Original Article

Variability of Faecal Calprotectin in Inflammatory Bowel Disease Patients: An Observational Case-control Study

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Abstract

Background and Aims: Several factors have been reported to affect faecal calprotectin [FC] values, and significant variation in FC concentrations has been observed in inflammatory bowel disease [IBD] patients. We aimed to evaluate FC variability in IBD patients, and to assess the robustness of a single stool punch.

Methods: This is a single-centre observational case-control study. Disease activity was assessed using endoscopic and clinical activity scores, as well as C-reactive protein levels. Stool samples were collected twice within a 1 to 6 days interval, and FC was measured on punches and homogenates by fluorometric enzyme immunocapture assay.

Results: In all, 260 stool samples were collected from 120 patients. Intrastool variability was low, with an intraclass correlation coefficient for single measures between three punches from a single stool sample of 0.91, and median coefficient of variation [CV] of 17%. CV of two stool samples a few days apart [intra-individual variability] were significantly higher [$p < 0.01$] with median CV of 36%. FC standard deviations correlated with mean FC levels either for intrastool or for intra-individual variability, with a Spearman's coefficient of rank correlation of 0.85 and 0.78, respectively [$p < 0.01$]. Disease type, location, activity, and FC levels did not influence variability.

Conclusions: A single stool punch is reliable for FC measurement, considering that intrastool variability is low. Intra-individual variability a few days apart is significantly higher. Therefore, decision-making strategies based on single measurements should consider this variability, to determine the minimum optimal variation to be achieved, rather than a cut-off, especially in high FC levels.

Key Words: Faecal calprotectin; variability; inflammatory bowel disease

1. Introduction

The heterodimeric calcium- and zinc-binding protein, calprotectin, mainly found intracellularly in neutrophils, accounts for about 60% of cytosolic proteins of these cells.¹ Its level in faeces is proportional to the amount of neutrophil migration into the bowel lumen from the inflamed wall.^{2,3} Faecal calprotectin [FC] is therefore a reliable biomarker of intestinal inflammation,^{3–8} and is currently the most sensitive noninvasive surrogate marker of disease activity in inflammatory bowel disease [IBD]. FC is nowadays widely used in the management of IBD patients.⁹

The differential diagnosis of IBD from irritable bowel syndrome [IBS] may be difficult based on clinical symptoms only, due to nonspecific clinical presentation of IBD, especially in Crohn's disease [CD]. FC is a valuable non-invasive diagnostic and monitoring marker in IBD patients, limiting the number of unnecessary endoscopies. Although there is no strong correlation between clinical scoring systems for IBD and FC levels, FC has a strong correlation with endoscopic and histological remission both in CD and in ulcerative colitis [UC].¹⁰ FC is increasingly used for patients in clinical remission to predict disease relapse^{11–13} or mucosal healing, and in active disease to monitor response to therapy.^{14–16}

FC values depend on disease type [CD or UC], disease activity [quiescent or active disease], and disease location. FC is more sensitive in assessing disease activity in UC than in CD,¹⁷ whereas in CD it is more sensitive for colonic or ileocolonic involvement compared with isolated ileal involvement.¹⁴ However, considerable overlaps in FC values exist among patients with different disease severity profile,^{14,18} and several studies found no correlation between baseline FC and response to treatment.^{15,19,20}

FC is highly resistant to bacterial and enzymatic degradation and is considered to be homogeneously distributed throughout the stool.²¹ In the presence of calcium, FC is stable in faeces at room temperature for up to 7 days.^{2,3,22} However, several factors have been reported to affect FC values, and a significant day-to-day^{23,24} and within-day²⁵ variation in FC concentrations has been observed in IBD patients, mainly in subgroups of patients [UC or CD and quiescent or active disease]. Known factors are colonic cleansing,²⁶ age,^{27,28} diet, or exercise.²⁸ In patients with active IBD, the presence of mucus or blood can vary widely between bowel movements, and could therefore influence FC levels.^{26,28} Stool consistency and the time of the day of collection have been reported to influence variability, with opposite results according to some studies. Due to this intra-individual variability, FC-based decision-making strategies based on single measurements of FC might be wrong. Hence, there is a need to better grasp the intra-individual variability to ensure correct interpretation of a single measurement during IBD follow-up. In most laboratories, FC measurement is currently done on a single stool punch defined as a small random sample of approximately 100 mg of faeces taken anywhere in the stool sample. An even distribution of calprotectin in the faeces is crucial to ensure the reliability of the test. However, there are no data about the reliability of a single stool punch of 100 mg. There is a growing interest in stool extraction methods and intrastool variability, because they might be relevant to FC-based decision-making strategies.

The increasing use of FC in clinical practice involves not only the clinical evaluation of disease activity on the dichotomous positive or negative interpretation of FC based on a single manufacturer's cut-off value, or a calculated cut-off value. Indeed, a modification of FC over time within the same patient is currently used in clinical practice as a marker of response to treatment or relapse.

2. Methods

2.1. Aim of the study

The aim of this study was to assess FC variability by evaluating intra- and intervariability of FC in a cohort of IBD and IBS patients [namely controls]. Intravariability included intrastool and intra-individual variability. The primary objective was to determine whether there is significant FC variability: [1] within a single bowel movement [intrastool variability]; [2] between two different bowel movements within a 1–6 days period [intra-individual variability]; and [3] between IBD patients [interindividual variability]. The secondary objective was to assess the robustness of using a single stool punch for FC measurement. Therefore, evaluation of intrastool variability was useful to determine whether FC is evenly distributed within the stool sample.

2.2. Study design and ethical considerations

This observational case-control study with prospective stool collection and retrospective analyses of the patient records was conducted in a single IBD tertiary referral centre, Erasme University Hospital [Université Libre de Bruxelles, Brussels, Belgium], after Ethics Committee agreement [reference number: 2014/229, approval date: June 17, 2014]. Each patient gave informed consent in accord with ethical guidelines of the 1975 Declaration of Helsinki. Patients with an established diagnosis of IBD or IBS were invited to participate in the study between March 2014 and August 2016.

2.3. Definitions

Intrastool variability was defined as FC variability between three punches in the same stool sample. Intra-individual variability was defined as FC variability between two stool samples collected a few days apart from the same patient. Interindividual variability was defined as the variability between IBD patients who differ by disease type [CD/UC], location [ileal/ileocolonic, and colonic CD] or activity [quiescent/active]. A punch was defined as a small random sample of approximately 100 mg of faeces taken anywhere in the stool sample, and a homogenate as a sample of 5 to 10 g of stool.

2.4. Data collection

All stool samples were collected prospectively. Clinical, endoscopic, and histological data were collected retrospectively by electronic chart review. Disease activity at the time of collection of the stool samples in IBD patients was assessed by clinical activity scores (Harvey–Bradshaw Index [HBI] in CD²⁹ and partial Mayo score in UC), C-reactive protein [CRP] levels, and endoscopic activity scores [presence/absence of ulcers for CD, endoscopic Mayo subscore in UC] when available. Active disease was defined as a Mayo score >2 for UC [3–5: mild; 6–10: moderate; 11–12: severe] or in the presence of endoscopic ulcers for CD. If a reliable endoscopy was not available, active disease was defined as a partial Mayo score >1 for UC [2–4: mild; 5–6: moderate; 7–9: severe] or a HBI >4 [5–7: mild; 8–16: moderate; >16: severe], or CRP above the normal range for CD. An endoscopy is defined as reliable for disease activity evaluation in case of quiescent disease if performed up to 2 years before stool sampling without treatment modification [escalation or de-escalation] or clinical relapse [based on symptoms and/or CRP levels]. In case of active disease, criteria are more stringent in terms of timing and it is then a case-by-case evaluation according to the changes in treatment occurring around the stool sampling.

2.5. Stool samples

Patients were instructed to collect two stool samples from two bowel movements within a 1 to 6 days interval, and to either return the samples to the hospital on the day of collection, or to store them at 4°C temperature and return them within maximum 5 days of collection. The presence of visible blood was recorded. Stool sampling was not allowed to be done during bowel cleansing or within 48 h of a colonoscopy, to avoid the impact of bowel cleansing on FC levels.²⁶ All samples were stored at -20°C before FC extraction, and measurements were done within 3 months of storage.

2.6. Faecal calprotectin extraction and measurement

Extraction was performed with both the conventional method [Roche device] and the homogenate method [see Supplementary materials, available as Supplementary data at ECCO-JCC online, for more precision about these methods]. FC was extracted from three punches of the first stool sample [Day 0] of more or less 100 mg of stool, and on two homogenates [Day 0 and Day 1–6] of 5g to 10 g of stool, depending on the quantity of faecal material available. For both sample types, i.e. punch and homogenate, the volume of extraction buffer was adapted to the stool weight so that the ratio weight/volume remained constant for all stool samples. Extracts were analysed using EliA Calprotectin fluorescence enzyme immunoassay on the Phadia® 250 [Thermo Fisher Scientific, Groot-Bijgaarden, Belgium] following manufacturer's instructions. Proposed cut-off value is ≤50 µg/g. Measuring ranges were 15–3000 µg/g. Samples scoring <15 µg/g were registered as 5 µg/g and those scoring ≥3000 µg/g as 3000 µg/g.

2.7. Statistical analyses

Data were analysed using MedCalc Statistical Software [version 18.5, MedCalc Software bvba, Ostend, Belgium]. Continuous variables were reported as medians with interquartile ranges [IQR] or ranges [minimum-maximum]. Categorical variables were reported as numbers [*n*] and proportions [%]. Comparisons of continuous variables were performed using a Mann-Whitney or Kruskal-Wallis test. Comparisons of categorical variables were performed using a Fisher's exact test or Pearson chi square test for trend. Extraction methods comparison for laboratory use of the homogenate was performed using the non-parametric Spearman rank correlation coefficient, Bland-Altman plot and the Passing-Bablok regression analysis. To compare FC values of different punches or homogenates from the same stool sample or from different stool samples, a Wilcoxon test was used. Intraclass correlation coefficients [ICC], which represent the degree of consistency among measurements, were calculated with 95% confidence interval [CI]. This would be equal to 1 if there was no within-stool variation. To measure variability, coefficients of variation [CV] were calculated. To assess intrastool variability,

coefficients of variation [CV] of the FC values of the three stool punches and the homogenate from the same sample from each patient were calculated. To assess intra-individual variability, CVs of the two samples within a 1 to 6 days interval from each patient were calculated. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Study population

A total of 122 patients were enrolled between March 2014 and August 2016. Two patients were excluded due to failure to return stool samples; 120 patients were included in the study for a total of 260 stool samples collected; 18 patients were included twice at 6 months to 1-year interval for a total of 138 inclusions; 16 patients failed to return the second stool sample a few days apart. Due to sometimes insufficient faecal material, all analyses (three punches and one homogenate on the first stool sample [Day 0] and one homogenate on the second stool sample [Day 1–6]) were not always possible.

The demographics of the study population and baseline characteristics of the IBD patients are summarised in **Tables 1** and **2**. Among the 77 CD patients, 24 had ileal disease, 40 ileocolonic disease, and 13 colonic disease. In UC patients, 20 had left UC and 14 had pancolic UC. Endoscopic assessment was available in 111/130 [85%] IBD inclusions.

3.2. Intravariability

3.2.1. Intrastool variability

To determine whether FC was evenly distributed within the stool and to assess the representativeness of small random stool samples [punches], FC levels in three punches from the same first stool sample [Day 0], from a single bowel movement for each inclusion, were compared and calculation of the CV of the three punches was done. In all, 99 of the 138 first stool samples were considered for punches analyses; one sample was excluded due to insufficient faecal material; and 38 samples were excluded due to FC value below 15 µg/g or equal to or above 3000 µg/g, that could have underestimated the variability. No statistical difference was shown in terms of FC value between the punches, either for the entire study population or within the six different subgroups of IBD patients [active ileal CD, quiescent ileal CD, active ileocolonic/colonic CD, quiescent ileocolonic/colonic CD, active UC, and quiescent UC] [*p* > 0.05]. A strong correlation (ICC: 0.9148; 95% confidence interval [CI]: 0.8835–0.9392) was found in terms of FC concentrations between the three punches. Median CVs were between 13% and 27%, depending on the subgroup of patients with a median CV of 17% [range: 3–113] for the entire study population [Supplementary Table 1, available as Supplementary data at ECCO-JCC online]; 39% of the patients had a CV below 10% and 61% were above 10%. There was no statistical difference in the

Table 1. Demographics of the study population.

	CD [<i>n</i> = 77]	UC [<i>n</i> = 34]	IBS [<i>n</i> = 9]	All patients [<i>n</i> = 120]	<i>p</i> -value*
Age [yr], median [range]	36 [15–83]	37 [23–74]	28 [16–62]	36 [15–83]	0.64
Male, <i>n</i> [%]	27 [35]	14 [41]	3 [33]	44 [37]	0.81
Disease duration [yr], median [IQR]	7 [4–14]	3 [1–7]	NA	6 [2–11] <i>n</i> = 111	<0.01
Current smoker, <i>n</i> [%]	26 [34]	5 [15]	2 [22]	33 [27]	0.11

CD, Crohn's disease; UC, ulcerative colitis; IBS, irritable bowel syndrome; *n*, number of patients; yr, years; IQR, interquartile range; NA, not applicable.

*p-value comparing different outcomes. Bold values are significant.

Table 2. Baseline characteristics of IBD patients

Disease location	Ileal CD [<i>n</i> = 24] <i>N</i> = 25	Ileocolonic/colonic CD [<i>n</i> = 53] <i>N</i> = 61	Left/pancolic UC [<i>n</i> = 34] <i>N</i> = 44	Total [<i>n</i> = 111] <i>N</i> = 130	<i>p</i> -value*
Ongoing treatment, <i>N</i> [%]					
5-ASA	4 [16]	14 [23]	32 [73]	50 [38]	<0.01
Systemic CS	2 [8]	11 [18]	10 [23]	23 [18]	0.59
Topic CS	1 [4]	7 [11]	14 [32]	22 [17]	<0.01
Thiopurines	8 [32]	13 [21]	5 [11]	26 [20]	0.13
Methotrexate	2 [8]	10 [16]	3 [7]	15 [12]	0.23
Anti-TNF	10 [40]	38 [62]	15 [34]	63 [48]	0.02
Previous surgery, <i>N</i> [%]	5 [20]	19 [47]	1 [2]	25 [19]	<0.01
Disease activity	Active	Inactive	Active	Inactive	Inactive
<i>N</i> [%]	13 [52]	12 [48]	31 [70]	13 [30]	51 [49]
HBI, median [IQR]	6 [2–6] <i>N</i> = 11	1 [0–4] <i>N</i> = 12	35 [57]	5 [4–8] <i>N</i> = 35	26 [43]
Partial Mayo score, median [IQR]					1 [0–2] <i>N</i> = 25
Endoscopic Mayo subscore, median [IQR]					5 [4–7] <i>N</i> = 29
Total Mayo score, median [IQR]					3 [2–3] <i>N</i> = 27
CRP [mg/l], median [IQR]	16 [3–50.3] <i>N</i> = 13	4.7 [2.2–7.7] <i>N</i> = 12	12 [5.4–34] <i>N</i> = 35	4.1 [2.2–7.1] <i>N</i> = 26	1 [0–1] <i>N</i> = 9
					1 [1–2] <i>N</i> = 9
					8 [7–10] <i>N</i> = 26
					6.6 [1.1–22.8] <i>N</i> = 31
					2 [0.6–3.9] <i>N</i> = 13
					9.8 [3.3–26] <i>N</i> = 79
					3.1 [1.4–6.7] <i>N</i> = 51

*n = number of patients; *N* = number of inclusions; CS: corticosteroid; 5-ASA: 5-aminosalicylic acid; TNF: tumour necrosis factor; CD: Crohn's disease; UC: ulcerative colitis; IBD: inflammatory bowel disease; HBI: Harvey-Bradshaw Index; IQR: interquartile range; CRP: C-reactive protein. *p-value comparing different outcomes. Bold values are significant.

CVs, either between the seven subgroups of patients [$p = 0.5744$] or between CD and UC [$p = 0.7079$], or between active and quiescent disease [$p = 0.3352$], or between low FC values [when at least two of the three punches were $<250 \mu\text{g/g}$] compared with higher FC values [$\geq 250 \mu\text{g/g}$] [$p = 0.8758$]. FC standard deviations correlated with mean FC levels [Spearman's coefficient of rank correlation = 0.854, $p < 0.0001$]. The variation was most pronounced in patients with high levels of FC [Figure 1]. Disease type, location, activity, and FC levels do not influence intrastool variability.

3.2.2. Intra-individual variability

To evaluate intra-individual variability, FC values of two stool samples from two different bowel movements, a few days apart for each inclusion, were compared and CVs between the two homogenates were calculated; 80 of the 122 inclusions that provided two stool samples were considered for analyses; 10 were excluded

due to insufficient faecal material; and 32 were excluded due to FC value below $15 \mu\text{g/g}$ or equal to or above $3000 \mu\text{g/g}$, that could have underestimated the variability. No statistical difference was shown in terms of FC value between the homogenates a few days apart, either for the entire study population or within the six different subgroups of IBD patients [$p > 0.05$]. ICC for single measures between the two homogenates was 0.7868 [95% CI: 0.6863–0.8578] and Spearman rank correlation coefficient was 0.743 [$p < 0.0001$] [Figure 2]. Median CVs of the two samples were between 27% and 58%, depending on the subgroup of patients with a median CV of 36% [range:0–123] for the entire study population [Supplementary Table 2, available as Supplementary data at ECCO-JCC online]; 14% of the patients had a CV below 10% and 86% were above 10%. We did not show any statistical difference in the CVs between the six subgroups of patients [$p = 0.8337$]. FC standard deviations correlated with mean

Figure 1. Correlation between mean faecal calprotectin [FC] concentrations of the three punches and the corresponding standard deviations.

Figure 2. Comparison plot of faecal calprotectin [FC] concentrations in $\mu\text{g/g}$ of the two homogenates a few days apart. The continuous line is the best fitted linear line, and the dotted line is $y = x$ and represents the ideal result where sample 1 = sample 2. Spearman rank correlation coefficient was 0.743 [$p < 0.0001$].

FC levels [Spearman’s coefficient of rank correlation = 0.781, $p < 0.0001$]. CVs of the two homogenates a few days apart were significantly higher compared with CVs of the three punches from the first stool sample [$p = 0.0001$].

3.3. Intervariability

Intervariability was evaluated between IBD patients who differed by disease type [CD/UC], location [ileal/colonic], or activity [quiescent/active] and IBS patients [defined as controls]. Median FCs for each group are represented in Table 3 and Figure 3. All IBS patients had FC below 29 $\mu\text{g/g}$. FC values were significantly higher in active UC compared with active CD [$p = 0.0031$]. The area under the curve for FC as a diagnostic test for IBD activity was 0.862 [$p < 0.0001$], with a Youden index of 0.59 for a cut-off value of 122 $\mu\text{g/g}$ [sensitivity 81%, specificity 78%]. CRP values were also significantly higher in active compared with quiescent IBD or IBS [$p < 0.0001$]; see Supplementary data, available at ECCO-JCC online, for more details about comparison of FC and CRP to predict disease activity.

Table 3. Median FC.

Groups	N	Median FC [$\mu\text{g/g}$]	Range
1 Quiescent ileal CD	12	17.5	5–643
2 Active ileal CD	13	490	59–1433
3 Quiescent ileocolic/colic CD	26	71	5–1247
4 Active ileocolic/colic CD	35	296	5–3000
5 Quiescent UC	13	21	5–656
6 Active UC	31	1614	5–3000
7 IBS	10	5	5–29
Total	140	163	5–3000

CD: Crohn’s disease; UC: ulcerative colitis; IBS: irritable bowel syndrome; FC: faecal calprotectin; N = number of inclusions.

4. Discussion

The present observational case-control study revealed that a single stool punch of 100 mg is reliable and representative of the entire stool sample for FC measurement, considering that intrastool variability is low and that a good correlation exists between the punches and the homogenate, a novel extraction method that assesses the robustness of using a single stool punch for FC measurement. However, CVs of two stool samples a few days apart [intra-individual variability] were significantly higher compared with CVs of the three punches, with median CVs ranging from 27% to 58% depending on the subgroup of IBD patients. Although intra-individual variability in FC levels occurs, the intrastool and intra-individual variation were not significant. The variability was not influenced by disease type, location, activity, or FC levels. It is of great importance to know the extent of FC variability to be able to correctly interpret the clinical significance of FC variation in the follow-up of IBD patients.

In most laboratories, FC measurement is currently done on a single stool punch. Even though volume-based extraction devices such as the faecal sample preparation kit [Roche device] [Roche Diagnostics GmbH®], which bypass the step of weighting every single faecal sample, are nowadays widely used, the reference method is still the weighting method that was used in this study. An even distribution of calprotectin in the faeces is crucial to ensure the reliability of such test results. In the study of Roseth *et al.*,²¹ the authors collected stool samples during 24 h and showed good correlation between three random stool samples of 5 g and the rest of the thoroughly blended stool, with a median CV of 21%. However, a 5-g sample is not comparable to a 100-mg sample, and we were among the first to show the reliability of a single stool punch of 100 mg, even more in the different subgroups of patients for FC measurement with a median CV of 17%. It is also important to stress that due to a lack of international standardisation for FC measurement and variability among FC measurement methods, it is recommended

Figure 3. Faecal calprotectin [FC] values according to disease type, location, and activity. FC is expressed in medians and 95% confidence intervals for medians. 1 = quiescent ileal CD; 2 = active ileal CD; 3 = quiescent ileocolonic/colonic CD; 4 = active ileocolonic/colonic CD; 5 = quiescent UC; 6 = active UC; and 7 = IBS [controls]. [1] is different from [2], [4], and [6]; [2] is different from [1], [3], [5], [6], and [7]; [3] is different from [2], [4], [6], and [7]; [4] is different from [1], [3], [5], [6], and [7]; [5] is different from [2], [4], and [6]; [6] is different from [1], [2], [3], [4], [5], and [7]; [7] is different from [2], [3], [4], and [6] [$p < 0.05$]. CD, Crohn’s disease; UC, ulcerative colitis.

to use the same method to follow a specific patient because results from different methods are not commutable.³⁰ Therefore, it is not recommended to extrapolate these results to other extraction devices or to other assays.

This study cohort is relatively large in comparison with other studies that have evaluated FC variability. Furthermore, active or inactive UC and CD patients, as well as a control group, were included in the present study, showing that the results are applicable for all IBD patients. Indeed in previous studies, significant variation in FC concentrations has been observed, mainly in subgroups of IBD patients [UC or CD with quiescent or active disease].^{25,31–33}

Our results in terms of intra-individual variability of FC are in agreement with other recent studies.^{25,31} Calafat *et al.*³¹ showed a median CV of 40% in samples collected during the same day, and Lasson *et al.*²⁵ showed a median CV of 52% and a strong correlation in terms of FC concentrations between two random samples collected during the same day [within-day variation] with an ICC of 0.79. Our median CV in samples collected a few days apart [day-to-day variation] was 36% with the same ICC of 0.79.

Some points in our study could be criticised. No instructions were given to our patients as to the time of day when they were to collect their samples. Indeed, unlike other studies, we did not require a sample from the first bowel movement. Regarding this issue, Lasson *et al.*²⁵ found that FC sampling should be taken from the first bowel movement of the morning, given a significant positive correlation between the level of FC and the time between bowel movements. However, Kristensen *et al.*³³ did not show significant intra-individual diurnal variation, even though this was on a limited number of patients [$n = 50$]. In the same way, Calafat *et al.*³¹ showed that the timing of stool sampling does not seem to be relevant, because the highest or lowest FC values may also vary during the day. Du *et al.*,³² although suggesting that the morning sample provided the lowest clinically significant intrastool FC variability, did not show any difference in terms of intrastool variability depending on the time of the day. Given the results of those studies, there is actually no demonstrated benefit in sampling the first stool in the morning for FC measurement. Moreover, we did not assess some macroscopic characteristics of stools, such as mucus or consistency, that might influence FC levels. However, Lasson *et al.*²⁵ were not able to find a correlation between stool consistency, content of blood, or time between bowel movements and CV. However, sampling error due to, for example, stool with non-homogeneous distribution of mucus or blood, was controlled by the fact that FC concentrations of the homogenates were very well correlated with the punches, which challenges the assumption that non-homogeneous distribution of mucus or blood may have an impact on FC variability.

In conclusion, the present study reveals that FC is a good marker of activity in IBD, in CD and in UC, regardless of location [ileal and ileocolonic/colonic CD]. A single stool punch is reliable for FC measurement, considering that intrastool variability is low. Intra-individual variability a few days apart is significantly higher. Therefore, decision-making strategies based on single measurements should take into account this variability, so as to determine the minimum optimal variation to be achieved in treat-to-target strategy, rather than a cut-off, especially in high FC levels.

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Conflict of Interest

All authors have no conflict of interest to disclose. The authors did not have any writing assistance in producing this manuscript.

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Author Contributions

All authors contributed to the concept and design of the study; PS and JS coordinated processing of samples; JK, MRB, and FB analysed the stool samples; collection, analysis, and interpretation of the data were performed by AC and JK; AC and JK performed the statistical analysis; AC made the draft of the manuscript with contribution from DF; all authors carried out critical revision of the manuscript for important intellectual content; all authors read and approved the final version of the manuscript.

Supplementary Data

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

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