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Accuracy of Faecal Calprotectin and Neutrophil Gelatinase B-associated Lipocalin in Evaluating Subclinical Inflammation in UlceRaTIVE Colitis—the ACERTIVE study

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Abbreviations: 5-ASA: 5-aminosalicylic acid or 'mesalazine'; AUC: area under the curve; AZT: azathioprine; FC: faecal calprotectin; IBD: inflammatory bowel disease; IBS: irritable bowel syndrome; IQR: interquartile range; MH: mucosal healing; NGAL: neutrophil gelatinase B–associated lipocalin; NPV: negative predictive value; PPV: positive predictive value; ROC: receiver operating characteristic; UC: ulcerative colitis; UCEIS: Ulcerative Colitis Endoscopic Index of Severity.

Abstract

Background and Aims: Mucosal healing and histological remission are different targets for patients with ulcerative colitis, but both rely on an invasive endoscopic procedure. This study aimed to assess faecal calprotectin and neutrophil gelatinase B–associated lipocalin as biomarkers for disease activity in asymptomatic ulcerative colitis patients.

Methods: This was a multicentric cross-sectional study including 371 patients, who were classified according to their endoscopic and histological scores. These results were evaluated alongside the faecal levels of both biomarkers.

Results: Macroscopic lesions [i.e. endoscopic Mayo score ≥1] were present in 28% of the patients, and 9% had active disease according to fht Ulcerative Colitis Endoscopic Index of Severity. Moreover, 21% presented with histological inflammation according to the Geboes index, whereas 15% and 5% presented with focal and diffuse basal plasmacytosis, respectively. The faecal levels of calprotectin and neutrophil gelatinase B–associated lipocalin were statistically higher for patients with endoscopic lesions and histological activity. A receiver operating characteristic–based analysis revealed that both biomarkers were able to indicate mucosal healing and histological remission with an acceptable probability, and cut-off levels of 150–250 μg/g for faecal calprotectin and 12 μg/g for neutrophil gelatinase B–associated lipocalin were proposed.

Conclusions: Faecal calprotectin and neutrophil gelatinase B–associated lipocalin levels are a valuable addition for assessment of disease activity in asymptomatic ulcerative colitis patients. Biological levels of the analysed biomarkers below the proposed thresholds can rule out the presence of macroscopic and microscopic lesions with a probability of 75–93%. However, caution should be applied whenever interpreting positive results, as these biomarkers present consistently low positive predictive values.

Key Words: Endoscopic and histological remission; faecal markers; ulcerative colitis

1. Introduction

Ulcerative colitis [UC] is a chronic inflammatory condition of the bowel characterized by a relapsing and remitting course.[1] This disease [IBD] causes a continuous mucosal inflammation of the colon that can affect the rectum and a variable extension of the colon.[1] The characteristic lesions found in the mucosa are intense basal plasmacytosis—defined as presence of plasma cells around or below the crypts—an increase in the diffuse transmucosal lamina propria cells, and a widespread architectural distortion of the mucosa or crypts.[2]

Mucosal healing [MH], routinely assessed by endoscopy, is considered a prognostic marker, as it has been described as able to predict disease outcomes such as steroid need, hospitalization, and colectomy.[3–5] Therefore, MH has emerged as an important clinical factor in disease progression, both for Crohn's disease and UC patients.[3,4,6,7]

However, from an histological point of view, recovery is often incomplete, and microscopic evidence of inflammation persists in 16–100% of the patients with endoscopically quiescent colitis.[5,8] In fact, histologic changes tend to lag behind clinical response and/or remission after starting UC treatment. This quiescent inflammation puts patients at risk of further disease relapses or of developing disease complications.[5,8] On the other hand, histological remission was shown to decrease colorectal cancer risk in UC.[7,9]

Many histological indices have been described since the 1950s for assessing the microscopic activity in UC patients; however, until recently none of them had been fully validated. Geboes *et al.* developed a reproducible six-graded classification system that accurately discriminates UC patients according to structural changes and chronic and acute inflammatory activity. Currently, the Geboes index [2000] is considered to be the best classification system for histological assessment of UC, having a kappa coefficient for inter-observer variation of 0.59–0.70.[10] Given that histological remission is considered to be a different target than the endoscopic MH in UC patients, the histological assessment of disease activity has been increasingly used by physicians.[11]

Faecal calprotectin [FC] is considered to be an excellent marker of intestinal inflammation, as it reflects the migration of neutrophils through the inflamed bowel wall to the mucosa.[2] Multiple studies have revealed that FC is a precise diagnostic tool—distinguishing IBD from non-IBD patients—and FC concentrations seem to correlate well with the degree of histological and endoscopic inflammation.[12–15]

Although not studied as well as FC, neutrophil gelatinase B–associated lipocalin [NGAL] has also been suggested as a potential surrogate marker for IBD diagnosis and monitoring. In fact, a few recent studies have demonstrated that NGAL is elevated in the serum of IBD patients (when compared with healthy controls or irritable bowel syndrome [IBS] patients) and have reported different cut-off values for predicting histological remission and MH.[16–19] Moreover, other studies have demonstrated that faecal NGAL levels are a reliable marker of UC activity.[20]

The primary aim of the present study was the evaluation of the accuracy of FC for assessing both MH and histological remission in UC patients without clinical symptoms. Its primary endpoints were: [i] to establish the prevalence of histologic inflammation and endoscopic activity in patients with clinically asymptomatic UC; and [ii] to evaluate the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of FC for assessing histological and/or endoscopic activity. Additionally, NGAL was quantified in a subset of patients and evaluated for the same purpose as FC.

2. Materials and Methods

2.1. Patients and study design

This was a nationwide and multicentre study: the patients included had a definitive diagnosis of UC, according to ECCO guidelines,[2] and were being followed in nine Portuguese IBD specialized centres. The inclusion criteria were: [i] UC patients older than 18 years old; and [ii] absence of symptoms according to the Montreal classification and Mayo partial score of <2. The exclusion criteria were the use of topic therapies and the presence of clinical symptoms.

All the patients enrolled in this study did so voluntarily and signed a written informed consent. The study was approved by the ethics committees of all hospitals involved and by the Portuguese Data Protection Authority [Comissão Nacional de Protecção de Dados]. The national coordinator of the Portuguese IBD group [GEDII] monitored the study.

All patients were subjected to blood analysis and collected faeces for the evaluation of FC and NGAL. A sigmoidoscopy was performed within 24 h of sample collection and without bowel preparation. During this examination sigmoid and rectum biopsies were taken. Endoscopic activity was evaluated by the Ulcerative Colitis Endoscopic Index of Severity [UCEIS][21] and by the Mayo endoscopic subscore.[22] Patients were considered in remission whenever UCEIS was equal or below 1, whereas MH was defined as a Mayo endoscopic subscore equal to 0.[23] Finally, 19 patients who had a FC level above 250 µg/g and a normal endoscopic exam were subjected to a complete colonoscopy later on, after signing a new informed consent.

2.2. Histological assessment

The rectum and sigmoid biopsies [two of each] taken during the sigmoidoscopy were subject to central reading by three independent pathologists blinded regarding patients' disease status and endoscopic results. Disagreements between the pathologists were resolved by a review using a multiheaded microscope and including a fourth pathologist [Karel Geboes] in order to reach a final score. The histological assessment was performed using the Geboes index.[10] In this study, histological remission was defined as a Geboes index inferior to 3.1. Moreover, the degree of basal plasmacytosis was evaluated and classified according to the following scale: 0, absent; 1, focal; 2, diffuse.[24]

2.3. Faecal calprotectin and neutrophil gelatinase B-associated lipocalin

A single stool sample was collected from each patient and divided into smaller subsamples, on which FC and NGAL were quantified following the specific protocols described below. FC was extracted and quantified from the stool samples of 364 and 371 patients, using the QB and the EliA kits respectively [see below]. Stools were kept at room temperature until extraction [within a maximum of 7 days after collection] in accordance with manufacturer's instructions ['Fecal sample preparation kit' of Roche Diagnostics, Germany]. Samples were then stored at –80°C until the assay was performed. FC values were determined from samples using two different assays: Quantum Blue, hereafter referred to as QB [Buhlmann®] and Automated Fluoroimmunoassay-EliA test, hereafter referred to as EliA [Phadia, ThermoFisher®], according to the manufacturers' instructions.

NGAL was extracted and quantified from the stool samples of 260 patients. For NGAL measurement, subsamples were kept at 4°C [for a maximum of 48 h] until shipment to the central laboratory [Department of Pharmacology and Therapeutics, Faculty of Medicine of University of Porto, Portugal], where stools were stored at -80°C. Faecal NGAL was measured by a quantitative enzyme immunoassay (Faecal NGAL [LCN2, Lipocalin-2] ELISA Kit, Epitope Diagnostics, San Diego, USA) according to the instructions provided by the manufacturer.

2.4. Statistical analysis

Categorical variables were described through absolute [*n*] and relative [%] frequencies, and continuous variables were described as mean and standard deviation, median, percentiles, and minimum/maximum values when appropriate. All the reported *p* values were two-sided, and *p* values below 0.05 were considered to be statistically significant. The ability of FC and NGAL to discriminate UC macroscopic and microscopic activity from remission was evaluated by plotting receiver operating characteristic [ROC] curves and computing the area under the curve [AUC]. All data was arranged, processed, and analysed with SPSS ® v.20.0 data [Statistical Package for Social Sciences]. Graphs were designed with Prism 6.

3. Results

3.1. Study population

This cohort enrolled 371 asymptomatic UC patients who were consecutively enrolled in this study, and whose demographic and clinical characteristics are depicted in Table 1. The female proportion of the population was 53%, while the patients' median [IQR] age was 47 [37–59] years; the median [IQR] period during which the patients had been followed-up at medical care centres was 7 [3–12] years. Regarding UC localization, 57% had left-sided colitis and 43% had extensive colitis. A total of 366 patients were medicated: 91% were on oral mesalazine [5-ASA], 1% was on steroids, 30% were on azathioprine [AZT], and 10% were on anti–tumour necrosis factor [TNF] therapy. Moreover, 77 patients were dependent on steroids, 15 were steroid-resistant, and 21 were intolerant to AZT.

3.2. UC overall assessment: histological analysis, endoscopic activity and biomarker levels

The overall assessment of the UC activity in the patients included in this study is shown in Table 2. Most of the patients were in remission according to the endoscopic examination: 91% according to UCEIS and 72% according to Mayo endoscopic subscore. These two approaches to the endoscopic results had an overall high correlation [Spearman's coefficient = 0.894, $p < 0.001$]. Moreover, most of the patients were also in remission according to the histological examination [79%], and basal plasmacytosis was absent in 80% of the patients. The median (interquartile range [IQR]) levels of C-reactive protein [CRP] were 2.0 mg/L [1.0–4.0]. Concerning FC levels, 47–69% of the studied patients were below the pre-established cut-off of 100 µg/g, depending on whether the quantification method was the QB or EliA, respectively. Moreover, the median [IQR] values of faecal NGAL were 9.00 µg/g [5.30–18.10].

The detailed histological assessment according to the Geboes index is shown in Table 3. Despite being clinically asymptomatic, 20% of the patients presented with crypt involvement, 11.6% presented with crypt destruction, and 11% presented with some degree of erosion and ulceration. Figure 1 illustrates the occurrence of histological inflammation stratified according to the Mayo endoscopic subscore, the UCEIS, and the basal plasmacytosis grade. As expected,

Table 1. Characteristics of the population enrolled in the study.

N		371
Median age [IQR, years]		47 [37–59]
Gender	Male, *n* [%]	174 [47]
	Female, *n* [%]	197 [53]
Median time of follow-up, years [IQR, years]		7 [3–12]
Localization [*n* = 369]		
Left side colitis, *n* [%]		211 [57%]
Extensive colitis, *n* [%]		158 [43%]
Smoking habits [*n* = 353]		
Smoker, *n* [%]		20 [6%]
Ex-smoker, *n* [%]		103 [29%]
Non-smoker, *n* [%]		230 [65%]
Steroid use, *n* [%]		
Steroid-dependence [*n* = 329]		77 [23%]
Steroid-resistance [*n* = 324]		15 [5%]
AZT intolerant [*n* = 312], *n* [%]		21 [7%]
Medication at the time of the study		
Steroid use [*n* = 366]		4 [1%]
AZT, [*n* = 365]		109 [30%]
Anti-TNF, [*n* = 366]		37 [10%]
Oral 5-ASA [*n* = 365]		333 [91%]
Extra-intestinal manifestations [*n* = 366], *n* [%]		74 [20%]
Arthralgia		64 [17%]
Arthritis		12 [3%]
Erythema nodosum		6 [2%]
Uveitis		4 [1%]

5-ASA: 5-aminosalicylic acid; AZT: azathioprine; IQR: interquartile range; TNF: tumour necrosis factor.

Table 2. Characteristics of the population enrolled considering the biomarkers measurements, endoscopic and histological activity.

Mayo Endoscopic Score [*n* = 370]	
0 – Normal or inactive disease	265 [72%]
1 – Mild disease [erythema, decreased vascular pattern, mild friability]	90 [24%]
2 – Moderate	14 [4%]
3 – Severe	1 [0.3%]
UCEIS [*n* = 371]	
Remission [0 or 1]	336 [91%]
Active disease [≥2]	35 [9%]
Histology [*n* = 370]	
<3.1	291 [79%]
≥3.1	79 [21%]
Basal plasmacytosis [*n* = 363]	
0 [absent]	289 [80%]
1 [focal]	54 [15%]
2 [diffuse]	20 [5%]
C-reactive protein, Median [IQR] [*n* = 364]	2.0 [1.0–4.0] mg/L
NGAL, Median [IQR] [*n* = 260]	9.00 [5.30–18.10] µg/g
FC [QB] Median [IQR] [*n* = 364]	114.00 [41.00–310.00] µg/g
<100 µg/g	172 [47%]
≥100 µg/g	192 [53%]
FC [EliA] Median [IQR] [*n* = 371]	36.40 [9.10–149.00] µg/g
<100 µg/g	255 [69%]
≥100 µg/g	116 [31%]

FC: faecal calprotectin; IQR: interquartile range; NGAL: neutrophil gelatinase B–associated lipocalin; UCEIS: Ulcerative Colitis Endoscopic Index of Severity.

the relative frequency of patients with histological inflammation increased with the severity of the other outcomes. Nevertheless, it should be noticed that there was a considerable fraction of patients among those whom presented with no endoscopic lesions [10%], an UCEIS below 2 [15%] or no basal plasmacytosis [10%], that still had a Geboes index of above 3.1 [Figure 1]. The agreement rate between the Geboes index and other outcomes used in this study is

Table 3. Histological classification according to the Geboes index.

	n	[%]
Grade 0		
0.0 – No abnormality	218	[58.8%]
0.1 – Mild abdnormality	96	[25.9%]
0.2 – Mild or moderate diffuse or multifocal abdormalities	52	[14.0%]
0.3 – Severe diffuse or multifocal abnormalities	5	[1.3%]
Grade 1 – Chronic inflammatory infiltrate		
1.0 – No increase	92	[24.8%]
1.1 – Mild but unequivocal increase	199	[53.6%]
1.2 – Moderate increase	55	[14.8%]
1.3 – Marked increase	25	[6.7%]
Grade 2 – Neutrophils and eosinophils in lamina propria		
2A – eosinophils		
2A.0 – No increase	193	[52.0%]
2A.1 – Mild but unequivocal increase	125	[33.7%]
2A.2 – Moderate increase	46	[12.4%]
2A.3 – Marked increase	7	[1.9%]
2B – Neutrophils		
2B.0 – None	308	[83.0%]
2B.1 – Mild but unequivocal increase	43	[11.6%]
2B.2 – Moderate increase	17	[4.6%]
2B.3 – Marked increase	3	[0.8%]
Grade 3 – Neutrophils in epithelium		
3.0 – None	297	[80.1%]
3.1 – <5% crypts involved	34	[9.2%]
3.2 – <50% crypts involved	29	[7.8%]
3.3 – >50% crypts involved	11	[3.0%]
Grade 4 – Crypt destruction		
4.0 – None	328	[88.4%]
4.1 – Probable – local excess of neutrophils in part of crypt	26	[7.0%]
4.2 – Probable – marked attenuation	7	[1.9%]
4.3 – Unequivocal crypt destruction	10	[2.7%]
Grade 5 – Erosion or ulceration		
5.0 – No erosion, ulceration, or granulation tissue	330	[88.9%]
5.1 – Recovering epithelium + adjacent inflammation	8	[2.2%]
5.2 – Probable erosion – focally stripped	12	[3.2%]
5.3 – Unequivocal erosion	15	[4.0%]
5.4 – Ulcer or granulation tissue	6	[1.6%]

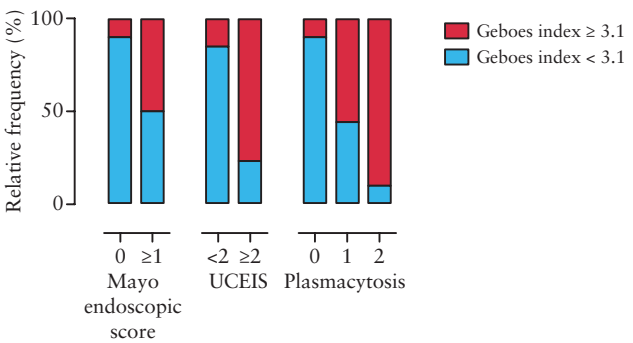

Figure 1. Histological inflammation stratified according to Mayo endoscopic subscore, Ulcerative Colitis Endoscopic Index of Severity [UCEIS], and basal plasmacytosis.

shown in Table 4: the highest accuracy rate [i.e. sum of true positives and true negatives] was found for the UCEIS [84%], whereas the accuracy rate concerning the Mayo endoscopic subscore was 79%.

Additionally, basal plasmacytosis was significantly associated with the presence of endoscopic lesions and histological activity [Supplementary Table 1]. In fact, 82% of patients without basal plasmacytosis had no endoscopic lesions, while 96% of them were in remission according to the UCEIS. Furthermore, 90% of patients without basal plasmacytosis had no histological inflammation, and the same percentage of patients with diffuse basal plasmacytosis had a Geboes index of ≥3.1.

3.3. Performance of FC and NGAL levels as UC biomarkers

The two different methods used to quantify the FC levels in patients' stools [QB and EliA] yielded considerably different results. In fact, the FC median [IQR] levels determined by QB were significantly superior to those depicted by EliA [Table 2, $p < 0.001$, Wilcoxon test]. However, upon categorizing the FC measurements into distinct grades, the intraclass correlation coefficient between QB and EliA methods was 0.710 [95% CI: 0.643–0.764].

Irrespective of the method used, the FC levels had a consistent significant difference when compared between patients with or without histological and endoscopic activity [Figure 2 and Supplementary Tables 2 and 3]. In fact, and as it can be seen in Figure 2, FC levels were significantly lower for patients without endoscopic lesions [Mayo endoscopic score of 0] when compared with those with lesions, as well as for patients with an UCEIS of <2. FC levels were also significantly lower for patients in histological remission when compared with those with a Geboes index ≥3.1

The ability of FC levels to assess the presence of macroscopic and microscopic lesions in asymptomatic patients was evaluated by plotting a ROC curve for each case and computing the respective AUC [Supplementary Figure 1 and Table 5]. The AUC values were quite similar between the QB and EliA methods, and all of them were significant; however, the AUC was smaller for the detection of endoscopic lesions when compared with the detection of histological inflammation and disease activity according to the UCEIS score. The sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV] and accuracy of the FC levels at different cut-offs for assessing histological and endoscopic activity are depicted in Table 5. For the same cut-off values, the QB method of quantifying FC had a higher sensitivity, whereas the EliA method had a higher specificity. The best accuracy ratios were obtained using the EliA method with a cut-off of 250 or 300 µg/g: 77%, 70%, and 80% for the detection of a Geboes index ≥3.1, Mayo endoscopic subscore ≥1, and UCEIS ≥2, respectively. Even though the PPVs were low in this situation [16–46%], the NPVs were considerably high and ranged from 74% for the detection of macroscopic lesions to 92% for the detection of an UCEIS above or equal to 2. Moreover, a ROC curve was built for the FC values considering the presence of either histological or endoscopic remission [according to UCEIS or Mayo endoscopic score], and the AUCs are indicated in Supplementary Table 4, as well as the accuracy and related statistics of each cut-off. In this scenario, both the FC NPV and accuracy decrease slightly, although the PPV is a bit higher, especially when considering the detection of disease activity concerning UCEIS. The AUC tends to be lower than that for the detection of each outcome individually, with the exception of the detection of endoscopic lesions.

The presence of NGAL was inspected and quantified in a subset of 260 patients. At this point, it is important to highlight that this subset was similar to the left-out patients regarding the most important disease characteristics and outcomes, and so the inclusion of all patients would likely yield similar results [results not shown]. Upon stratifying NGAL concentrations according to the various analysed outcomes, the results have shown that NGAL levels were significantly lower for patients with no endoscopic lesions, with an UCEIS <2, or in histological remission, when compared with their counterparts [Figure 3 and Supplementary Table 4].

The ability of NGAL to assess macroscopic and microscopic lesions was evaluated by plotting a ROC for each case and computing the AUCs [Supplementary Figure 1 and Table 6]. All AUCs were statistically significant and ranged from 0.635 to 0.713. The analyses of the NGAL cut-offs for each case are depicted in Table 6: the highest accuracy ratio was obtained for a cut-off of 12 µg/g. For the detection of a Mayo endoscopic subscore ≥1, NGAL levels above 12 µg/g had an accuracy of 64% and a NPV of 77%; regarding the detection of UCEIS ≥2, the same cut-off had an accuracy ratio of 64% and an NPV of 93%; finally, for the detection of a Geboes index ≥3.1, the ratio was 62% and the NPV was 83%. As with the FC, the PPVs regarding NGAL cut-offs were considerably lower [between 15% and 43%]. Moreover, a ROC curve was built to evaluate the performance of NGAL in the detection of either histological or endoscopic remission [according to UCEIS or Mayo endoscopic score]. AUC, accuracy, and related statistics are indicated in Supplementary Table 4: when compared against their counterparts in the specific analysis of each outcome, both accuracy and PPV tend to be higher, whereas NPV tends to be lower. The AUC is generally lower than that for the detection of each outcome individually.

The AUCs of the ROC curves using the CRP for the detection of the outcomes mentioned above were also computed [Supplementary Figure 1]. However, the AUC was in all cases non-significant and below that of the faecal markers.

Cut-off values of 150 µg/g for the EliA method, 250 µg/g for the QB method and 12 µg/g for NGAL were chosen as the ones presenting a better balance between sensitivity, specificity, PPV and NPV. The overall accuracy and kappa index for FC and NGAL using these

Table 4. Comparison between the histological score and the other outcomes.

	Accuracy [%]	Kappa [95% CI]
Mayo endoscopic subscore [0 vs. ≥1]	79%	0.429 [0.325; 0.533]
UCEIS [<2 vs. ≥2]	84%	0.394 [0.276; 0.512]
FC – QB [<250 vs. ≥250 µg/g]	70%	0.200 [0.092; 0.308]
FC – EliA [<150 vs. ≥150 µg/g]	75%	0.283 [0.171; 0.395]
NGAL [<12 vs. ≥12 µg/g]	62%	0.130 [0.014; 0.245]

EliA: Automated Fluoroimmunoassay-EliA test; FC: faecal calprotectin; NGAL: neutrophil gelatinase B–associated lipocalin; QB: Quantum Blue assay; UCEIS: Ulcerative Colitis Endoscopic Index of Severity.

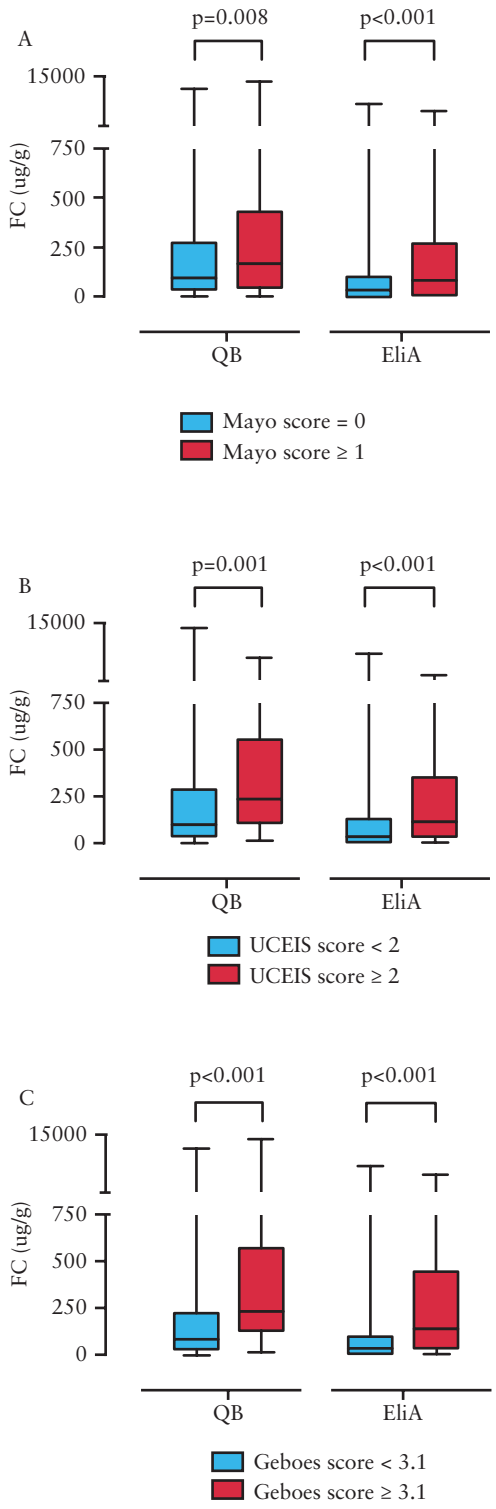

Figure 2. Minimum to maximum box-and-whiskers plots representing the faecal calprotectin [FC] levels stratified by various histological and clinical outcomes: [A] Mayo endoscopic subscore [0 vs. ≥1]; [B] Ulcerative Colitis Endoscopic Index of Severity [UCEIS] [<2 vs. ≥2]; and [C] Geboes index [<3.1 vs. ≥ 3.1].

thresholds are depicted in Table 4, and are below those observed for the UCEIS and Mayo endoscopic subscore. Interestingly, both biomarkers were correlated with the CRP levels in a weak although significant fashion [Spearman correlation coefficients: 0.160, p = 0.002 [for FC measured with QB], 0.135, p = 0.010 (for FC measured with EliA], and 0.195, p = 0.002 [for NGAL]). Moreover, NGAL was correlated with FC irrespective of the extraction method used (Spearman correlation coefficients: 0.544, p < 0.001 [for FC measured with QB], 0.597, p < 0.001 [for FC measured with EliA]).

Combining the two studied biomarkers—FC and NGAL—seems like a rational approach to increase their detection potential. However, such combination did not seem to yield better results than the utilization of each marker alone [Supplementary Table 6]. In fact, although there is an increase in the accuracy [mostly due to an increase in specificity], this difference does not seem to hold any significance on the daily clinical practice, as NPVs remained fairly unchanged.

3.4. Sigmoidoscopy vs. colonoscopy

To further explore the relation between FC levels and macroscopic lesions, patients who had FC levels above 250 µg/g and a normal sigmoidoscopy were invited to do a complete colonoscopy a few months after [data not shown]. Nineteen patients accepted the invitation to do so. Interestingly, in 16 of those patients macroscopic lesions were detected in or above the sigmoid colon, and in 12 of those 16 there was also an increase in the Geboes index.

The choice of a sigmoidoscopy as the patient's initial endoscopic examination instead of a colonoscopy had the rational of avoiding mechanical bowel preparation [known to cause structural changes and inflammation, and therefore prone to causing some bias in the results].[25,26] Nonetheless, a sigmoidoscopy is unable to detect lesions above the lower third of the colon, and therefore one could raise the question of whether a more complete examination would change the results. In order to address that issue, patients were stratified according to their disease location—left-side or extensive colitis. These groups were individually analysed regarding FC and NGAL cut-offs [Supplementary Tables 7 and 8], and the results showed that the 95% CI for sensitivity, specificity, PPV, NPV, and accuracy were, in the vast majority of cases, overlapping between them.

4. Discussion

The gold standard for monitoring disease activity in UC patients still includes a colonoscopy, usually followed by the histological analysis of sampled biopsies. However, colonoscopies are invasive and time-consuming procedures, are relatively expensive, and cause discomfort to the patients. For these reasons, the optimization and validation of biomarkers that can accurately diagnose or monitor disease progression in UC [and other IBD] patients is highly desirable. Among the many biomarkers that have been explored in this context, FC—a calcium-binding protein that composes up to 60% of the neutrophils' cytosol—stands out as a particularly promising one. The presence of calprotectin in faeces reflects the leakage of neutrophils into the bowel lumen, usually caused by an inflammatory process that alters the mucosal architecture. Hence, FC is expected to mirror the integrity of the bowel mucosa.

Many studies have been made in the last two decades to measure the value of FC in diagnosing and monitoring disease activity in IBD patients.[27–29] However, the vast majority of these studies have focused on the ability of FC to discriminate IBD patients from healthy volunteers or patients with irritable bowel syndrome [IBS], or on predicting disease activity in IBD patients. This study has the particularity of focusing on asymptomatic UC patients [N = 371], and reporting on the value of FC for assessing endoscopic and

Table 5. Assessment of ulcerative colitis outcomes by faecal calprotectin.

Assessment of endoscopic lesions [Mayo endoscopic subscore ≥1]					
AUC [QB] = 0.590 [95% CI: 0.524–0.657], $p = 0.008$					
AUC [EliA] = 0.635 [95% CI: 0.570–0.699], $p < 0.001$					
[QB/EliA] µg/g	**Sen [%]**	**Spe [%]**	**PPV [%]**	**NPV [%]**	**Accuracy [%]**
QB/EliA ≥ 50	77/59	32/63	31/39	79/79	45/62
QB/EliA ≥ 100	61/47	51/75	33/42	77/78	54/67
QB/EliA ≥ 150	55/37	62/80	35/42	78/76	60/68
QB/EliA ≥ 200	45/32	67/83	34/44	76/76	61/69
QB/EliA ≥ 250	37/28	74/86	36/44	75/75	64/70
QB/EliA ≥ 300	36/23	78/88	39/43	76/74	66/69
Assessment of activity [UCEIS ≥ 2]					
AUC [QB] = 0.701 [95% CI: 0.607–0. 795], $p = 0.001$					
AUC [EliA] = 0.717 [95% CI: 0.627–0.807], $p < 0.001$					
QB/EliA ≥ 50	88/71	32/59	12/15	96/95	37/60
QB/EliA ≥ 100	77/54	50/71	14/16	95/94	52/70
QB/EliA ≥ 150	74/43	60/77	16/16	96/93	61/74
QB/EliA ≥ 200	65/34	67/80	17/15	95/92	67/76
QB/EliA ≥ 250	44/31	73/84	14/17	93/92	70/79
QB/EliA ≥ 300	41/26	76/86	15/16	93/92	73/80
Assessment of histological activity					
AUC [QB] = 0.732 [95% CI: 0.674–0.791], $p < 0.001$					
AUC [EliA] =0.735 [95% CI: 0.676–0.795], $p < 0.001$					
QB/EliA ≥ 50	92/67	36/63	28/33	94/88	48/63
QB/EliA ≥ 100	81/57	55/76	33/39	91/87	60/72
QB/EliA ≥ 150	74/48	65/82	37/42	90/85	67/75
QB/EliA ≥ 200	62/42	71/85	37/43	87/84	69/76
QB/EliA ≥ 250	46/38	76/88	35/46	84/84	70/77
QB/EliA ≥ 300	44/32	79/89	37/45	84/83	72/77

AUC: area under the curve; EliA: Automated Fluoroimmunoassay-EliA test; NPV: negative predictive value; PPV: positive predictive value; QB: Quantum Blue assay; Sen: sensitivity; Spe: specificity.

histological activity in patients that have a definitive diagnosis but no clinical activity of the disease. Additionally, the faecal NGAL levels were also accounted for in this context [in 260 patients of this cohort].

As expected—given the absence of symptoms—most patients were in MH according to the Mayo endoscopic subscore [72%], in remission according to the UCEIS [91%], or had no microscopic signs of inflammation [Geboes <3.1] [79%]. However, the agreement between these different evaluations was only moderate [79–84%]. In fact, there were a number of patients with a Geboes index above 3.1 who were considered to be in remission according to the Mayo endoscopic subscore and the UCEIS, and who had no basal plasmacytosis. Additionally, 15% of the patients with diffuse basal plasmacytosis were considered to be in MH [Mayo endoscopic subscore = 0], whereas 50% had a UCEIS <2. These results support the previously published notion that endoscopic findings fail to indicate the presence of microscopic activity and basal plasmacytosis, particularly in mild UC cases.[24,30] Histological remission is a clinically relevant end-point that must be taken into consideration, as histological inflammation may be a better predictor of clinical relapses than endoscopic lesions in UC.[5] Moreover, and in the particular case of patients in clinical remission, the histology grade has the strongest association with the risk of clinical relapse.[31]

The current literature concerning the utility of FC as an IBD biomarker agrees on its potential, but strongly disagrees on the cut-off values proposed—in fact, one can easily find variable FC cut-offs within the same context and for the same purpose.[28,29] This is likely to be in part due to the variety of commercial kits currently available for quantifying FC, which are based on a similar method [ELISA] but may differ in specific details [e.g. monoclonal vs. polyclonal antibodies]. To tackle this issue, two different FC quantification kits were used in this study: the QB and the EliA. And whereas the results had a high intraclass correlation index, they were rather distant in terms of average and median values. Furthermore, the stratification of patients according to the pre-established cut-off of 100 µg/g yielded different-sized groups for each of the two methods. Notwithstanding, and despite the absolute levels quantified, QB and EliA had a similar variation for the different grades of histological activity and endoscopic lesions in UC asymptomatic patients. So, different cut-offs have to be optimized and validated for each kit and within each context.

Overall, the ROC analysis of the FC levels revealed that FC is a good predictor of macroscopic and microscopic activity in asymptomatic UC patients. As cut-off values, and considering the best balance between specificity, sensitivity, PPV, NPV, and accuracy, one should consider 150 µg/g for the EliA method, and 250 µg/g for the QB method. Given the low prevalence of inflammatory activity in the asymptomatic patients, these cut-off values present considerably high NPVs. So, negative test results can exclude the presence of endoscopic activity and microscopic inflammation with an appreciable degree of certainty, which can spare the patient from an unnecessary colonoscopy. On the other hand, the PPVs tend to be low and never reach 50%. For that reason, a positive result [i.e. a test result above the proposed thresholds] must be interpreted with caution, and should not be used as the sole basis to justify a complete colonoscopy. Such an invasive procedure should only be carried out

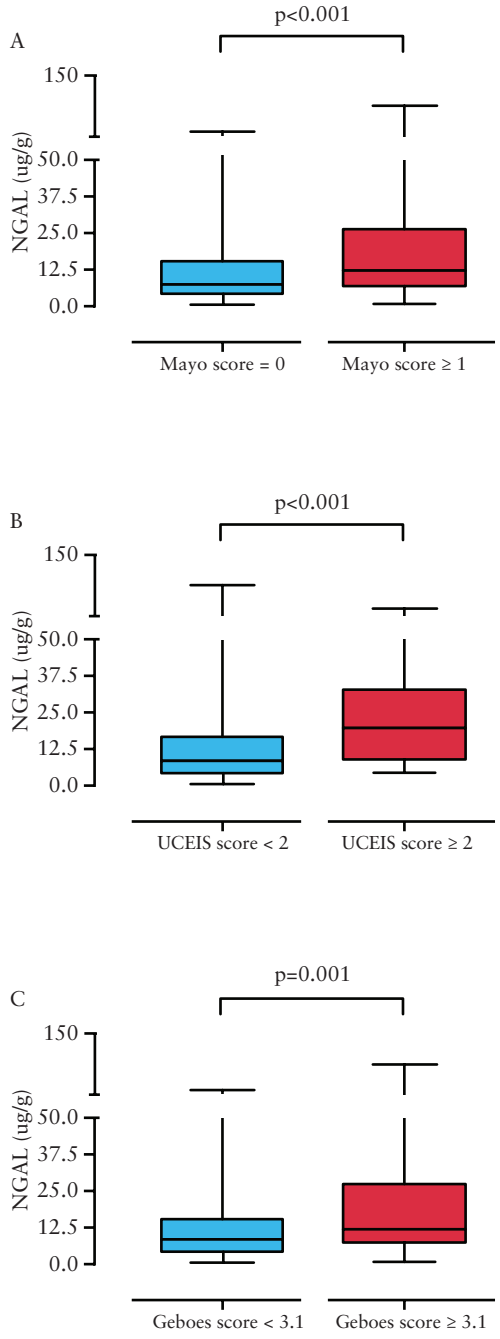

Figure 3. Minimum to maximum box-and-whiskers plots representing the neutrophil gelatinase B–associated lipocalin [NGAL] levels stratified by various histological and clinical outcomes: [A] Mayo endoscopic subscore [0 vs. ≥1]; [B] Ulcerative Colitis Endoscopic Index of Severity [UCEIS] [<2 vs. ≥2]; and [C] Geboes index [<3.1 vs. ≥3.1].

in the presence of other indicators [high levels of serum markers or exacerbation of clinical symptoms, for instance].

Interestingly, Guardiola *et al.*[32] have recently proposed that an FC concentration of lower than 155 µg/g can indicate the absence of acute inflammatory infiltrate with a NPV of 89% in UC patients considered to be in clinical and endoscopic remission. Our study validates and expands this previous report, using a much larger cohort [371 vs. 59], a validated histological index, and including the UCEIS and the basal plasmacytosis assessment.

Additionally, two other studies have reported the potential of FC to predict MH and/or disease flares in patients with quiescent UC.[33,34] However, these studies have not included the histological assessment of the patients. Given the importance of the histological remission as a primary end-point in UC, particularly in the case of patients considered to be in clinical remission,[5,31] we believe that our study offers a more inclusive approach to studying the usefulness of FC in assessing asymptomatic patients. Moreover, in one of those studies,[33] the FC cut-off proposed [50 to 13.9 µg/g] was much lower then the ones we report here. Whereas the use of topical medication in 15% of the patients included in that study may have contributed to the different values obtained, the use of a different commercial kit to quantify the FC is likely the reason for the generally lower FC levels reported, consequently resulting in a lower cut-off. This reinforces the idea postulated earlier that each kit requires a specific optimization and validation of the cut-off[s].

As a secondary outcome of this study, the potential of faecal NGAL for assesings MH and histological remission was evaluated in 260 patients. The AUCs of the NGAL levels computed for the Geboes index, the Mayo endoscopic subscore, and the UCEIS were significant and ranged from 0.653 to 0.713. The accuracy ratios were lower than those obtained for FC. Still, a cut-off of 12 µg/g can be useful in the assessment of disease activity. Whereas the number of false positives was rather high, the number of false negatives was very low, and therefore a test result below 12 µg/g can exclude the presence of histological activity with a probability of 83%, the presence of endoscopic lesions with a probability of 77%, and an UCEIS equal to or above 2 with a probability of 93%.

Combining the two biomarkers did not increase the accuracy of the test. Such a result was not unexpected: in fact, as the source of calprotectin and NGAL is the same—the neutrophils—both biomarkers are expected to reflect the leakage of these cells into the lumen, and therefore they should be redundant. Moreover, the AUCs for the CRP assessment of endoscopic and histological outcomes were non-significant and below those of FC and NGAL, suggesting that faecal markers are likely a better approach for detecting active disease at least in asymptomatic UC patients. Nevertheless, the combination of FC and/or NGAL with biomarkers of a different nature merits further attention in the future.

The utilization of a sigmoidoscopy as the initial patient's examination could be considered a limitation, as lesions above the lower third of the colon could not be detected. However, a sigmoidoscopy avoids bowel preparation, which could affect the colon inflammation. Moreover, the 95% CI intervals for sensitivity, specificity, PPV, NPV, and accuracy regarding FC and NGAL cut-offs are, in the vast majority of cases, overlapping for patients with left-side or extensive colitis. This demonstrates that the presence of lesions undetected in the sigmoidoscopy do not affect the overall results, and therefore the examination performed was adequate and sufficient for this study.

The main strengths of this study were the considerable large size of the cohort, the collection of stool samples for FC and NGAL analysis in a period of 24 h prior to the sigmoidoscopy, the use of two different assays for quantifying the FC, the use of a well-known and strong index for the histological assessment [Geboes index], and the fact that the biopsies were analysed by independent pathologists blinded to the disease status. The more recent validated histological indices were indeed not yet published when this study was designed.[35,36] As for limitations, one should account for the fact that FC quantification was based on a single sample—De Vos *et al.* have recently shown that two consecutive measurements of calprotectin are more specific than a single one for the prediction of relapses.[37]

Table 6. Assessment of ulcerative colitis outcomes by NGAL

[NGAL] µg/g	Sen [%]	Spe [%]	PPV [%]	NPV [%]	Accuracy [%]
Assessment of endoscopic lesions [Mayo endoscopic subscore ≥1] AUC = 0.653 [95% CI: 0.583–0.723], $p < 0.001$					
Ngal ≥ 7	77	40	36	80	52
Ngal ≥ 10	62	62	42	79	62
Ngal ≥ 12	53	69	43	77	64
Assessment of activity [UCEIS ≥ 2] AUC = 0.713 [95% CI: 0.618–0. 809], $p < 0.001$					
Ngal ≥ 7	93	38	15	98	44
Ngal ≥ 10	67	58	15	94	58
Ngal ≥ 12	59	64	16	93	64
Assessment of histological activity AUC = 0.689 [95% CI: 0.616–0.763], $p < 0.001$					
Ngal ≥ 7	84	40	28	90	50
Ngal ≥ 10	60	59	29	84	59
Ngal ≥ 12	51	66	29	83	62

AUC: area under the curve; NGAL: neutrophil gelatinase B–associated lipocalin; NPV: negative predictive value; PPV: positive predictive value; Sen: sensitivity; Spe: specificity.

Moreover, this was a cross-sectional study, and therefore the progression of the disease—along with that of the FC and NGAL levels—was not accounted for.

In conclusion, this study reports the presence of histological inflammation and even macroscopic lesions among asymptomatic UC patients, and points out that FC and faecal NGAL levels can be useful for dismissing the presence of histological and endoscopic activity, therefore reducing the number of unnecessary colonoscopies. FC and NGAL are, therefore, clinically relevant biomarkers, as they can aid the physician in the follow-up assessment of UC patients without clear symptoms. Their utility to predict the presence of lesions is, however, limited, and their utilization for this purpose must be complemented by the use of other markers. Moreover, further studies are needed to validate the proposed cut-off values, as well as to extend them with a follow-up approach, in order to determine whether these [or other] values could also be useful for the anticipation and prevention of flares.

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Conflict of Interest

FM served as a speaker and received honoraria from Merck Sharp & Dohme, Abbvie, Vifor, Falk, Laboratorios Vitoria, Ferring, Hospira, and Biogen.

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Author Contributions

Fernando Magro: Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; study supervision; critical revision of the manuscript for important intellectual content. Rosa Coelho: acquisition of data; analysis and interpretation of data. Cláudia Camila Dias: statistical analysis. Joanne Lopes: histological analysis. Paula Borralho: histological analysis. Joana Afonso: Calprotectin and NGAL analysis. Karel Geboes: supervisor of the histological analysis; critical revision of the manuscript for important intellectual content. Fátima Carneiro: histological analysis; critical revision of the manuscript for important intellectual content. All other authors: recruitment of patients and collection of samples. All authors read and approved the final version of the manuscript.

Supplementary Data

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

References

1. Silverberg MS, Satsangi J, Ahmad T, *et al*. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005;**19** Suppl A:5A–36A.
2. Dignass A, Eliakim R, Magro F, *et al*. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis. Part 1: definitions and diagnosis. *J Crohns Colitis* 2012;**6**:965–90.
3. Froslie KF, Jahnsen J, Moum BA, Vatn MH, Group I. Mucosal healing in inflammatory bowel disease: results from a Norwegian population–based cohort. *Gastroenterology* 2007;**133**:412–22.
4. Colombel JF, Rutgeerts P, Reinisch W, *et al*. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011;**141**:1194–201.
5. Bryant RV, Winer S, Travis SP, Riddell RH. Systematic review: histological remission in inflammatory bowel disease. Is 'complete' remission the new treatment paradigm? An IOIBD initiative. *J Crohns Colitis* 2014;**8**:1582–97.
6. Vatn MH. Mucosal healing: impact on the natural course or therapeutic strategies. *Dig Dis* 2009;**27**:470–5.
7. Rutter M, Saunders B, Wilkinson K, *et al*. Severity of inflammation is a risk factor for colorectal neoplasia in ulcerative colitis. *Gastroenterology* 2004;**126**:451–9.
8. Riley SA, Mani V, Goodman MJ, Dutt S, Herd ME. Microscopic activity in ulcerative colitis: what does it mean? *Gut* 1991;**32**:174–8.
9. Gupta RB, Harpaz N, Itzkowitz S, *et al*. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* 2007;**133**:1099–105; quiz 340–1.

10. Geboes K, Riddell R, Ost A, *et al*. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. *Gut* 2000;**47**:404–9.
11. Marchal Bressenot A, Riddell RH, Boulagnon-Rombi C, *et al*. Review article: the histological assessment of disease activity in ulcerative colitis. *Aliment Pharmacol Ther* 2015;**42**:957–67.
12. von Roon AC, Karamountzos L, Purkayastha S, *et al*. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol* 2007;**102**:803–13.
13. Sipponen T, Savilahti E, Kolho KL, *et al*. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. *Inflamm Bowel Dis* 2008;**14**:40–6.
14. Mao R, Xiao YL, Gao X, *et al*. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. *Inflamm Bowel Dis* 2012;**18**:1894–9.
15. Canani RB, Terrin G, Rapacciuolo L, *et al*. Faecal calprotectin as reliable non-invasive marker to assess the severity of mucosal inflammation in children with inflammatory bowel disease. *Dig Liver Dis* 2008;**40**:547–53.
16. Oikonomou KA, Kapsoritakis AN, Theodoridou C, *et al*. Neutrophil gelatinase-associated lipocalin (NGAL) in inflammatory bowel disease: association with pathophysiology of inflammation, established markers, and disease activity. *J Gastroenterol* 2012;**47**:519–30.
17. Yeşil A, Gönen C, Senateş E, *et al*. Relationship between neutrophil gelatinase-associated lipocalin (NGAL) levels and inflammatory bowel disease type and activity. *Dig Dis Sci* 2013;**58**:2587–93.
18. de Bruyn M, Arijs I, De Hertogh G, *et al*. Serum neutrophil gelatinase B–associated lipocalin and matrix metalloproteinase-9 complex as a surrogate marker for mucosal healing in patients with Crohn's disease. *J Crohns Colitis* 2015;**9**:1079–87.
19. de Bruyn M, Arijs I, Wollants WJ, *et al*. Neutrophil gelatinase B–associated lipocalin and matrix metalloproteinase-9 complex as a surrogate serum marker of mucosal healing in ulcerative colitis. *Inflamm Bowel Dis* 2014;**20**:1198–207.
20. Nielsen OH, Gionchetti P, Ainsworth M, *et al*. Rectal dialysate and fecal concentrations of neutrophil gelatinase-associated lipocalin, interleukin-8, and tumor necrosis factor-alpha in ulcerative colitis. *Am J Gastroenterol* 1999;**94**:2923–8.
21. Travis SP, Schnell D, Krzeski P, *et al*. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the ulcerative colitis endoscopic index of severity (UCEIS). *Gut* 2012;**61**:535–42.
22. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *New Engl J Med* 1987;**317**:1625–9.
23. Peyrin-Biroulet L, Sandborn W, Sands BE, *et al*. Selecting therapeutic targets in inflammatory bowel disease (STRIDE): determining therapeutic goals for treat-to-target. *Am J Gastroenterol* 2015;**110**:1324–38.
24. Bessissow T, Lemmens B, Ferrante M, *et al*. Prognostic value of serologic and histologic markers on clinical relapse in ulcerative colitis patients with mucosal healing. *Am J Gastroenterol* 2012;**107**:1684–92.
25. Driman DK, Preiksaitis HG. Colorectal inflammation and increased cell proliferation associated with oral sodium phosphate bowel preparation solution. *Human Pathol* 1998;**29**:972–8.
26. Bucher P, Gervaz P, Egger JF, Soravia C, Morel P. Morphologic alterations associated with mechanical bowel preparation before elective colorectal surgery: a randomized trial. *Dis Colon Rectum* 2006;**49**:109–12.
27. Roseth AG, Aadland E, Jahnsen J, Raknerud N. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion* 1997;**58**:176–80.
28. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. *Clin Exp Gastroenterol* 2016;**9**:21–9.
29. Lehmann FS, Burri E, Beglinger C. The role and utility of faecal markers in inflammatory bowel disease. *Therap Adv Gastroenterol* 2015;**8**:23–36.
30. Kim DB, Lee KM, Lee JM, *et al*. Correlation between histological activity and endoscopic, clinical, and serologic activities in patients with ulcerative colitis. *Gastroenterol Res Pract* 2016;**2016**:5832051.
31. Zenlea T, Yee EU, Rosenberg L, *et al*. Histology grade is independently associated with relapse risk in patients with ulcerative colitis in clinical remission: a prospective study. *Am J Gastroenterol* 2016;**111**:685–90.
32. Guardiola J, Lobaton T, Rodriguez-Alonso L, *et al*. Fecal level of calprotectin identifies histologic inflammation in patients with ulcerative colitis in clinical and endoscopic remission. *Clin Gastroenterol Hepatol* 2014;**12**:1865–70.
33. Langhorst J, Boone J, Lauche R, Rueffer A, Dobos G. Fecal lactoferrin, calprotectin, PMN-elastase, CRP and white blood cell count as an indicator for mucosal healing and clinical course of disease in patients with mild to moderate ulcerative colitis: post hoc analysis of a prospective clinical trial. *J Crohns Colitis* 2016;**10**:786–94.
34. Yamaguchi S, Takeuchi Y, Arai K, *et al*. Fecal calprotectin is a clinically relevant biomarker of mucosal healing in patients with quiescent ulcerative colitis. *J Gastroenterol Hepatol* 2016;**31**:93–8.
35. Mosli MH, Feagan BG, Zou G, *et al*. Development and validation of a histological index for UC. *Gut* 2015, published online Oct 16 DOI: 10.1136/gutjnl-2015-310393
36. Marchal-Bressenot A, Salleron J, Boulagnon-Rombi C, *et al*. Development and validation of the Nancy histological index for UC. *Gut* 2015, published online Oct 13 DOI: 10.1136/gutjnl-2015-310187
37. De Vos M, Louis EJ, Jahnsen J, *et al*. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. *Inflamm Bowel Dis* 2013;**19**:2111–7.