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**Original Article**

# Fecal Calprotectin Diagnostic Level Gradient Along the Small Bowel in Patients With Crohn's Disease

**Offir Ukashi,[a,b] Uri Kopylov,[a,b] Bella Ungar,[a,b] Adi Talan Asher,[a,b] Eyal Shachar,[a,b] Tal Engel,[a,b] Ahmad Albshesh,[a,b] Doron Yablecovitch,[a,b] Adi Lahat,[a,b,c] Rami Eliakim,[a,b] Shomron Ben-Horin,[a,b] For the Israeli IBD Research Nucleus (IIRN)**

[a]Gastroenterology Institute, Sheba Medical Center Tel Hashomer, Ramat Gan, Israel
[b]The Faculty of Medical and Health Sciences, Tel-Aviv University, Tel Aviv, Israel
[c]Department of Gastroenterology, Assuta Ashdod Hospital, Ashdod, Israel

Corresponding authors: Shomron Ben-Horin, Gastroenterology Institute, Sheba Medical Center Tel Hashomer, Ramat Gan, 52621, Israel. Tel: +972-35-305-000; Fax: +972-35-304-408; Email: shomron.benhorin@gmail.com; Offir Ukashi, Gastroenterology Institute, Sheba Medical Center Tel Hashomer, Ramat Gan, 52621, Israel. Tel: +972-35-305-000; Fax: +972-35-304-408; Email: offirukashi@gmail.com

## Abstract

**Background and Aims:** Fecal calprotectin (FC) is known to be a sensitive biomarker of colonic inflammation but to a lesser degree of small bowel (SB) inflammation. Moreover, data on FC's diagnostic levels in different SB segments are scarce. We aimed to examine FC's diagnostic levels along the SB axis in CD.

**Methods:** This was a post hoc aggregated analysis of 5 prospective studies of adult CD patients who underwent FC testing and SB video capsule endoscopy. Lewis score (LS) inflammation in different SB segments was tested for correlation with FC level after the exclusion of colonic disease. The diagnostic levels of FC for SB inflammatory topographical gradient were assessed using a receiver operating characteristic.

**Results:** Two hundred and fourteen patients were included (age: 30 [24-43] year-old, males-57%). For a similar SB inflammatory activity (LS ≥ 135), FC levels incrementally increased from proximal to distal SB segments (63 [30-121] vs 190 [78-549], $p = 0.005$) and from distal SB segment to the colon (190 [78-549] vs 542 [185-1000], $p = 0.010$). The best FC cutoffs to identify isolated mild proximal/distal SB inflammation (LS ≥ 135) were 77 µg/g and 123 µg/g, respectively. A cutoff of 234 µg/g was best to detect more significant proximal inflammation (LS ≥ 350) when only mild distal SB inflammation was present. In sensitivity analyses, this proximal-to-distal FC gradient was maintained when LS ≥ 350 and LS ≥ 790 were used as the inflammatory reference values. Unlike FC, the magnitude of CRP elevation was unrelated to the topography of inflammation along the SB axis.

**Conclusions:** FC may serve as a topographical biomarker of CD-activity, with its sensitivity to identify mucosal inflammation increases from proximal to distal SB segments.

**Key Words:** Fecal calprotectin; Crohn's disease; proximal small bowel inflammation; Lewis score

## 1. Introduction

The diagnosis of suspected Crohn's disease (CD) and monitoring of disease activity usually incorporates multiple invasive and noninvasive tests (ie, inflammatory biomarkers, ileo-colonoscopy, video capsule endoscopy [VCE], intestinal ultrasound [IUS], and cross-sectional imaging).[1–3]

Fecal calprotectin (FC) is considered the most sensitive biomarker of intestinal inflammation in patients with inflammatory bowel disease (IBD),[2,4] and the current guidelines endorse it as an intermediate medium-term test[5,6] for disease monitoring in CD.[3,7] In this context, FC has been proven to accurately distinguish between IBD and irritable bowel syndrome, serve as a noninvasive surrogate for disease activity in IBD, assess response to treatment, and predict postoperative disease recurrence.[8] However, FC's diagnostic accuracy was found to be better in UC than in CD, possibly reflecting its established correlation to colonic mucosal inflammation.[9]

In contrast, the existing literature on the correlation of FC with small bowel (SB) inflammation is conflicting, and its diagnostic yield in patients with SB-CD is still debatable.[10–16] Moreover, to the best of our knowledge, studies to date have focused on FC's diagnostic accuracy for colonic inflammation versus SB (or ileal) inflammation as one segment,[11–13] but there are hitherto no studies exploring the correlation of FC levels with inflammation in the different segments of the SB, from proximal to distal. Providing such data may not only explain some of the conflicting aforementioned results but also better inform clinicians on how to interpret and act upon FC results in patients with suspected SB-CD activity. This is particularly important in light of the known worse prognosis of proximal SB-CD[17,18] and the high prevalence (ie, 80%) of SB involvement in CD, wherein exclusive proximal SB inflammation is seen in up to 30% of patients.[19]

Therefore, in this study, we aimed to examine the levels of FC vis-à-vis disease activity along the SB as detected by VCE

and to evaluate the correlation between FC and inflammatory activity in different SB segments.

## 2. Methods

### 2.1. Study design and population

This was a post hoc analysis aggregating data from adult patients (>18 years old) with CD who were included in 5 prospective studies conducted at Sheba Medical Center between 2013 and 2023.[6,20] The complete protocols of the studies that have not yet been fully published are available online (NCT04612621; https://clinicaltrials.gov/ct2/show/ NCT04612621), (NCT03889613; https://clinicaltrials.gov/ct2/show/ NCT03889613, NCT03555058; https://clinicaltrials.gov/ct2/show/ NCT03555058 and NCT06362174; and https://clinicaltrials.gov/ct2/show/ NCT06362174).

In all 5 studies, patients underwent baseline C-reactive protein (CRP), FC, and VCE studies within 4 weeks. Some studies also included a baseline ileo-colonoscopy and/or magnetic resonance enterography (MRE) and/or IUS (see below). The present study analyzed only available baseline SB-VCE and FC measures (at the study enrollment timepoint only and not at subsequent timepoints during follow-up). Only a single assessment per patient was included; thus, if patients were enrolled in more than one of the aforementioned studies, only the earlier assessment was counted in this analysis while the rest were not. Prior to VCE ingestion, SB patency was assessed by patency capsule (PC) ingestion. Patients with unpassed PC (ie, failure to extract PC in stool or its presence on abdominal X-ray within 30 hours) were excluded. As detailed in Supplementary Figure 1, patients were also excluded if any technical problems were encountered during the VCE procedure, if they had any high-risk features for capsule retention upon clinical/radiological assessment, if they withdrew consent, or if they met any per-study-specific exclusion criteria.

### 2.2. Assessment of mucosal inflammation

#### 2.2.1. SB activity assessment

The VCEs were read and interpreted by experienced readers. The SB was divided into 3 tertiles based on capsule transit time, as determined by the embedded capsule Rapid Reader software.[21] Segmental Lewis score (LS) was calculated based on the customary parameters, including villous appearance (ie, edematous vs normal), the number and extent of mucosal ulcers, and the presence of stenosis[21] for each tertile of the SB (ie, proximal, middle, and distal SB tertile LS). The tertiles' inflammatory activity was classified as the following: LS < 135: no inflammation/clinically insignificant inflammation, LS ≥ 135: presence of mucosal inflammation,[21] LS ≥ 350: clinically significant inflammation,[6] and LS ≥ 790: moderate-to-severe inflammation.[21] Data on the use of nonsteroidal anti-inflammatory drugs (NSAIDs) was collected and the use of these drugs was prohibited for 1 month before VCE ingestion.

#### 2.2.2. Assessment of colonic disease involvement

The presence of colonic inflammation was determined by at least one of the following procedures if they were conducted ≤6 months prior to SB-VCE ingestion: colonoscopy, cross-sectional imaging (ie, MRE or computed tomography enterography), IUS or pan-enteric capsule (PillCam Crohn's capsule).

#### 2.2.3. Criteria and definitions of disease anatomical involvement among the study's patients

To increase statistical power, and in line with the L4 classification of proximal CD which is associated with worse prognosis,[17,18] the 2 proximal tertiles of the SB were combined and designated 'Proximal SB' and compared with the third tertile (designated 'distal SB') for median FC levels. Similarly, we combined colonic and SB and colonic disease in these comparisons, given the dominance of colonic inflammation in driving FC results.

Patients, therefore, were categorized based on the following definitions into 5 subgroups based on disease anatomical extent:

- *No inflammation*—All 3 SB tertile's LS < 135, and no inflammation was detected in the colon.
- *Proximal SB inflammation*—proximal and/or middle SB tertile's LS ≥ 135, while no inflammation was detected in the distal SB or the colon.
- *Distal SB inflammation*—distal SB tertile's LS ≥ 135, while no inflammation was detected in the proximal SB or the colon.
- *Pan-SB inflammation*—the presence of both proximal and distal SB inflammation, while no inflammation was detected in the colon.
- *Colonic inflammation*—the presence of colonic inflammation with or without any extent of SB inflammation.

### 2.3. Data extraction

The following baseline characteristics were extracted from the studies' CRFs: age (years), sex (male/female), body mass index (BMI; kg/m$^2$), current smoking (yes/no), disease duration (years), age at diagnosis, disease anatomical extent, presence of perianal disease, presence of extra-intestinal manifestations, disease-behavior by Montreal classification (inflammatory [B1], stricturing [B2], and penetrating [B3][22]), history of CD-related hospitalization, history of CD-related intestinal resection, current use of biologics, CRP (normal range of 0-5 $\frac{mL}{L}$) and FC levels were measured by standard laboratory techniques. FC concentrations were reported as continuous values between the range of 30-1000 $\frac{\mu g}{g}$ (Buhlmann Laboratories AG, Basel, Switzerland).

### 2.4. Statistical analysis

Discrete and continuous variables were presented as proportions (%) and as median and interquartile range (IQR), respectively, following Shapiro–Wilk test for normal distribution testing. Pairwise median FC/CRP level comparisons between each subgroup to the other (ie, no inflammation, proximal SB inflammation, distal SB inflammation, pan-SB inflammation, and colonic inflammation) were performed using the Mann–Whitney U test. Correlations between each of the tertile's LS to FC and CRP levels were obtained using Spearman's correlation. William's test was used to assess for differences between correlations for dependent samples (psych package in R, version 3.3-1).

A receiver operating characteristic (ROC) curve was constructed, and the area under the curve (AUC) was calculated to explore the discriminatory accuracy of FC in different anatomical extent disease activity. Youden's most accurate points were computed for each ROC curve, as well as sensitivity, specificity, negative predictive value, positive predictive value,

positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio (DOR; ie, the odds ratio of a specific binary classifier, the designated Youden cutoff).[23] Sensitivity analyses were conducted to examine the trend regarding FC levels along the SB axis, with different cutoffs of disease activity as gauged by VCE-detected inflammatory severity (ie, LS ≥ 350 and LS ≥ 790). An additional sensitivity analysis was conducted to examine the diagnostic yield of FC to identify a clinically significant proximal SB inflammation (ie, LS ≥ 350[6]) in a specific clinical scenario, wherein upon initial work-up, no colonic but only mild distal inflammation was detected. All statistical tests were 2-sided, and $p = 0.05$ was considered statistically significant. Statistical analyses were conducted using SPSS software (IBM SPSS Statistics for Windows, version 26; IBM, Armonk, NY, 2019).

### 2.5. Study ethics and patient consent

This study was carried out in accordance with the ethical guidelines of the Declaration of Helsinki. The study was approved by the Sheba Medical Center ethics committee. Approval was granted for Helsinki protocol SMC-13-0218, SMC-18-4945, SMC-22-9902, SMC-20-7436, and SMC-17-4710. A written informed consent was obtained from all patients.

## 3. Results

### 3.1. Patients' baseline characteristics

Out of 340 patients included in the 5 prospective studies, 126 patients were excluded (66 due to unpassed PC or a technical fault, 31 due to withdrawal, pregnancy, or treatment changes, and 29 due to unavailable timely VCE or FC results or other technical causes; see Supplementary Figure 1 for details). Thus, 214 patients with CD were included (median age 30 [24-43]) year-old, male – 122/214 [57%]). Table 1 summarizes patients' baseline features. Most of the patients had inflammatory disease phenotype (60.9%) followed by stricturing disease phenotype (24.5%), and penetrating disease phenotype (14.6%).

Nearly half of the patients (96/214) had pan-SB inflammation, whereas the rest had isolated distal SB activity, proximal SB activity, colonic disease involvement, or no inflammation at all ($n = 48$, 18, 34, and 18, respectively).

### 3.2. FC levels in different disease locations

Analysis of the median values of FC levels in different disease locations along the gastrointestinal tract showed that FC levels gradually and significantly diminished with more proximal locations of disease activity (Figure 1). Specifically, patients with inflammation involving the colon had a higher median FC level compared to patients with even extensive SB inflammation (542 [185-1000] µg/g vs 223 [118-501] µg/g for colonic vs pan-SB inflammation, respectively, $p = 0.027$, Table 2). Furthermore, while the median FC level was higher in patients with distal SB inflammation compared to patients with proximal SB inflammation ($p = 0.005$), no statistically significant difference in FC levels was observed between proximal SB inflammation versus no inflammation state (63 [30-121] µg/g vs 30 [30-54] µg/g, respectively, $p = 0.068$). Patients with distal SB inflammation had a comparable median FC level regardless if they had additional proximal SB inflammation or not (223 [118-501] µg/g vs. 190 [78-549] µg/g, respectively, $p = 0.348$).

**Table 1** Baseline patients' characteristics.

| | CD patients with SB VCE and FC measure ($n = 214$) |
|---|---|
| *Demographics* | |
| Age (years old) median (IQR) | 30 (24-43) |
| Male *n* (%) | 122 (57%) |
| Body mass index (kg/m$^2$)^ | 23.1 (21.1-26.8) |
| Current smoking *n* (%)^ | 30 (14.1%) |
| *Disease related features*^ | |
| Disease duration (years) | 3 (1-7) |
| Age at diagnosis | 24 (19-34) |
| *Anatomical extent*^ | |
| Ileum (L1) *n* (%) | 124 (58.2%) |
| Colon (L2) *n* (%) | 4 (1.9%) |
| Ileo-colon (L3) *n* (%) | 85 (39.9%) |
| Proximal gastrointestinal involvement (L4) *n* (%) | 22 (10.3%) |
| Perianal involvement *n* (%) | 32 (15.2%) |
| Disease phenotype^ | 16 (88.9%) |
| B1 inflammatory *n* (%) | 129 (60.9%) |
| B2 stricturing *n* (%) | 52 (24.5%) |
| B3 penetrating *n* (%) | 31 (14.6%) |
| Extra-intestinal manifestations *n* (%)^ | 45 (21.3%) |
| Previous CD-related intestinal resection *n* (%)^ | 38 (17.8%) |
| Previous use of corticosteroids *n* (%)^ | 127 (59.9%) |
| Current use of biologics *n* (%)^ | 88 (41.3%) |
| *Baseline clinical and laboratory measures* | |
| C-reactive protein (ml/L) median (IQR)^ | 52 (24-110) |
| fecal-calprotectin (µg/g) median (IQR) | 182 (62-552) |
| *Baseline LS values (median [IQR])* | |
| Proximal tertile LS | 0 (0-225) |
| Middle tertile LS | 0 (0-225) |
| Distal tertile LS | 225 (135-768) |
| Conventional LS | 337 (225-900) |

Abbreviations: CD, Crohn's disease; VCE, video capsule endoscopy; IQR, interquartile range; LS, Lewis score; SB, small bowel; FC, fecal calprotectin.
^Data were missing for <3% of the cohort.

In sensitivity analyses, FC discriminatory capability to distinguish between proximal SB inflammation and no inflammation was increased when the presence of segmental inflammatory activity was defined by a higher endoscopic activity score of LS ≥ 350 ($p = 0.09$) which was previously suggested as more clinically significant inflammation,[6] and increased further to become statistically significant when inflammation was defined by an even higher cutoff of LS ≥ 790 corresponding to moderate-to-severe inflammation ($p < 0.001$, Supplementary Figure 2).

In correlation analyses among CD patients without colonic inflammation, the magnitude of the correlation of FC levels to LS inflammatory score values consistently increased along the SB axis, with the highest correlation between the distal SB tertile's LS and FC levels ($r = 0.634$, $p < 0.001$), followed by the middle SB tertile's LS to FC levels ($r = 0.379$, $p < 0.001$; $p < 0.001$) and FC levels to the proximal SB tertile's LS ($r = 0.260$, $p < 0.001$; $p < 0.001$). The FC to LS correlation

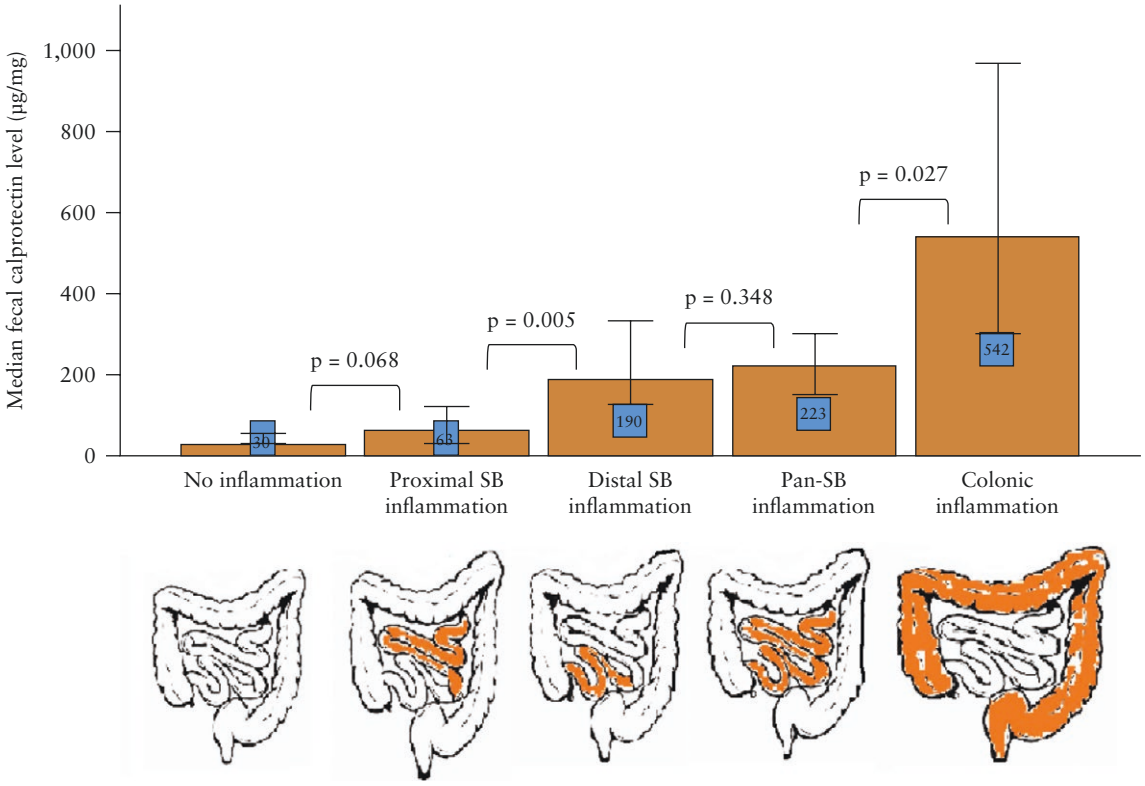


**Figure 1** Median fecal calprotectin level in different anatomical extent disease activity among patients with Crohn's disease. Disease inflammation was defined as a Lewis score ≥135.

was numerically higher in the middle compared to the proximal SB tertile ($p$ = 0.085, Figure 2).

### 3.3. CRP levels in different disease locations

As depicted in Supplementary Figure 3, patients with active inflammation of any anatomical extent had a higher median CRP level compared to patients without any inflammation (eg, no inflammation vs proximal SB inflammation, $p$ = 0.001), but still within the normal range (Table 2). However, contrasting with the FC gradient along the small and large intestines, the median CRP levels were comparable and unaffected by the specific segment inflicted with inflammation.

CRP levels to LS correlations in the same population were comparable throughout the SB as depicted in Figure 2 and were not associated with the location of active inflammation.

### 3.4. Diagnostic yield of FC

Table 3 details the diagnostic measures of FC in different segments of the small and large intestines. ROC analyses showed that for the same FC cutoff of 123 µg/g, the AUC and the DOR to detect distal SB inflammation versus no inflammation/proximal SB inflammation were 0.79 and 12.4 (4.1-38), an AUC of 0.82 with a DOR of 15.1 (5.5-41.3) for distinguishing distal SB to pan-SB inflammation from no inflammation/proximal SB inflammation, and AUC of 0.825 with DOR of 16.8 (6.2-45.7) to discriminate distal SB to colonic inflammation from no inflammation/proximal SB inflammation. Thus, 96% of the patients with FC of ≥123 µg/g had a distal SB and/or colonic inflammation. FC cutoff of 77 µg/g had a DOR of 8 (1.4-45.4) to best discriminate between an isolated proximal SB inflammation to no inflammation state.

In the specific clinical scenario where no colonic but mild distal inflammation (LS ≥ 135) was detected, FC of 234 µg/g was the best discriminative cutoff to identify clinically significant proximal SB inflammation (LS ≥ 350) with a DOR of 6 (2.6-13.7), on top of some distal SB inflammation (Figure 3).

## 4. Discussion

In this post hoc aggregated analysis of five prospective studies, we examined for the first time the diagnostic yield of FC for diagnosing CD inflammatory activity along the entire gastrointestinal tract, including the proximal SB, utilizing VCE as the reference method. We found that FC levels incrementally increased hand-in-hand with the extension of inflammation from proximal to distal parts of the SB. This longitudinal anatomical correlation was unique to FC and was not shared by CRP levels in the same population, which were unaffected by disease location.

Mucosal healing (MH) has been increasingly accepted as a paramount treatment goal in patients with CD, as it has been associated with improved long-term outcomes.[5] However, long-term MH monitoring in CD requires multiple invasive tests (mostly ileo-colonoscopy and VCE)[1–3] or imaging studies (MRE or IUS), which better gauge transmural inflammation rather than mucosal inflammation, *per se*. Therefore, surrogate inflammatory biomarkers (ie, CRP and FC) have become mainstay biomarkers for this clinical scenario,[2,5,24] with a higher performance of FC compared to CRP.[5] FC is the most abundant cytosolic protein in neutrophils and plays an important role in inflammatory processes. During gut inflammation, chemokines and cytokines lead to increased gut barrier permeability, which results in neutrophil migration to inflamed sites along the gastrointestinal tract, and subsequently to high levels of FC in stool.[4] Yet, the current literature on the correlation of FC to SB inflammation is conflicting, and

**Table 2** Median values of C-reactive protein (CRP) and fecal calprotectin (FC) in different inflamed SB and colon segments.

| Index | No inflammation (*n* = 18) | Proximal SB inflammation[#] (n = 18) | Distal SB inflammation[*] (*n* = 48) | Pan-SB inflammation (*n* = 96) | Colonic inflammation (*n* = 34) |
|---|---|---|---|---|---|
| CRP | 0.64 (0.50-1.73) | 4.53 (1.91-11.50) | 4.06 (1.40-9.11) | 3.13 (1.53-8.19) | 4.85 (1.52-14.44) |
| FC | 30 (30-54) | 63 (30-121) | 190 (78-549) | 223 (118-501) | 542 (185-1000) |

Abbreviations: SB, small bowel.
[#]Proximal SB inflammation was defined as proximal and/or middle SB tertile Lewis score of ≥ 135, while no inflammation was detected in the distal SB or colon.
[*]Distal SB inflammation was defined as distal SB tertile Lewis score of ≥ 135, while no inflammation was detected in the proximal SB or colon.

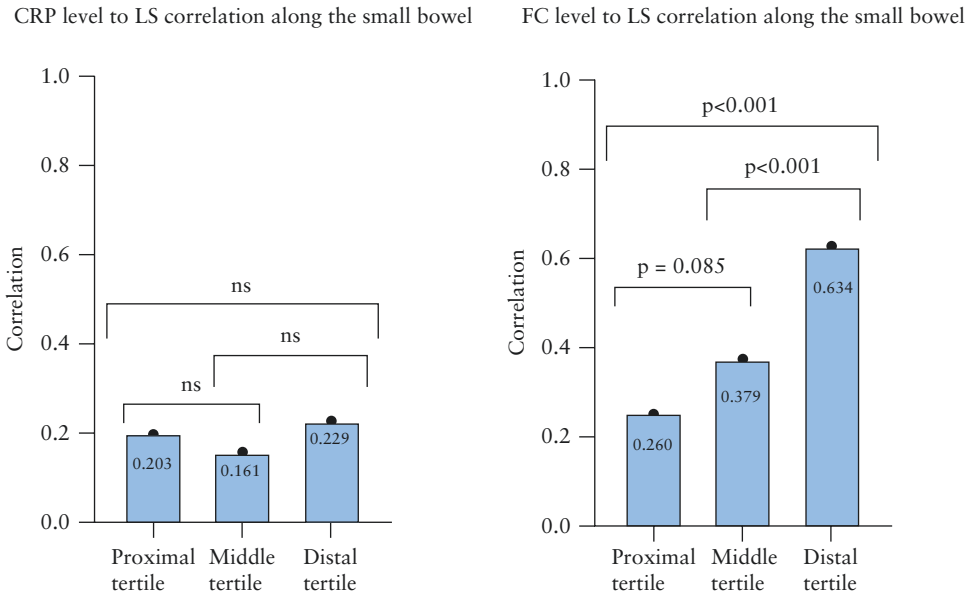


**Figure 2** Inflammatory biomarkers to Lewis score (LS) correlation along the small bowel in Crohn's disease patients without colonic inflammation.

clinically relevant FC cutoffs for distinguishing inflammatory activity restricted to the SB in CD patients are still debatable. Older studies have demonstrated a nonsignificant correlation between FC and SB mucosal activity, with the insufficient diagnostic performance of FC for isolated SB involvement in this population (sensitivity: 59%, specificity: 41%,[10] AUC: 0.52[11]), and a poor sensitivity to detect ileal-exclusive involvement in CD.[12] Contrasting these data, recently published studies showed an improved diagnostic yield of FC for SB-exclusive lesions in CD, as detected by both SB-VCE[13,25] and ileo-colonoscopy,[26] and even comparable FC levels in both SB and colon segments.[13,26] However, none of these studies explored FC performance for detecting inflammation in different SB segments or along the SB axis, as they all addressed the SB as a single entity. This shortcoming is clinically relevant given the known worse outcome of proximal SB-CD compared with distal SB-CD.[17]

Moreover, not only does the ability of FC to diagnose SB inflammation remain debatable, but the FC cutoffs which are best suited for this goal have been variable, and ranged from 76 µg/g to 236 µg/g and 265 µg/g in different studies.[13,25] FC cutoffs of 50[16] and 100[17] µg/g had the highest DOR values to identify inflammation in SB-CD, as determined by previously performed meta-analyses.[14,15] Some of the variability may stem from the heterogeneity of reference modalities used to determine SB inflammation. FC cutoffs of 95 µg/g and 72 µg/g were ideal for detecting ileal-active and inactive disease,[12,26] respectively, using ileo-colonoscopy as the reference method, whereas a cutoff of 170 µg/g had the best diagnostic performance for detecting SB inflammation as determined by retrograde enteroscopy (up to 210 cm proximal to the ileocecal valve/intestinal anastomosis).[27]

The fact that the aforementioned studies produced varying optimal cutoffs may also be explained by the lack of consideration of the anatomical extent of CD activity within the SB, which was neither reported nor considered. Moreover, VCE is known to disclose proximal disease involvement better than MRE[28] and can certainly ascertain it beyond the reach of ileocolonoscopy, so using other tools for reference may introduce additional heterogeneity. Therefore, we elected to use VCE as the reference method and investigated for the first time the diagnostic yield of FC for inflammation along the SB axis, using subgroups of patients with different disease anatomical extents. We found a higher sensitivity of FC for the same level of inflammatory activity in the distal compared to the proximal parts of the SB, corresponding to the gradual increment of FC cutoffs that best detected proximal SB inflammation, distal SB inflammation, and colonic disease involvement among the study population (ie, 77, 123, and 291 µg/g, respectively). Thus, a lower cutoff of 77 µg/g will be optimal to

**Table 3** Diagnostic measures of fecal calprotectin in different inflamed segments of the small bowel and colon among patients with Crohn's disease (CD) using a receiver operating characteristic (ROC) analysis.

| Disease anatomical extent | Comparative population | AUC (95% CI) | Youden index | Sen | Spec | PPV | NPV | PLR (95% CI) | NLR (95% CI) | DOR (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| [#]Proximal SB inflammation^,[+] | No inflammation | 0.67 (0.50-0.86) | 77 | 50% (26-74%) | 89% (65-98%) | 82% (53-95%) | 64% (52-74%) | 4.5 (1.13-18.00) | 0.56 (0.34-0.92) | 8.0 (1.4-45.4) | 0.027 |
| Isolated distal SB inflammation^,[+] | No inflammation or exclusive proximal SB inflammation | 0.79 (0.69-0.89) | 123 | 67% (52-79%) | 86% (70-95%) | 86% (73-94%) | 66% (56-75%) | 4.8 (2.08-11.10) | 0.39 (0.25-0.59) | 12.4 (4.1-38.0) | <0.001 |
| Distal ± proximal SB inflammation ^,[+] | No inflammation or exclusive proximal SB inflammation | 0.82 (0.74-0.90) | 123 | 71% (63-78%) | 86% (70-95%) | 95% (90-98%) | 42% (36-50%) | 5.1 (2.25-11.58) | 0.34 (0.25-0.45) | 15.1 (5.5-41.3) | <0.001 |
| Distal SB inflammation and/or colonic inflammation ± proximal SB inflammation^ | No inflammation or exclusive proximal SB inflammation | 0.825 (0.75-0.90) | 123 | 73% (66-79%) | 86% (70-95%) | 96% (92-98%) | 39% (33-46%) | 5.26 (2.32-11.92) | 0.31 (0.24-0.41) | 16.8 (6.2-45.7) | <0.001 |
| Clinically significant distal SB inflammation (LS cutoff ≥350) | No inflammation or exclusive proximal SB inflammation using LS cutoff ≥350. | 0.828 (0.768-0.887) | 146 | 81% (71-89%) | 72% (63-81%) | 68% (60-74%) | 84% (77-90%) | 2.94 (2.12-4.09) | 0.26 (0.16-0.42) | 11.4 (5.5-23.5) | <0.001 |
| Moderate-to-severe distal SB inflammation (LS cutoff ≥790) | No inflammation or exclusive proximal SB inflammation using LS cutoff ≥790. | 0.854 (0.796-0.913) | 258 | 83% (68-93%) | 78% (70-84%) | 52% (44-61%) | 94% (89-97%) | 3.72 (2.65-5.22) | 0.22 (0.11-0.43) | 16.9 (6.8-41.8) | <0.001 |
| [+]Clinically significant proximal SB inflammation[*] in patients with mild distal SB inflammation ^ | [+]Mild distal SB inflammation in patients without Clinically significant proximal SB inflammation[*] | 0.73 (0.64-0.82) | 234 | 76% (60-88%) | 66% (56-75%) | 47% (39-55%) | 87% (80-92%) | 2.23 (1.62-3.07) | 0.37 (0.21-0.64) | 6.0 (2.6-13.7 | <0.001 |
| Colonic inflammation | No inflammation or any extent of small bowel inflammation | 0.68 (0.57-0.78) | 291 | 71% (53-85%) | 65% (58-72%) | 28% (22-34%) | 92% (87-95%) | 2.02 (1.5-2.71) | 0.45 (0.27-0.77) | 4.4 (2.0-9.9) | <0.001 |

**Abbreviations:** AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; DOR, diagnostic odds ratio; CI, confidence interval; SB, small bowel; Sen, sensitivity; Spec, specificity; PLR, positive likelihood ratio; NLR, negative likelihood ratio.
[*]Lewis score (LS) ≥ 350.
[+]Without colonic inflammation.
^LS cutoff ≥ 135.
[#]Proximal SB inflammation was defined as proximal and/or middle SB tertile LS of ≥ 135, while no inflammation was detected in the distal SB or the colon.

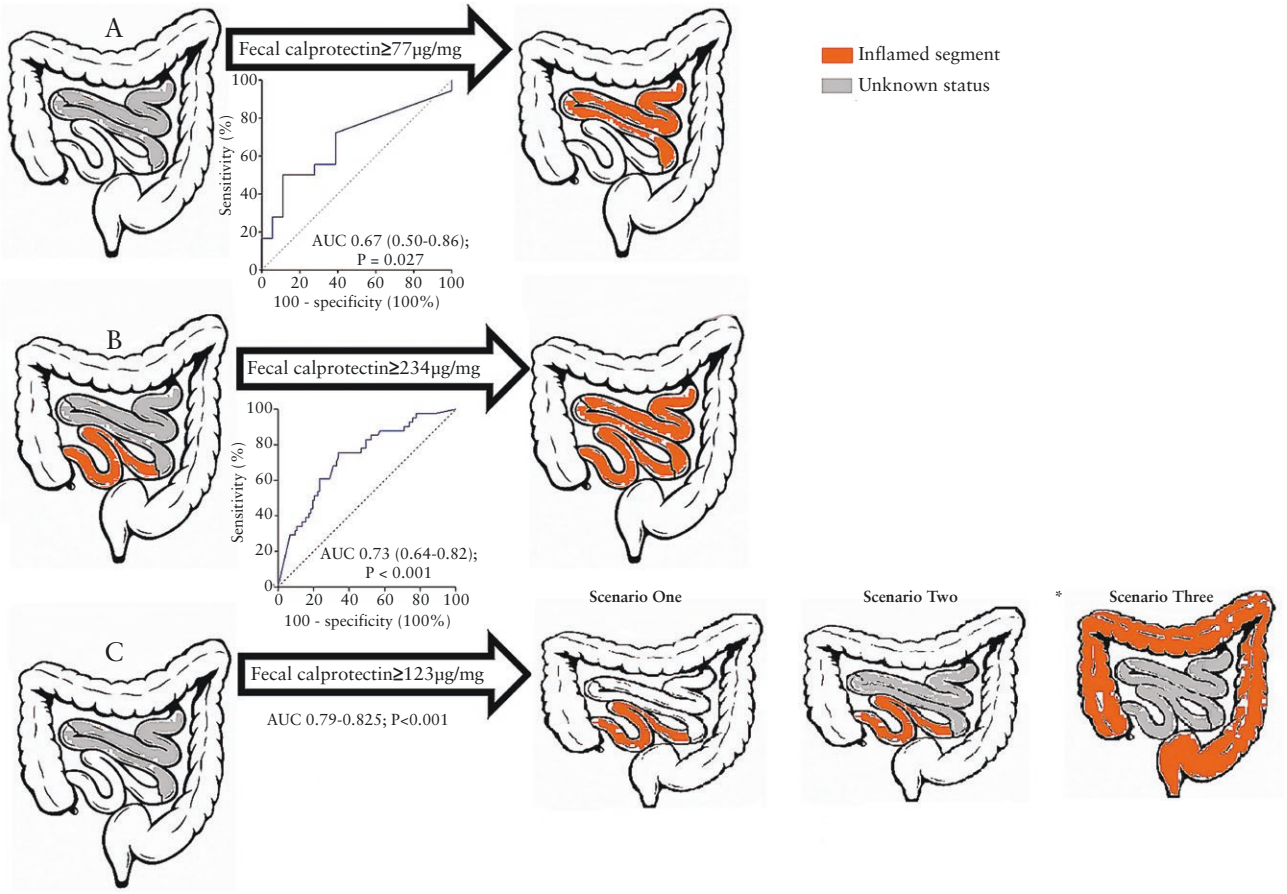


**Figure 3** The ideal cutoffs of fecal calprotectin (FC) based on receiver operating characteristic analysis to identify inflammation in distinct locales along the small and large intestines: (**A)** An isolated mild proximal small bowel (SB) inflammation (Lewis score [LS] ≥ 135), (**B)** A clinically significant proximal SB inflammation (LS ≥ 350) among Crohn's disease patients without colonic inflammation but with mild distal SB inflammation (LS ≥ 135). (**C)** Distal SB, pan-SB, and colonic inflammation. Abbreviations: AUC, area under the curve. *Colonic inflammation with/without SB inflammation of any extent.

inform on any possible proximal SB inflammation (LS ≥ 135) as opposed to a cutoff of 123 µg/g which was the best discriminator for more distal inflammation in the SB. Among CD patients who do have mild distal SB inflammation (LS ≥ 135) but no colonic inflammation, an FC of 234 µg/g had the best diagnostic value to identify clinically significant proximal SB inflammation (LS ≥ 350 which was previously shown to correlate with future flare risk,[6] AUC-0.73). Therefore, these 2 FC cutoffs may be utilized as a discriminative tool in the decision to proceed with VCE ingestion, when no inflammation or when only mild distal SB involvement is detected during ileo-colonoscopy, respectively.

The present study corroborated the superiority of FC over CRP as a better surrogate marker for mucosal inflammation. This was in line with the findings by Li et al. that showed a better correlation of FC to ileal/ileo-colonic mucosal inflammation than CRP to the latter (0.674 vs 0.560),[26] and with the STRIDE-II recommendations.[5] While FC levels and the FC to disease activity correlation (ie, LS) gradually increased along the SB and colon inflammatory topography, this phenomenon was not shared by the median CRP levels, which were comparable and within the normal range regardless of the specific inflamed locale along the gastrointestinal tract. Notably, the use of NSAIDs was prohibited 1 month before VCE ingestion, while the use of proton pump inhibitors was allowed.

This study has several limitations. First, as an aggregated post hoc analysis, this study may be subject to biases inherent in the original study designs. Second, there was no standardization in the assessment of colonic inflammation, and subsequently, we could not use a mucosal activity scoring system (eg, SES-CD) in the colon. However, the diagnostic yield of FC in patients with colitis has been well-established in the past, and the aim of this study was to evaluate its diagnostic yield along the SB axis. Third, we had a relatively small number of patients with LS ≥ 350 (previously suggested as more clinically significant inflammation[6]) and LS ≥ 790 (defined as moderate-to-severe mucosal inflammation[21]). Therefore, mucosal inflammation was defined as LS ≥ 135 in line with the conventional validated LS cutoff which defines a score ≥135 as an absence of MH. Still, the gradual trend of FC levels along the SB axis was numerically maintained, even for LS ≥ 350 and LS ≥ 790, despite the small number of patients in these subgroups. Fourth, our study had a relatively small number of patients with isolated proximal SB-CD, which limits the generalization of our findings, especially regarding the LS cutoffs of 350 and 790. However, the correlation analysis between LS and FC along the SB, including all patients with proximal disease involvement, was consistent with our primary findings, thus strengthening our conclusions. Finally, we excluded patients who failed to pass the PC to prevent VCE retention events. These patients might have significant

inflammatory and stricturing features, resulting in higher levels of FC. However, this exclusion criteria applies to any VCE research that excludes patients with failed PC. Since we did not have data regarding the exact location where the PC became lodged or whether this was due to delayed colonic transit, we could not topographically assess this data in line with the aim of this study.

In conclusion, the present study shows that for the same severity of mucosal inflammation, FC diagnostic levels incrementally increase with proximal to distal gradient of inflamed parts of the SB. We also found that isolated proximal SB inflammation in patients without any inflammation on ileocolonoscopy should be suspected at a relatively low FC cutoff of 77 µg/g, while in patients with mild distal SB activity, a higher FC level of 234 µg/g should prompt further work-up with VCE ingestion, as this cutoff may indicate clinically significant proximal SB inflammation. If corroborated by future studies, these novel findings may help inform clinicians on the astute interoperation of FC test results and better guide subsequent clinical decisions.

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## Conflict of Interest

Shomron Ben-Horin has received advisory board and/or consulting fees from Abbvie, Takeda, Janssen, Celltrion, Pfizer, GSK, Ferring, Novartis, Roche, Gilead, NeoPharm, Predicta Med, Galmed, Medial Earlysign, BMS and Eli Lilly, holds stocks/options in Predicta Med, Evinature & Galmed, and received research support from Abbvie, Takeda, Janssen, Celltrion, Pfizer, & Galmed. Uri Kopylov received speaker and consultancy fees from Abbvie, BMS, Elly Lilly, Celtrion, Medtronic, Janssen, Pfizer, Roche, and Takeda, research support from Abbvie, Elli Lilly, Medtronic Takeda, and Janssen. Rami Eliakim received consultant and speaker fees from Janssen, Abbvie, Takeda, and Medtronic. Bella Ungar received consultation fees from Neopharm, Takeda, Janssen, and AbbVie. Ahmad Albshesh received speaking and lecturing fees from Takeda, Janssen, and AbbVie. The remaining authors declare that they have no conflicts of interest.

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## Author Contributions

S.B.H. conceived the study and participated in the study design and in drafting of the manuscript; O.U. participated in the study conception and design, acquired and analyzed the data, and drafted the manuscript. O.U., U.K., B.U., A.T.A., E.S., T.E., A.A., D.Y., A.L., R.E., and S.B.H. participated in the acquisition of data, in data interpretation, and in critical revision of the manuscript for important intellectual property. All authors have approved the final draft submitted.

## Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

## Supplementary Data

Supplementary data are available online at *ECCO-JCC* online.

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