Fecal Calprotectin for Small Bowel Crohn’s Disease: Is It a Cutoff Issue?

Cristina Romero-Mascarell 1,2,3, Gloria Fernández-Esparrach 1,2,3, Cristina Rodríguez-De Miguel 1, Maria Carme Masamunt 4, Sonia Rodríguez 5, Jordi Rimola 5, Miguel Urpi 3, Gherzon Simon Casanova 1, Ingrid Ordóñez 3, Elena Ricart 2,3,4, Berta Caballol 2,3,4, Agnès Fernández-Clotet 2,3,4, Julià Panés 2,3,4, Josep Llach 1,2,3 and Begoña González-Suárez 1,2,3,*

Abstract: (1) Background: Fecal calprotectin (FC) correlates well with colonic inflammatory activity of Crohn’s disease (CD); data about relation of FC and small bowel (SB) lesions are still contradictory. The main aim was to analyze the relationship between FC levels and SB inflammatory activity in patients with established or suspected Crohn’s disease, assessed by small bowel capsule endoscopy (SBCE) or magnetic resonance enterography (MRE). (2) Methods: Two cohorts of patients were included: 1. Prospective data were collected from patients with established or suspected CD who underwent SBCE and FC (Cohort A); 2. A retrospective cohort of patients who underwent MRE and FC determination (Cohort B). Different cutoffs for FC were tested in both cohorts. (3) Results: 83 patients were included and 66 were finally analyzed. A total of 69.6% had SB lesions seen by SBCE and MRE, (n = 25) or MRE (n = 21). FC mean levels were 605.74 ± 607.07 µg/g (IQ range: 99.00–878.75), being significantly higher in patients with SB lesions compared to patients without lesions (735.91 ± 639.70 µg/g (IQ range: 107.75–1366.25) vs. 306.35 ± 395.26 µg/g (IQ range: 78.25–411.0), p < 0.005). For cohort A, 25 out of 35 patients had SB lesions and a significant correlation between Lewis Score and FC levels was achieved (R² = 0.34; p = 0.04). FC sensitivity (S), specificity (E), positive predictive value (PPV), and negative predictive values (NPV) for predicting SB lesions were 80%, 50%, 80%, and 50%, respectively, for FC > 100 µg/g. For cohort B, inflammatory SB activity, measured by MaRIA score, was detected in 21 out of 31 patients (67.7%). Patients with positive findings in MRE had significantly higher values of FC than those with no lesions (944.9 ± 672.1 µg/g vs. 221 ± 212.2 µg/g, p < 0.05). S, E, PPV, and NPV of FC were 89%, 50%, 77.2%, and 71.4% for FC levels > 100 µg/g. The higher sensitivity and specificity of FC were used for the detection of SB lesions with SBCE and MRE was obtained for an FC cutoff >265 µg/g and >430 µg/g, respectively. (4) Conclusions: FC has a good correlation with the presence of SB lesions, assessed by SBCE and MRE, in patients with established or suspected Crohn’s disease. However, the ideal cutoff is here proven to be higher than previously reported. Multicenter and large prospective studies are needed in order to establish definitive FC cutoff levels.

Keywords: Crohn’s disease; inflammatory bowel disease; small bowel capsule endoscopy; magnetic resonance enterography; fecal calprotectin

1. Introduction

Crohn’s disease is a chronic inflammatory bowel disease affecting any segment of the gastrointestinal tract, with small bowel (SB) being the most common location (in up
to 30% of cases, it is the only segment affected). Proximal SB disease, not accessible with ileocolonoscopy, is present in 10% of patients [1,2]. In these patients, and in the absence of obstructive symptoms or known stenosis, small bowel capsule endoscopy (SBCE) is the first diagnostic tool [3–5]. Contrarily, Magnetic Resonance Enterography (MRE) is the initial method of choice in patients with obstructive symptoms or known SB stenosis [6–8], being a complementary and/or alternative technique to SBCE although it has less sensitivity for detecting early lesions in the SB [9].

Assessment of inflammatory activity in Crohn’s disease has been traditionally based on signs and symptoms through clinical scores (CDAI, Harvey–Bradshaw), as well as serological biomarkers. However, symptoms are not pathognomonic of Crohn’s disease, and serological biomarkers as C-reactive protein or erythrocyte sedimentation rate have shown a low sensitivity for detecting SB inflammation [10–14]. Therefore, other alternatives as fecal biomarkers have been explored in the last years.

Calprotectin is a calcium-binding protein with antimicrobial, antiproliferative, and proinflammatory properties. Its concentration in the feces is directly proportional to the presence of neutrophils in the intestinal lumen making it useful for detecting inflammation in inflammatory bowel disease in clinical practice [15–17].

Fecal calprotectin (FC) has a good correlation with the endoscopic activity in colonic and ileocolonic Crohn’s disease, considering FC levels higher than 250 µg/g as pathological [18–22]. Regarding SB, values higher than 100 µg/g are associated with the detection of lesions by SBCE [23–26]. However, it is controversial if FC can accurately predict endoscopic activity changes in the SB [27–29], and some studies show a worse correlation for ileal CD than for colonic or ileocolonic disease [30–32].

Up to now, we have scarce data about the correlation between FC levels and the inflammatory activity in SB measured by MRE [33].

The aim of our study was to assess the correlation of FC levels, clinical scores, and serological markers with inflammatory activity in the SB of patients with established or suspected CD assessed by SBCE or MRE.

2. Materials and Methods

2.1. Study Design

Two cohorts of patients with established or suspected CD using clinical, radiological, endoscopic, and histological criteria were included: 1. A prospective cohort of patients referred to the endoscopy unit of the Hospital Clinic of Barcelona for SBCE between 2013–2015. The same day of SBCE, the patients were instructed to collect a stool sample within a week, which should be returned to the laboratory for FC analysis (Cohort A).

2. A retrospective cohort of patients selected from a prospectively recorded database who were submitted to a MRE between 2013–2015 and had a FC determination within 3 months (Cohort B).

General exclusion criteria were the following: age less than 18 years old, patients with active colonic CD, perianal disease or pouchitis, treatment with nonsteroidal anti-inflammatory drugs, any contraindication for SBCE, or severe comorbidities. Those patients with FC sample collected more than 3 months apart from the MRE or those who had modified their CD treatment throughout the recruitment period were also excluded. Additionally, in Cohort A, the presence of any contraindication for SBCE was an exclusion criterion.

During the follow-up period, clinical relapse was defined as the presence of gastrointestinal symptoms compatible with CD, requiring hospitalization or a treatment step-up. For the prospective cohort, visits were scheduled every 4–6 months according to daily clinical practice and, for the retrospective cohort, medical records were reviewed.

2.2. Small Bowel Capsule Endoscopy Procedure

SBCE was performed with Pillcam SB2 or SB3 (Given Imaging Inc., Yonqneam, Israel), and Endocapsule EC (Olympus, Tokyo, Japan). PillCam SB2 and Endocapsule have a single camera and capture 2 frames per second. PillCam SB3 also has a single camera plus an
adaptative frame rate between 2–6 frames per second according to the capsule movement. To improve the visualization of the SB, patients were given 1L of ascorbic acid plus PEG (Moviprep®, Norgine VB, Amsterdam, The Netherlands) the day before.

Images were analyzed by 2 gastroenterologists with experience in assessment of SBCE (BG-S and CR-M) with the Rapid Reader 7 software for PillCam and Olympus Endocapsule System 10 for the Olympus capsules, respectively. Standard terminology was used for the description of findings [4]. For the analysis, SB was systematically divided into 3 segments: jejunum, ileum, and terminal ileum.

Severity of SB lesions was graded using the Lewis Score, a quantitative index based on characteristics of villous edema, ulcers, and stenosis. A score lower than 135 was considered as normal or non-clinically significant mucosal inflammatory changes, between 135 and 790 was considered as mild, and ≥790 was defined as moderate to severe disease [34].

Quality of SBCE images was evaluated based on the proportion of the mucosa visualized without debris, liquid, or bubbles. It was categorized as excellent (>90%), good (≥75%), fair (50–75%), or poor (<50%) [35].

Capsule retention was defined as the failure of the passage of the capsule from the GI for more than 2 weeks. Patients with clinical suspected but not confirmed SB stenosis were previously submitted to a dissolvable Agile Patency Capsule (APC).

2.3. Magnetic Resonance Enterography Procedure

All examinations were performed using a standardized clinical protocol on a 3.0-T MR unit (TrioTim: Siemens Medical Solution, Erlangen, Germany). Patients fasted for at least 6h and, before MRE, ingested one liter of Polietilenglicol water solution or 2.5% mannitol solution, as intraluminal contrast agent. Protocol of MRE was described elsewhere [9,36,37]. MRE images were assessed by two expert radiologists (SR and JR) with more than 10 years of experience in the evaluation of IBD.

SB wall thickening (>3 mm), edema, hyperenhancement, comb sign (increased mesenteric vascularity adjacent to the inflamed intestinal loop), or presence of ulcers were considered signs of active CD. Strictures were defined as a luminal narrowing less than 10 mm. The presence of fistulas or abscesses was also registered [7,8].

The MaRIA score of each SB segment (jejunum, proximal ileum, and terminal ileum) was calculated to quantify the severity of the SB lesions Active disease was defined as MaRIA ≥7, whereas severe disease was defined as MaRIA ≥11 [38,39].

2.4. Biomarkers and Clinical Disease Activity

Clinical disease activity (CDAI) was calculated at the time of SBCE in cohort A whereas the registered index closest to MRE was used in cohort B.

Biomarkers such as platelet count, serum levels of hemoglobin, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were determined by routine laboratory analysis performed at the same period of time.

2.5. Fecal Calprotectin

Stool samples were collected at patients’ home in a specific FC collection container, transported to laboratory and stored at −20 °C. FC levels were analyzed by a quantitative immunochromatography test (ScheBo Quick-Prep, Buhlman Laboratories®), according to the manufacturer’s instructions [40].

2.6. Statistical Analysis

Continuous variables are expressed as mean plus standard deviation and interquartile range. Categorical data are expressed as frequencies and percentages. Chi-square test was used to compare categorical variables. Bivariate correlations were analyzed using Spearman’s correlation coefficient. Performance characteristics of FC levels were calculated using different cutoff. Receiver operator characteristics (ROC) curves were constructed in order to assess the diagnostic accuracy of FC to detect SB lesions. The area under the curve
(AUC) and optimal operating point with its sensitivity and specificity and 95% confidence intervals were calculated.

A *p* value less than 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows software 21.0 (SPSS Inc., Chicago, IL, USA).

### 3. Results

A total of 83 patients with established or suspected CD were eligible for the study: 52 patients in Cohort A and 31 patients in Cohort B. In cohort A, 17 patients (32%) were excluded: 3 without FC, 1 with a positive Agile Patency Capsule test, and 13 for other reasons. Finally, 66 patients (35 with SBCE and 31 with MRE) with FC were included. (Figure 1)

![Study flow chart](chart.png)

Figure 1. Study flow chart.

A total of 46 out of 66 patients (69.6%) had SB lesions seen by SBCE (*n* = 21) or MRE (*n* = 21). FC mean levels were 605.74 ± 607.07 µg/g (IQ range: 99.00–878.75). FC levels were significantly higher in patients with SB lesions compared to patients without lesions (735.91 ± 639.70 µg/g (IQ range: 107.75–1366.25) vs. 306.35 ± 395.26 µg/g (IQ range: 78.25–411.0) *p* = 0.007).

#### 3.1. Cohort A

Indications for SBCE were suspected CD (*n* = 21) and assessment of SB inflammatory activity in established CD patients (*n* = 14). Demographic, clinical, and analytical characteristics are summarized in Table 1. No cases of capsule retention were registered.
### Table 1. Demographic, clinical, and analytical characteristics.

|                                      | Whole Cohort N = 66 | SBCE N = 35 | MRE N = 31 |
|-------------------------------------|----------------------|-------------|------------|
| Female gender                       | 40 (60.6%)           | 23 (65.7%)  | 17 (54.8%) |
| Age (years)                         | 39.3 +/- 12.7        | 40.1 +/- 12.2 | 38.3 +/- 13.4 |
| Active smokers                      | 20 (30.3%)           | 7 (20%)     | 13 (41.9%) |
| Suspected/Established CD            | 24/42                | 21/14       | 3/28       |
| Duration of CD (months)             | 83.06 ± 106.45       | 75.86 ± 119.33 | 91.19 ± 91.02 |
| Previous surgery                    | 10 (15.2%)           | 5 (14.3%)   | 5 (16.1%)  |
| Treatment                           |                      |             |            |
| No treatment                        | 29 (43.9%)           | 19 (54.3%)  | 10 (32.2%) |
| Anti-TNFs                           | 3 (4.5%)             | 1 (2.9%)    | 2 (6.5%)   |
| Thiopurines                         | 11 (16.6%)           | 7 (20%)     | 4 (12.9%)  |
| Mesalazine                          | 3 (4.5%)             | 0           | 3 (9.7%)   |
| Corticosteroids                     | 6 (9.1%)             | 4 (11.4%)   | 2 (6.4%)   |
| Anti-TNFs + thiopurines             | 14 (21.2%)           | 4 (11.4%)   | 10 (32.2%) |
| CDAI score                          |                      |             |            |
| >150                                | 23 (34.8%)           | 11 (31.4%)  | 12 (38.7%) |
| <150                                | 38 (57.6%)           | 24 (68.6%)  | 14 (45.2%) |
| Age at CD onset                     |                      |             |            |
| <18 years                           | 6 (9.1%)             | 2 (5.7%)    | 4 (12.9%)  |
| 18–40 years                         | 23 (34.8%)           | 7 (20%)     | 16 (51.6%) |
| >40 years                           | 13 (19.7%)           | 5 (14.3%)   | 8 (25.4%)  |
| Age of suspected CD                 | 24 (36.4%)           | 21 (60%)    | 3 (9.7%)   |
| Disease location                    |                      |             |            |
| Ileal                               | 29 (43.9%)           | 9 (25.7%)   | 20 (64.5%) |
| Colonic                             | 1 (1.5%)             | 1 (2.9%)    | 0          |
| Ileo-colonic                        | 7 (10.6%)            | 1 (2.9%)    | 6 (19.4%)  |
| Upper GI tract                      | 1 (1.5%)             | 1 (2.9%)    | 0          |
| Ileal + upper GI tract              | 4 (6.1%)             | 2 (5.7%)    | 2 (6.5%)   |
| Suspected CD                        | 24 (36.4%)           | 21 (60%)    | 3 (9.7%)   |
| CD phenotype                         |                      |             |            |
| Inflammatory                        | 33 (50%)             | 11 (31.4%)  | 22 (71%)   |
| Stricturing                         | 3 (4.5%)             | 1 (2.9%)    | 2 (6.5%)   |
| Penetrating                         | 3 (4.5%)             | 1 (2.9%)    | 2 (6.5%)   |
| Stricturing and penetrating         | 3 (4.5%)             | 1 (2.9%)    | 2 (6.5%)   |
| Suspected CD                        | 24 (36.4%)           | 21 (60%)    | 3 (9.7%)   |
| ESR/hour                            | 12.15 ± 9.61         | 10.91 ± 6.88 | 13.63 ± 12.07 |
| CRP (mg/L)                          | 0.39 ± 0.67          | 0.16 ± 0.34 | 0.64 ± 0.84 |
| Hb (g/L)                            | 133.03 ± 20.56       | 135.17 ± 13.33 | 130.61 ± 26.51 |

SBCE: small bowel capsule endoscopy, MRE: magnetic resonance enterography. CRP: C-reactive protein; ESR: erythrocyte sedimentation rate, Hb: hemoglobin.

Quality of SBCE images was considered good or excellent in all of patients. Three out of thirty-five patients (8.6%) had an incomplete SBCE study, but in all of them, SB lesions compatible with CD were observed.

SB lesions were detected in 25 out of 35 patients (71.4%): 10 in patients with established CD (10/14, 71.4%) and 15 in patients with suspected CD (15/21, 71.4%). The type of lesions and severity, assessed by Lewis score, are described in Table 2. Sensitivity, specificity, negative predictive value, and positive predictive value related to the different FC cut-offs are shown in Table 3. Using the standard cut-off of 100 µg/g, 20 out the 25 lesions were detected.
Table 2. Small bowel capsule endoscopy findings and Lewis score.

| Lesions                        | Patients (n = 35) | Established Crohn's Disease (n = 14) | Suspected Crohn's Disease (n = 21) |
|-------------------------------|------------------|-------------------------------------|------------------------------------|
| Isolated jejunum ulcers       | 2                | 1                                   | 1                                  |
| Jejunum + ileal ulcers        | 12               | 3                                   | 9                                  |
| Ileal ulcers                  | 11               | 6                                   | 5                                  |
| No lesions                    | 10               | 4                                   | 6                                  |

| Severity (Lewis score)        |                  |                                     |                                     |
|-------------------------------|------------------|-------------------------------------|------------------------------------|
| Normal (<135)                 | 10               | 4                                   | 6                                  |
| Mild (135–790)                | 15               | 4                                   | 11                                 |
| Moderate to severe (>790)     | 10               | 4                                   |                                    |

Table 3. Performance characteristics of Fecal Calprotectin for the detection of small bowel lesions with small bowel capsule endoscopy.

|                  | S     | E   | PPV  | NPV  |
|------------------|-------|-----|------|------|
| FC ≥ 50 µg/g     | 92%   | 20% | 74%  | 50%  |
| FC ≥ 100 µg/g    | 80%   | 50% | 80%  | 50%  |
| FC ≥ 500 µg/g    | 40%   | 90% | 40%  | 90%  |

S: sensibility; E: specificity, PPV: positive predictive value, NPV: negative predictive value.

FC levels were 503.37 ± 541.10 µg/g (IQ range 99–611). Patients with positive findings in SBCE had significantly higher levels of FC than patients without lesions (611 +/− 596.6 µg/g vs. 233 +/− 207.5 µg/g, p < 0.05). FC values associated with presence of SB lesions were higher among patients with established CD than in those with suspected disease (700 + 588.63 µg/g (IQ range: 155.25–1390) vs. 584.50 + 625.08 µg/g (IQ range 100.50–809).

A subgroup of 14 patients (41.1%) had proximal lesions (isolate jejunal or jejunal and ileal lesions). No differences were observed regarding FC levels in this group of patients compared with those having isolated ileal lesions (626.9 +/− 563.2 vs. 591.9 +/− 664.2 µg/g; p = 0.8).

There were no differences in severity or in serological markers levels between the group of patients with lesions and without (Table 4).

Table 4. Small bowel capsule endoscopy cohort: clinical, serological biomarkers, and Fecal Calprotectin levels related to small bowel activity.

| Lesions Detected by SBCE (N = 25) | No Lesions by SBCE (N = 10) | p     |
|-----------------------------------|-----------------------------|-------|
| FC mean (µg/g)                    | 611.5 +/− 596.6             | 233 +/− 207.5 | 0.009 |
| CDL score mean (range)            | 103.2 +/− 102.8             | 148 +/− 62.9 | 0.20  |
| CRP (µg/L) mean (range)           | 0.2 +/− 0.4                 | 0.04 +/− 0.05 | 0.20  |
| ESR (mm/h) mean (range)           | 10.1 +/− 6.1                | 13.57 +/− 9.1 | 0.25  |

SBCE: small bowel capsule endoscopy; FC: Fecal Calprotectin; CDL: Crohn’s Disease Activity Index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Regarding the severity of lesions, a low positive correlation between Lewis score and FC levels was found (R²: 0.34; p = 0.04) (Figure 2).
Table 4. Small bowel capsule endoscopy cohort: clinical, serological biomarkers, and Fecal Calprotectin levels related to small bowel activity.

| Lesions Detected by SBCE (N = 25) | No Lesions by SBCE (N = 10) | p       |
|-----------------------------------|-----------------------------|---------|
| FC mean (μg/g)                    | 611.5 +/− 596.6             | 233 +/− 207.5 | 0.009  |
| CDAI score mean (range)           | 103.2 +/− 102.8             | 148 +/− 62.9 | 0.20  |
| CRP (mg/L) mean (range)           | 0.2 +/− 0.4                 | 0.04 +/− 0.05 | 0.20  |
| ESR (mm/h) mean (range)           | 10.1 +/− 6.1                | 13.57 +/− 9.1 | 0.25  |

SBCE: small bowel capsule endoscopy; FC: Fecal Calprotectin; CDAI: Crohn's Disease Activity Index; CRP: c-reactive protein; ESR: erythrocyte sedimentation rate.

Figure 2. Correlation between fecal calprotectin levels and severity of inflammation measured with Lewis score.

The area under the curve (AUC) for the diagnostic accuracy of FC to detect SB lesions was 0.76, (95% CI, 0.622–0.908; p = 0.005). A cut-off >265 μg/g showed the highest sensitivity and specificity values of FC for the detection of SB lesions with SBCE (sensitivity 67%; specificity 70%) (Figure 3).

Figure 3. Receiver operating curve analysis for fecal calprotectin levels and presence of small bowel capsule endoscopy lesions.

3.2. Cohort B

A total of 31 patients were included in the MRE cohort. MRE indication was suspected CD in three patients and assessment of SB disease activity in 28 patients with established CD. Demographic, clinical, and analytical characteristics are shown in Table 1.

FC levels were <100 μg/g in 7 patients (41.2%), between 100–500 μg/g in 8 patients, and 16 patients (61.5%) had FC levels >500 μg/g, identifying lesions in MRE in 3 (14.2%), 5 (23.8%), and 13 (61.9%) (p = 0.04). Patients with positive findings in MRE had significantly higher values of FC than those with no lesions (944.9 ± 672.1 μg/g vs. 221 ± 212.2 μg/g, p < 0.05). Sensitivity, specificity, negative predictive value, and positive predictive value related to the different FC cutoffs are shown in Table 5.
Inflammatory SB activity, measured by MaRIA score, was detected in 21 out of 31 patients (67.7%). Two MRE studies were not evaluated due to lack of SB distension (Table 6).

Table 6. Magnetic Resonance Enterography findings and MaRIA Score.

| Lesions          | Patients (n = 31) | Established Crohn’s Disease (n = 28) | Suspected Crohn’s Disease (n = 3) |
|------------------|------------------|-------------------------------------|---------------------------------|
| Isolated jejunum | 0                | 0                                   | 0                               |
| Jejunum + ileal  | 0                | 0                                   | 0                               |
| Ileal            | 19               | 19                                  | 0                               |
| No lesions       | 12               | 9                                   | 3                               |
| Severity (MaRIA score) |
| MaRIA ≥ 7        | 2                | 3                                   | 0                               |
| MaRIA ≥ 11       | 19               | 19                                  | 0                               |
| No activity      | 8                | 4                                   | 3                               |

Otherwise, no differences were observed in CRP and CDAI index between both groups. A moderate positive correlation between MaRIA score and FC levels was found ($R^2$: 0.5; $p = 0.004$) (Figure 4).

![Figure 4](image_url). Correlation between fecal calprotectin levels and lesions at magnetic resonance enterography (MaRIA Index).

Receiver operating curves (ROC) were performed in order to assess the diagnostic accuracy of the FC to detect MRE lesions. The AUC referred to the accuracy in diagnosing inflammatory activity in MRE was 0.78 (95% CI, 0.663–0.898; $p = 0.000$) showing a moderate accuracy for FC to predict lesions in MRE (Figure 5).
Figure 4. Correlation between fecal calprotectin levels and lesions at magnetic resonance enterography (MaRIA Index).

Figure 5. Receiver operating curve analysis for fecal calprotectin levels and magnetic resonance enterography lesions.

3.3. Follow-Up

Follow-up was 40.8 ± 18.3 months. During this period of time, 16 (24.24%) patients presented a relapse (5 in cohort A and 11 in cohort B). A multivariate analysis was performed and baseline FC levels were not a predictive factor for relapse in the follow-up.

4. Discussion

In this study, we analysed the correlation of SB lesions and FC in CD patients assessed by SBCE and MRE. A recent study of our group showed a significantly higher sensitivity of SBCE for detecting superficial and proximal SB lesions compared to MRE (76.6% vs. 44.7%, \( p = 0.001 \)) [9]. However, MRE allows evaluation of transmural lesions and provides an accurate assessment of structuring and penetrating complications, being considered complementary to SBCE in the study of the SB [4,7,8,41]. The present study shows an FC cut-off higher than previously reported, in order to identify lesions in SB for SBCE and MRE (265 \( \mu g/g \) and 430 \( \mu g/g \), respectively) [18–22]. Two previous meta-analyses and systematic reviews studied the correlation between FC levels and inflammatory activity in the SB so far. Kopylov et al. [24] published a meta-analysis in 2016 that included seven studies (four retrospective); an FC cut-off of 50 \( \mu g/g \) had a high sensitivity (83%), with low specificity (53%) to detect SB lesions assessed by SBCE; a higher specificity was observed with FC > 200 \( \mu g/g \). The study suggested that patients with FC levels < 50 \( \mu g/g \), present a low probability to have lesions in the SBCE. This analysis had been after updated by Jung et al. [26] including 14 studies (6 retrospectives) showing similar results; a FC cut-off of 100 \( \mu g/g \) showed a sensitivity and specificity of 73%. They proposed to use this cut-off as a tool to screen SB Crohn’s disease assessed by SBCE. Contrarily, our results showed that a higher cut-off had a better performance for detecting SB lesions by SBCE with a positive correlation with Lewis score. The reason for this discrepancy may be related to the high number of retrospective studies included in the meta-analysis and the use of different diagnostic criteria in the previous publications.

None of the previous published studies differentiate levels of FC depending on suspected or established Crohn’s disease. After performing in our cohort, a separate analysis for suspected and established disease patients, we observed that FC levels associated with presence of SB lesions in the first group of patients were higher than those registered for established Crohn’s disease. Otherwise, we didn’t find any difference regarding FC levels associated with relapse. The higher sensitivity and specificity of the FC levels for the detection of SB lesions with MRE was obtained for a FC cut-off > 430 \( \mu g/g \) (Sensitivity 72%, specificity 73%).

The higher sensitivity and specificity of the FC levels for the detection of SB lesions with MRE was obtained for a FC cut-off > 430 \( \mu g/g \) (Sensitivity 72%, specificity 73%).
levels and disease location (isolate jejunal or jejunal and ileal lesions), mentioned by other authors [42].

Few studies have evaluated the correlation between FC and SB lesions assessed by MRE. Recently, Cerrillo et al. [33] performed a prospective study including 120 consecutive patients with Crohn’s disease, and a significant positive correlation was found between MaRIA score and FC levels. A FC cut-off of 166.5 µg/g showed 90% sensitivity and 74% specificity for the diagnosis of inflammatory activity in SB. However, in this study patients with colonic involvement were also included, and higher FC levels could be due to the presence of colonic disease. Contrarily, our study was performed in a population with isolated SB lesions, resulting the higher sensitivity and specificity of the FC for the detection of SB lesions with MRE for a cut-off >430 µg/g.

In the present study, other biomarkers, such as CRP and ESR, have shown to be poor predictors for SB lesions in CD, demonstrating a low sensitivity and specificity, as previously reported in the literature [12–14,31].

Previous studies suggested FC as a marker of clinical recurrence. Kennedy A et al. [43] published a retrospective study showing that high FC levels are associated with increased disease progression. In this study, neither SBCE lesions, MRE lesions, baseline Lewis score, or FC level were seen as predictive factors for relapse in the follow-up. This fact may be explained by a small number of patients presenting clinical relapse.

Two main limitations should be pointed out in our study: on one hand, one of the cohorts is retrospective and, secondly, the small sample size may prevent obtaining more definitive results. Strengths of the present study are a partial prospective design and consecutive inclusion of patients with suspected or known small bowel Crohn’s disease.

5. Conclusions

This study suggests that FC has a good correlation with the presence of SB lesions, assessed by SBCE and/or MRE, in patients with established or suspected Crohn’s disease. However, the ideal cut-off seems to be higher than previously reported. Multicenter and prospective studies are needed in order to establish definitive FC cutoff levels.

Author Contributions: Conceptualization, B.G.-S. and C.R.-M.; methodology, B.G.-S., S.R., J.R. and G.F.-E.; formal analysis, B.G.-S., C.R.-D.M. and G.F.-E.; data curation, A.F.-C., G.S.C., C.R.-M. and B.C.; writing—original draft preparation, B.G.-S., C.R.-M. and G.F.-E.; writing—review and editing, B.G.-S. and G.F.-E.; visualization, C.R.-M., M.C.M., M.U. and G.S.C.; supervision, I.O., E.R., J.P. and J.L.; project administration, B.G.-S. and C.R.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study protocol was approved by the ethics committee of the Hospital Clinic of Barcelona (Code HCB/2014/0532; Approval date: 25 May 2014).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: Dr. Fernández-Esparraz reports non-financial support from Medtronic and non-financial support from Norgine Iberia, during the conduct of the study; personal fees and non-financial support from Norgine Iberia; personal fees and non-financial support from Olympus Spain; and personal fees from CDx Diagnostics, outside the submitted work. Dr. Jordi Rimola acts as central reader for Robarts Clinical Research and Genentech and as a consultant for Abbvie, Robarts, Takeda, and Boehringer Ingelheim. Dr. Ingrid Ordás has received consulting fees from Abbvie and lecture fees from Abbvie, Takeda and Janssen. Dr. Julia Panés acts as an advisor for Janssen, Celtrio, MSD, TiGenix, and Takeda. Dr. Elena Ricart has served as a speaker or has received research or education funding or advisory fees from MSD, Abbvie, Takeda, Ferring, Pfizer, Janssen, Fresenius Kabí. Dr. Begoña González-Suárez reports speaker fees from Olympus Spain, Medtronic, and Norgine Iberia; Non-financial support for clinical investigation from Medtronic.
References

1. Cosnes, J.; Gower Rousseau, C.; Seksik, P.; Cortot, A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 2011, 140, 1785–1794.e4. [CrossRef] [PubMed]

2. Torres, J.; Mehandru, S.; Colombel, J.F.; Peyrin-Biroulet, L. Crohn’s disease. *Lancet* 2017, 389, 1741–1755. [CrossRef]

3. Annesi, V.; Daperno, M.; Rutter, M.D.; Amiot, A.; Bossuyt, P.; East, J.; Ferrante, M.; Götz, M.; Katsanos, K.H.; Kießlich, R.; et al. European evidence based consensus for endoscopy in inflammatory bowel disease. *J. Crohn’s Colitis* 2013, 7, 982–1018. [CrossRef] [PubMed]

4. Pennazio, M.; Spada, C.; Eliakim, R.; Keuchel, M.; May, A.; Mulder, C.J.; Rondonotti, E.; Adler, S.N.; Albert, J.; Baltes, P.; et al. Small-bowel capsule endoscopy and device-assisted enteroscopy for diagnosis and treatment of small-bowel disorders: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2015, 47, 352–376. [CrossRef]

5. Rondonotti, E.; Spada, C.; Adler, S.; May, A.; Despott, E.J.; Koulouzidis, A.; Panter, S.; Domagk, D.; Fernandez-Urrien, I.; Rahmi, G.; et al. Small-bowel capsule endoscopy and device-assisted enteroscopy for diagnosis and treatment of small-bowel disorders: European Society of Gastrointestinal Endoscopy (ESGE) Technical Review. *Endoscopy* 2018, 50, 423–446. [CrossRef]

6. Panes, J.; Bouhnik, Y.; Reinsch, W.; Stoker, J.; Taylor, S.A.; Baumgart, D.C.; Danese, S.; Halligan, S.; Marineck, B.; Matos, C.; et al. Imaging techniques for assessment of inflammatory bowel disease: Joint ECCO and ESGAR evidence-based consensus guidelines. *J. Crohn’s Colitis* 2013, 7, 556–585. [CrossRef]

7. Maaser, C.; Sturm, A.; Vavricka, S.R.; Kucharzik, T.; Fiorino, G.; Annesi, V.; Calabrese, E.; Baumgart, D.C.; Bettenworth, D.; Borralho Nunes, P.; et al. ECCO-ESGAR Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. *J. Crohn’s Colitis* 2019, 13, 144–164. [CrossRef]

8. Sturm, A.; Maaser, C.; Calabrese, E.; Annesi, V.; Fiorino, G.; Kucharzik, T.; Vavricka, S.R.; Verstockt, B.; van Rheenen, P.; Tolan, D.; et al. Ecco-esgar guideline for diagnostic assessment in ibd part 2: Ibd scores and general principles and technical aspects. *J. Crohn’s Colitis* 2013, 13, 273–284. [CrossRef]

9. González-Suárez, B.; Rodriguez, S.; Ricart, E.; Ordás, I.; Rimola, J.; Díaz-González, Á.; Romero, C.; de Miguel, C.R.; Jáuregui, A.; Araujo, I.K.; et al. Comparison of Capsule Endoscopy and Magnetic Resonance Enterography for the Assessment of Small Bowel Lesions in Crohn’s Disease. *Inflamm. Bowel Dis.* 2018, 24, 775–780. [CrossRef]

10. Plavšić, I.; Hauser, G.; Tkalić, M.; Pletikosić, S.; Salkić, N. Diagnosis of Irritable Bowel Syndrome: Role of Potential Biomarkers. *Gastroenterol. Res. Pract.* 2015, 2015, 490183. [CrossRef]

11. Däbritz, J.; Musci, J.; Foell, D. Diagnostic utility of faecal biomarkers in patients with irritable bowel syndrome. *World J. Gastroenterol.* 2014, 20, 363–375. [CrossRef] [PubMed]

12. Sands, B.E. Biomarkers of Inflammation in Inflammatory Bowel Disease. *Gastroenterology* 2015, 149, 1275–1285.e2. [CrossRef] [PubMed]

13. Vermeire, S.; Van Assche, G.; Rutgeerts, P. C-Reactive Protein as a Marker for Inflammatory Bowel Disease. *Inflamm. Bowel Dis.* 2004, 10, 661–665. [CrossRef] [PubMed]

14. Ma, C.; Battat, R.; Khanna, R.; Parker, C.E.; Feagan, B.G.; Jairath, V. What is the role of C-reactive protein and fecal calprotectin in evaluating Crohn’s disease activity? *Best Pract. Res. Clin. Gastroenterol.* 2019, 38, 38–39. [CrossRef] [PubMed]

15. Foell, D.; Wittkowski, H.; Roth, J. Monitoring disease activity by stool analyses: From occult blood to molecular markers of intestinal inflammation and damage. *Gut* 2009, 58, 859–868. [CrossRef]

16. Kopylov, U.; Rosenfeld, G.; Bressler, B.; Seidman, E. Clinical utility of fecal biomarkers for the diagnosis and management of inflammatory bowel disease. *Inflamm. Bowel Dis.* 2014, 20, 742–756. [CrossRef]

17. Konikoff, M.R.; Denson, L.A. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. *Inflamm. Bowel Dis.* 2006, 12, 524–534. [CrossRef]

18. Lin, J.F.; Chen, J.M.; Zuo, J.H.; Yu, A.; Xiao, Z.J.; Deng, F.H.; Nie, B.; Jiang, B. Meta-analysis. *Inflamm. Bowel Dis.* 2014, 20, 1407–1415. [CrossRef]

19. Mosli, M.H.; Zou, G.; Garg, S.K.; Feagan, S.G.; MacDonald, J.K.; Chande, N.; Sandborn, W.J.; Feagan, B.G. 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. [CrossRef]

20. Kopylov, U.; Yablecovitch, D.; Lahat, A.; Neuman, S.; Levhar, N.; Greener, T.; Klang, E.; Rozendorn, N.; Amitai, M.M.; Ben-Horin, S.; et al. Detection of Small Bowel Mucosal Healing and Deep Remission in Patients With Known Small Bowel Crohn’s Disease Using Biomarkers, Capsule Endoscopy, and Imaging. *Am. J. Gastroenterol.* 2015, 110, 1316–1323. [CrossRef]

21. Rodriguez-Moranta, F.; Lobatón, T.; Rodrigo-Arano, L.; Guardiola, J. Calprotectina fecal en el diagnóstico de enfermedades inflamatorias. *Gastroenterol. Hepatol.* 2013, 36, 400–406. [CrossRef] [PubMed]

22. Guardiola, J.; Lobatón, T.; Cerrillo, E.; Ferreiro-Iglesias, R.; Gisbert, J.P.; Doménech, E.; Chaparro, M.; Esteve, M.; Rodriguez-Moranta, F. Recomendaciones del Grupo Español de Trabajo en Enfermedad de Crohn y Colitis Ulcerosa (GETECCU) sobre la utilidad de la determinación de calprotectina fecal en la enfermedad inflamatoria intestinal. *Gastroenterol. Hepatol.* 2018, 41, 514–529. [CrossRef] [PubMed]

23. Aggarwal, V.; Day, A.S.; Connor, S.; Leach, S.T.; Brown, G.; Singh, R.; Friedman, A.; Zekry, A.; Craig, P.I. Role of capsule endoscopy and fecal biomarkers in small-bowel Crohn’s disease to assess remission and predict relapse. *Gastrointest. Endosc.* 2017, 86, 1070–1078. [CrossRef]
24. Kopylov, U.; Yung, D.E.; Engel, T.; Avni, T.; Battat, R.; Ben-Horin, S.; Plevris, J.N.; Eliakim, R.; Koulaouzidis, A. Fecal calprotectin for the prediction of small-bowel Crohn’s disease by capsule endoscopy: A systematic review and meta-analysis. Eur. J. Gastroenterol. Hepatol. 2016, 28, 1137–1144. [CrossRef] [PubMed]

25. Xiang, B.; Dong, Z.; Dai, C. The diagnostic and predictive value of fecal calprotectin and capsule endoscopy for small-bowel Crohn’s disease: A systematic review and meta-analysis. Rev. Esp. Enferm. Dig. 2021, 113, 193–201. [PubMed]

26. Jung, E.S.; Lee, S.P.; Kae, S.H.; Kim, J.H.; Kim, H.S.; Jang, H.J. Diagnostic accuracy of fecal calprotectin for the detection of small intestinal Crohn’s disease through capsule endoscopy: An updated meta-analysis and systematic review. Gut Liver 2021, 15, 732–741. [CrossRef]

27. Jensen, M.D.; Kjeldsen, J.; Nathan, T. Fecal calprotectin is equally sensitive in Crohn’s disease affecting the small bowel and colon. Scand. J. Gastroenterol. 2011, 46, 694–700. [CrossRef]

28. Arai, T.; Takeuchi, K.; Miyamura, M.; Ishikawa, R.; Yamada, A.; Katsumata, M.; Igarashi, Y.; Suzuki, Y. Level of Fecal Calprotectin Correlates With Severity of Small Bowel Crohn’s Disease, Measured by Balloon-assisted Enteroscopy and Computed Tomography Enterography. Clin. Gastroenterol. Hepatol. 2017, 15, 56–62. [CrossRef]

29. Simon, E.G.; Wardle, R.; Thi, A.A.; Eldridge, J.; Samuel, S.; Moran, G.W. Does fecal calprotectin equally and accurately measure disease activity in small bowel and large bowel Crohn’s disease? A systematic review. Intest. Res. 2019, 17, 160–170. [CrossRef]

30. Sipponen, T.; Savilahti, E.; Kolho, K.L.; Nuutinen, H.; Turunen, U.; Färkkilä, M. 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. [CrossRef]

31. Schoepfer, A.M.; Beglinger, C.; Straumann, A.; Trummler, M.; Vavricka, S.R.; Bruegger, L.E.; Seibold, F. 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. [CrossRef] [PubMed]

32. Stawczyk-Eder, K.; Eder, P.; Lykowska-Szuber, L.; Krela-Kazmierczak, I.; Klimczak, K.; Szymczak, A.; Szachta, P.; Katulska, K.; Linke, K. Is faecal calprotectin equally useful in all Crohn’s disease locations? A prospective, comparative study. Arch. Med. Sci. 2015, 11, 353–361. [CrossRef]

33. Coimbra, A.J.; Rimola, J.; O’Byrne, S.; Lu, T.T.; Bengtsson, T.; De Crespiigny, A.; Luca, D.; Rutgeerts, P.; Bruining, D.H.; et al. Magnetic resonance enterography is feasible and reliable in multicenter clinical trials in patients with Crohn’s disease, and may help select subjects with active inflammation. Aliment. Pharmacol. Ther. 2016, 43, 61–72. [CrossRef] [PubMed]

34. Gralnek, I.M.; Defranchis, R.; Seidman, E.; Leighton, J.A.; Legnani, P.; Lewis, B.S. Development of a capsule endoscopy scoring index for small bowel mucosal inflammatory change. Aliment. Pharmacol. Ther. 2008, 27, 146–154. [CrossRef]

35. Simon, E.G.; Wardle, R.; Thi, A.A.; Eldridge, J.; Samuel, S.; Moran, G.W. Does fecal calprotectin equally and accurately measure disease activity in small bowel and large bowel Crohn’s disease? A systematic review. Intest. Res. 2019, 17, 160–170. [CrossRef]

36. Rimola, J.; Rodríguez, S.; García-Bosch, O.; Ordás, I.; Ayala, E.; Aceituno, M.; Pellisé, M.; Ayuso, C.; Ricart, E.; Donoso, L.; et al. Magnetic resonance for assessment of disease activity and severity in ileocolonic Crohn’s disease. Gut 2009, 58, 1113–1120. [CrossRef] [PubMed]

37. Coorevits, L.; Baert, F.J.; Vanpoucke, H.J.M. Faecal calprotectin: Comparative study of the Quantum Blue rapid test and an established ELISA method. Clin. Chem. Lab. Med. 2013, 51, 825–831. [CrossRef]

38. Tillack, C.; Seidener, J.; Brand, S.; Göke, B.; Reiser, M.F.; Schaefer, C.; Diepolder, H.; Ochsenschlüssel, T.; Herrmann, K.A. Correlation of magnetic resonance enteroclysis (MRE) and wireless capsule endoscopy (CE) in the diagnosis of small bowel lesions in Crohn’s disease. Inflamm. Bowel Dis. 2008, 14, 1219–1228. [CrossRef] [PubMed]

39. García-Bosch, O.; Ordás, I.; Aceituno, M.; Rodríguez, S.; Ramírez, A.M.; Gallego, M.; Ricart, E.; Rimola, J.; Panes, J. Comparison of Diagnostic Accuracy and Impact of Magnetic Resonance Imaging and Colonoscopy for the Management of Crohn’s Disease. J. Crohn’s Colitis 2016, 10, 663–669. [CrossRef] [PubMed]

40. Coorevits, L.; Baert, F.J.; Vanpoucke, H.J.M. Faecal calprotectin: Comparative study of the Quantum Blue rapid test and an established ELISA method. Clin. Chem. Lab. Med. 2013, 51, 825–831. [CrossRef] [PubMed]

41. Tillack, C.; Seidener, J.; Brand, S.; Göke, B.; Reiser, M.F.; Schaefer, C.; Diepolder, H.; Ochsenschlüssel, T.; Herrmann, K.A. Correlation of magnetic resonance enteroclysis (MRE) and wireless capsule endoscopy (CE) in the diagnosis of small bowel lesions in Crohn’s disease. Inflamm. Bowel Dis. 2008, 14, 1219–1228. [CrossRef] [PubMed]

42. D’Arcangelo, G.; Imondi, C.; Terrin, G.; Catassi, G.; Alois, M. Is Fecal Calprotectin a Useful Marker for Small Bowel Crohn Disease? J. Pediatr. Gastroenterol. Nutr. 2021, 73, 242–246. [PubMed]

43. Kennedy, N.A.; Jones, G.R.; Plevris, N.; Patenden, R.; Arnott, I.D.; Lees, C.W. Association Between Level of Fecal Calprotectin and Progression of Crohn’s Disease. Clin. Gastroenterol. Hepatol. 2019, 17, 2269–2276.e4. [CrossRef] [PubMed]