Clinical remission and a FC-QPOCT <280 μg/g (or FC-ELISA levels < 250 μg/g) almost excludes "endoscopic activity" defined as Mayo endoscopic subscore ≥2, whereas the presence of clinical activity and FC-QPOCT ≥280 μg/g (or FC-ELISA ≥ 250 μg/g) almost exclude "endoscopic remission" defined as Mayo endoscopic subscore ≤1 (Table 4).

Our study has several limitations. First, because of the fact that this is the first study measuring FC levels with a rapid QPOCT in patients with UC, there is a need to validate our results externally. Second, this is a transversal study in which most of the patients with "endoscopic remission" were in long-lasting remission. One of the situations in which FC would be most useful is in evaluating response to treatment. However, there is a lack of a follow-up of FC levels in our patients with UC.

In conclusion, our results indicate that FC is a reliable surrogate marker of endoscopic activity in UC. Its accuracy in predicting endoscopic remission improves when using FC in combination with clinical data. This could be especially useful to confirm endoscopic remission in patients in symptomatic remission. Finally, the excellent correlation observed between the FC-ELISA test and the rapid quantitative test allows us to use FC more easily in our clinical practice. Therefore, it seems reasonable to incorporate FC in the management of patients with UC.

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REFERENCES

1. D'Haens G, Sandborn WJ, Feagan BG, et al. A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology*. 2007;132:763–786.
2. Regueiro M, Rodemann J, Kip KE, et al. Physician assessment of ulcerative colitis activity correlates poorly with endoscopic disease activity. *Inflamm Bowel Dis*. 2011;17:1008–1014.
3. Fagan EA, Dyck RF, Maton PN, et al Serum levels of C-reactive protein in Crohn's disease and ulcerative colitis. *Eur J Clin Invest.* 1982;12:351–359.
4. Solem CA, Loftus EV Jr, Tremaine WJ, et al. Correlation of C-reactive protein with clinical, endoscopic, histologic, and radiographic activity in inflammatory bowel disease. *Inflamm Bowel Dis.* 2005;11:707–712.
5. Froslie KF, Jahnsen J, Moum BA, et al. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. *Gastroenterology*. 2007;133:412–422.
6. Meucci G, Fasoli R, Saibeni S, et al. Prognostic significance of endoscopic remission in patients with active ulcerative colitis treated with oral and topical mesalazine: a prospective, multicenter study. *Inflamm Bowel Dis.* 2012;18:1006–1010.
7. Colombel JF, Rutgeerts P, Reinisch W, et al. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology*. 2011;141:1194–1201.
8. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. *Gastroenterology*. 2011;140:1817–1826.
9. Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion*. 1997;58:176–180.
10. Roseth AG, Aadland E, Grzyb K. Normalization of faecal calprotectin: a predictor of mucosal healing in patients with inflammatory bowel disease. *Scand J Gastroenterol.* 2004;39:1017–1020.
11. 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;14:40–46.
12. D'Inca R, Dal Pont E, Di Leo V, et al. Calprotectin and lactoferrin in the assessment of intestinal inflammation and organic disease. *Int J Colorectal Dis.* 2007;22:429–437.
13. Hanai H, Takeuchi K, Iida T, et al. Relationship between fecal calprotectin, intestinal inflammation, and peripheral blood neutrophils in patients with active ulcerative colitis. *Dig Dis Sci.* 2004;49:1438–1443.

14. Sipponen T, Karkkainen P, Savilahti E, et al. Correlation of faecal calprotectin and lactoferrin with an endoscopic score for Crohn's disease and histological findings. *Aliment Pharmacol Ther.* 2008;28: 1221–1229.
15. Fagerberg UL, Loof L, Lindholm J, et al. Fecal calprotectin: a quantitative marker of colonic inflammation in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* 2007;45:414–420.
16. Jones J, Loftus EV Jr, Panaccione R, et al. Relationships between disease activity and serum and fecal biomarkers in patients with Crohn's disease. *Clin Gastroenterol Hepatol.* 2008;6:1218–1224.
17. Sipponen T, Bjorkesten CG, Farkkila M, et al. Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment. *Scand J Gastroenterol.* 2010;45:325–331.
18. 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;105:162–169.
19. Schoepfer AM, Beglinger C, Straumann A, et al. Ulcerative colitis: correlation of the Rachmilewitz endoscopic activity index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. *Inflamm Bowel Dis.* 2009;15:1851–1858.
20. 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.
21. 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
22. Xiang Y, Ouyang Q, Li GD, et al. Clinical value of fecal calprotectin in determining disease activity of ulcerative colitis. *World J Gastroenterol.* 2008;14:53–57.
23. 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;18:2218–2224.
24. Dolci A, Panteghini M. Comparative study of a new quantitative rapid test with an established ELISA method for faecal calprotectin. *Clin Chim Acta.* 2012;413:350–351.
25. Wassell J, Wallage M, Brewer E. Evaluation of the Quantum Blue® rapid test for faecal calprotectin. *Ann Clin Biochem.* 2012;49(pt 1):55–58.
26. Sydora MJ, Sydora BC, Fedorak RN. Validation of a point-of-care desk top device to quantitate fecal calprotectin and distinguish inflammatory bowel disease from irritable bowel syndrome. *J Crohns Colitis.* 2012;6:207–214.
27. Kolho KL, Turner D, Veereman-Wauters G, et al. Rapid test for fecal calprotectin levels in children with Crohn's disease. *J Pediatr Gastroenterol Nutr.* 2012 [epub ahead of print].
28. Rutter M, Saunders B, Wilkinson K, et al. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology.* 2004;126:451–459.
29. Daperno M, Castiglione F, Ridder L, et al. Results of the 2nd part Scientific Workshop of the ECCO (II): measures and markers of prediction to achieve, detect, and monitor intestinal healing in inflammatory bowel disease. *J Crohns Colitis.* 2011;5:484–498.
30. Pineton de Chambrun G, Peyrin-Biroulet L, Lémann M, et al. Clinical implications of mucosal healing for the management of IBD. *Nat Rev Gastroenterol Hepatol.* 2010;7:15–29.