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Original Article

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Histological Outcomes and Predictive Value of Faecal Markers in Moderately to Severely Active Ulcerative Colitis Patients Receiving Infliximab

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Abstract

Background and Aims: Histological healing has emerged as a promising therapeutic goal in ulcerative colitis. This is especially important in the context of biological therapies. The objectives of the present study were to investigate the ability of infliximab to induce histological remission in ulcerative colitis [UC] patients and to explore the utility of faecal calprotectin and lactoferrin in predicting histological activity.

Methods: Multi-centre, single-cohort, open-label, 52-week trial including moderately to severely biological-naïve UC patients receiving intravenous infliximab [5 mg/kg]. The primary outcome was the proportion of patients with histological remission [Geboes index ≤ 3.0] after 8 weeks of treatment, scored by two independent pathologists.

Results: Twenty patients were included. The rate of histological remission increased from 5% at baseline to 15% and 35% at Week 8 and Week 52, respectively. At Week 8, 40% of patients were in clinical remission [Mayo ≤ 2] and 45% achieved mucosal healing [Mayo endoscopy subscore 0–1].

At Week 52, 25% of patients had clinical, endoscopic and histological remission. Faecal calprotectin and lactoferrin showed the highest correlation with histological activity at Week 8 (area under the curve [AUC] 94%, $p = 0.017$; and 96%, $p = 0.013$, respectively) and both markers revealed an excellent positive predictive value for this outcome at this time point [100%, $p = 0.017$; and 94%, $p = 0.013$, respectively].

Conclusions: Infliximab was able to induce histological remission. There was a good agreement between histology and faecal biomarkers. Faecal calprotectin and lactoferrin were good predictors of histological remission. Our data support inclusion of histology as a treatment target complementary to endoscopy in clinical trials when evaluating therapeutic response in UC.

Keywords: Histopathology; infliximab; ulcerative colitis.

1. Introduction

Therapeutic goals in inflammatory bowel diseases [IBD] aim at resolution of clinical symptoms and endoscopic mucosal healing.[1,2,3] The latter is associated with prolonged clinical remission and lower rates of hospitalisation and colectomy.[4,5,6]

The assessment of histological inflammation has emerged as a promising endpoint in UC.[7] However, the link between histological disease activity and other measures of clinical disease activity is not yet well established. Some authors suggest that the presence of histological inflammation is a better predictor of future clinical relapse than endoscopic activity.[8] Several studies showed that patients with residual microscopic active inflammation seem to be more prone to relapse when compared with patients with normal histology.[9,10,11] Histological recovery in UC is often incomplete, and microscopic evidence of inflammation is common even in patients with quiescent colitis assessed clinically and by sigmoidoscopy.[7,8,9,10,11,12] On the other hand, histological remission seems to be associated with low rate of hospitalisation.[13]

Given the fact that the rectum is always involved in UC and inflammatory activity is diffuse and restricted to the mucosa, the collection of samples from the rectal and sigmoid mucosa is a potentially useful tool for evaluating disease severity.

Histologically, active disease is defined by the presence of neutrophils in conjunction with epithelial cell damage.[14] Several histological scores have been proposed to measure histological disease activity.[8] The Geboes index, including five domains, was tested for reproducibility and is used in clinical trials.[15] This instrument has a more elaborated grading of crypt lesions and surface epithelial damage than other proposed indexes.[14]

Faecal calprotectin and lactoferrin have emerged as relevant biomarkers in the diagnosis and monitoring of intestinal inflammation.[16,17,18,19] A significant correlation between the faecal level of calprotectin and the degree of inflammation as assessed by endoscopic and histological criteria has been reported.[20] Similarly, lactoferrin has been shown to correlate well with clinical and endoscopic grading of IBD disease activity.[18,21,22,23] Still, more robust prospectively obtained evidence is essential to confirm the role of these parameters as surrogate markers of histological inflammation.

The prognostic relevance of microscopic inflammation needs further research. This is especially important in the context of biological therapies. The aim of this study was to investigate the ability of infliximab, a tumour necrosis factor [TNF]-alpha antagonist, to induce histological remission and the agreement of this outcome with other indicators of disease activity. In addition, we explored the utility of faecal calprotectin and lactoferrin in predicting histological activity in this setting.

2. Materials and Methods

2.1. Study design and participants

This was a multi-centre, single-cohort, open-label trial designed primarily to investigate histological outcomes among moderately to severely UC patients receiving infliximab. Study participants were recruited from eight IBD centres in Portugal. The trial was conducted in accordance with the Declaration of Helsinki and ethical principles of Good Clinical Practice and was approved by the local ethics committees. All participants gave their written informed consent. The trial is registered at [Clinicaltrials.gov] [NCT01408810].

Adult male or female biological-naïve patients with moderately to severely active UC [defined as Mayo score ≥ 6 and endoscopic sub-score > 2][24] were enrolled. In addition, patients had to meet eligibility criteria to start infliximab according to the local Summary of Product Characteristics as follows: inadequate response to steroids at the equivalent of 40 mg/day of prednisolone or less, with or without 5-aminosalycilates [5-ASA], or steroid-dependence defined as inability to reduce steroids below 10 mg/day within 3 months of its start or relapse within 3 months of stopping steroids; or inadequate response/intolerance to thiopurines [after exposure for at least 3 months]. Patients with any contraindication to infliximab, severe anaemia [haemoglobin < 8.0 g/dl], non-removed adenomatous polyps, history of demyelinating diseases, opportunistic infections, and known viral infections [cytomegalovirus, human immunodeficiency virus, hepatitis B virus, or hepatitis C virus] were excluded. History of malignancy [including lymphoproliferative disorders] in the previous 5 years, history of latent or active tuberculosis, or signs or symptoms suggestive of active tuberculosis and topical treatment with 5-ASA or steroids were other exclusion criteria. Patients who were expected to undergo colectomy within 12 weeks after inclusion were also excluded.

2.2. Procedures

The study consisted of a 2-week screening period, a visit for infliximab administration [baseline], and clinical appointments at Weeks 8, 30, and 52. Before the introduction of infliximab, all patients underwent physical and neurological examinations and were characterised regarding disease extent and location [Montreal classification].[25] In addition, patients were screened for tuberculosis [chest X-ray and purified protein derivative skin test].[26] A cut-off of 5 mm was used for diagnosis of latent tuberculosis, according to local guidelines for immunosuppressed patients.[27]

Eligible patients started intravenous infliximab [Remicade®, 5 mg/kg] with subsequent infusions at Weeks 2, 6 and every 8 weeks thereafter. Patients receiving 5-ASA concomitantly maintained this treatment at the same dose until the end of the study. The dose of

corticosteroids had to be stable within the first 8 weeks. However, the physician was allowed to decrease or increase the dose according to the patient's condition. Thereafter, the dose was tapered at a dose ≤ 5 mg/week until complete discontinuation at Week 20. In case of clinical relapse [Mayo score > 2], intensified infliximab regimen [every 6 weeks] or re-introduction of corticosteroids was allowed. C-reactive protein [CRP] was assessed at each time point.

Demographic and clinical variables of interest were captured into an electronic case report form.

2.2.1. Histological assessment

The primary outcome was the proportion of patients with histological remission, measured with Geboes index, after 8 weeks of treatment with infliximab. Histological remission was defined as Geboes index ≤ 3.0.[15] The Geboes index consists of a scoring system with five distinctive grades [each with subgrades], corresponding to different aspects of inflammatory activity in the mucosa. Grade 0 corresponds to structural [architectural] change, grade 1 to chronic inflammatory infiltrate, grade 2 to lamina propria eosinophils [2A] and neutrophils [2B], grade 3 to neutrophils in the epithelium, grade 4 to crypt destruction, and grade 5 to erosion or ulceration. Higher subgrade scores reflect a more severe condition.[15]

During sigmoidoscopy, rectum and sigmoid biopsies [two of each] were taken for histological examination before introduction of infliximab, at Weeks 8, 30, and 52. The specimens were fixed in neutrally buffered formalin [4%] and were mailed to the central pathologist [Pathology Department of Centro Hospitalar São João]. Histological activity was graded by two independent pathologists [using the Geboes index][15] blinded for patient's disease status and endoscopic score. The biopsy with most severe inflammatory activity in rectum and sigmoid [Geboes index] was selected for the analysis of the histological score. Disagreements between the two pathologists concerning the histological score were solved by a third pathologist [KG] in a review of the sections, using a multi-headed microscope. The Geboes score remained blinded to all investigators.

2.2.2. Clinical and endoscopic assessments

UC clinical activity was assessed by Mayo score at the same time points as the evaluation of histological activity. Clinical remission was defined as Mayo score ≤ 2, with no individual subscore exceeding 1.[26] Clinical response was defined as a reduction in the Mayo score of at least 3 points and a decrease of at least 30% from the baseline score, accompanied by a decrease of at least 1 point in the rectal bleeding scale or an absolute rectal bleeding score of 0 or 1.[7] At each centre, the same gastroenterologist performed endoscopies using standardised procedures for assessment of Mayo score.

Mucosal healing was determined from the endoscopy subscore of the Mayo score. Mucosal healing was defined as endoscopy subscore of 0 or 1.[28]

2.2.3. Faecal markers assessment

Stool samples for determination of calprotectin and lactoferrin levels were collected at all study visits. Samples were kept at 4°C [maximum 48 h] until shipment to the central laboratory [Department of Pharmacology and Therapeutics, Faculty of Medicine of University of Porto]. For faecal calprotectin assessment, stools were extracted within a maximum of 7 days after collection in accordance with manufacturer's instructions ['Faecal sample preparation kit' of Roche Diagnostics, Germany] and stored at -80°C until the assay was performed. For faecal lactoferrin evaluation, once at the laboratory, stools were immediately stored at -80°C. Faecal calprotectin was measured by a quantitative enzyme immunoassay [EK-CAL ELISA kit-Bühlmann Laboratories AG, Switzerland]. All measurements were performed in duplicate samples, as recommended by ELISA manufacturers. Intra-assay variation was calculated to verify measurements repeatability. A good coefficient of variability was obtained [3.2%].

Faecal lactoferrin was also measured by a quantitative enzyme immunoassay [IBD-SCAN test -Techlab, Blacksburg, VA, USA]. All measurements were performed by single analysis of two dilutions [1:100 and 1:1000] for each stool sample, as recommended by manufacturers. Dilution that best fitted the standard curve was selected. It was not possible to assess intra-assay variation.

2.3. Statistical analysis

This was an exploratory study, with no formal hypothesis testing. Due to logistical constraints, it was only possible to enrol up to 20 patients. Descriptive statistics were used to describe patients included in the study. Continuous variables were summarised as median and interquartile ranges [IQR] and categorical variables as absolute and relative frequencies. Mann-Whitney testing was used to compare subgroups regarding biomarkers results. The rate of agreement between histological and other outcomes was obtained through the sum of true positives and true negatives over the overall sample.

Receiver operating characteristics [ROC] curves were generated and the area under the curve [AUC] was calculated to summarise the predictive ability of calprotectin and lactoferrin [at pre-established cut-offs] regarding histologically active disease [Geboes > 3]. Calprotectin and lactoferrin remission were considered at a concentration of < 100 µg/g[29] and ≤ 7.25 µg/g,[30] respectively.

The level of statistical significance was set at 0.05. Statistical Package for Social Sciences [SPSS] v.20.0® [IBM, Milan, Italy] was used for all analyses.

3. Results

3.1. Study population

A total of 20 patients were included in the study from February to November 2011. There were no drop-outs during the follow-up period. Table 1 summarises patients' baseline demographic and clinical characteristics. Patient median age was 40 years and the majority were female [65%]; 19 [95%] patients had active histological disease [Geboes index > 3.0] and all patients had endoscopic Mayo subscore > 1; 19 [95%] patients had calprotectin levels ≥ 100 µg/g and all patients had lactoferrin levels > 7.25 µg/g.

Six patients needed intensified infliximab regimen during the follow-up period [three patients required intensification before Week 30] [Figure 1]. The proportion of patients on corticosteroids and azathioprine over time and respective doses are shown as Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online.

3.2. Evolution of histological markers of inflammation

Table 2 shows the evolution of neutrophils in lamina propria and epithelium, crypt destruction, and erosion or ulceration [Geboes grades 2 to 5]. For all these domains, there was a gradual decrease in the most severe subgrades over time. At Weeks 30 and 52, 50% and 45% of patients, respectively, had no neutrophils in the lamina propria [subgrade 2B0]. Of all patients who achieved Geboes ≤ 3.0, none was subgrade 2B.1, 2B.2, or 2B.3 [data not shown]. The rate of subgrade 3.3 [> 50% crypts involved] decreased from 65% [13/20]

at baseline to 10% [2/20] at Week 52, and the rate of subgrade 4.3 [unequivocal crypt destruction] decreased from 80% [16/20] to 25% [5/20], respectively. The proportion of patients with no erosions or ulceration [subgrade 5.0] increased from 5% [1/20] at baseline to 45% [9/20] at Week 52 [Table 2].

Table 1. Baseline demographic and clinical characteristics of patients.

N	20
Age [years], median [IQR]	40 [37–51]
Female, *n* [%]	13 [65]
Smoking status, *n* [%]	
Ex-smoker	7 [35]
Non-smoker	13 [65]
Disease location, *n* [%]	
Left sided colitis	13 [65]
Pancolitis	7 [35]
Extra-intestinal manifestations, *n* [%]	7 [35]
Corticosteroids, *n* [%]	15 [75]
Corticosteroids dose [mg/day], mean	35
Azathioprine, *n* [%]	17 [85]
Geboes index > 3.0, *n* [%]	19 [95]
Total Mayo score, median [IQR]	8.5 [8–10]
Mayo endoscopic subscore > 1, *n* [%]	20 [100]
CRP [mg/l], median [IQR]	5.38 [2.40–13.00]
CRP < 3 mg/l, *n* [%]	8 [40]
Faecal calprotectin levels [µg/g], median [IQR]	624.13 [64.8–1679.9]
Faecal calprotectin levels = 100 µg/g, n [%]	19 [95]
Faecal lactoferrin levels [µg/g], median [IQR]	271.90 [14.9–988.6]
Faecal lactoferrin levels > 7.25 µg/g, n [%]	20 [100]

Data are expressed as number and percentage or median and interquartile range [IQR].

CRP, C-reactive protein; IQR, interquartile range.

3.3. Association of histological activity with other outcomes

The rate of histological remission increased gradually over time [Table 3]. At Week 8, 15% [3/20] of patients had Geboes index ≤ 3.0, compared with 5% [1/20] at baseline. At Week 52, 35% [7/20] of patients achieved histological remission. Two out of three patients with Geboes ≤ 3.0 at Week 8 maintained histological remission up to Week 52. Five out of six patients with Geboes ≤ 3.0 at Week 30 were still in histological remission at Week 52. The rates of clinical remission and clinical response followed the same trend as histology. At Week 52, 50% [10/20] of patients were in clinical remission and 90% [18/20] had clinical response. Although 45% [9/20] of patients achieved mucosal healing at Week 8, this rate remained steady between Weeks 30 and 52 [55%; 11/9]. At Weeks 30 and 52, 25% [5/20] of patients achieved clinical, endoscopic, and histological remission.

At Week 8, 35% [7/20] of patients reached calprotectin remission compared with 5% [1/20] at baseline. The rate of lactoferrin remission increased over time, with 35% [7/20] of patients achieving this outcome at Week 52.

No relevant variation was found over time when combining clinical, histological, endoscopic and biomarkers remission.

At Week 8, two out of three patients with Geboes index ≤ 3.0 were also in clinical remission, this rate increasing to five out of six patients at Week 30 [agreement rate of 75%] and slightly decreasing to five out of seven at Week 52 [Table 4]. The agreement between clinical response and histological remission over time was low [45-50%].

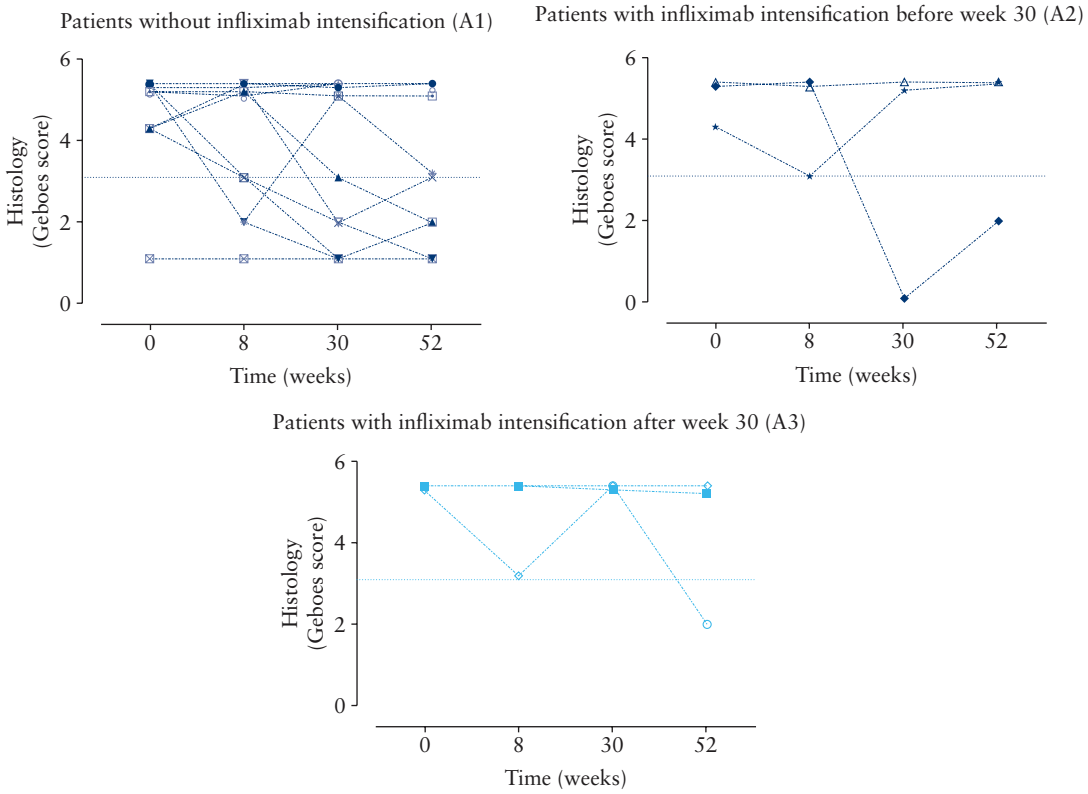

Figure 1. Individual patient evolution of histology [Geboes index] over time based on the need for infliximab intensification. After intensification, two out of six patients achieved histological remission.

Table 2. Evolution of neutrophils in lamina propria and epithelium, crypt destruction, and erosion or ulceration [Geboes grades 2-5].

N = 20	Baseline	Week 8	Week 30	Week 52
Grade 2 Neutrophils and eosinophils in lamina propria				
2B Neutrophils				
0 None	1 [5%]	2 [10%]	10 [50%]	9 [45%]
1 Mild but unequivocal increase	9 [45%]	10 [50%]	7 [35%]	9 [45%]
2 Moderate increase	8 [40%]	7 [35%]	3 [15%]	2 [10%]
3 Marked increase	2 [10%]	1 [5%]	0 [0%]	0 [0%]
Grade 3 Neutrophils in epithelium				
3.0 None	1 [5%]	3 [15%]	6 [30%]	8 [40%]
3.1 < 5% crypts involved	0 [0%]	5 [25%]	5 [25%]	3 [15%]
3.2 < 50% crypts involved	6 [30%]	3 [15%]	4 [20%]	7 [35%]
3.3 > 50% crypts involved	13 [65%]	9 [45%]	5 [25%]	2 [10%]
Grade 4 Crypt destruction				
4.0 None	1 [5%]	8 [40%]	10 [50%]	10 [50%]
4.1 Probable: local excess of neutrophils in part of crypt	2 [10%]	2 [10%]	3 [15%]	5 [25%]
4.2 Probable: marked attenuation	1 [5%]	0 [0%]	1 [5%]	0 [0%]
4.3 Unequivocal crypt destruction	16 [80%]	10 [50%]	6 [30%]	5 [25%]
Grade 5 Erosion or ulceration				
5.0 No erosion, ulceration, or granulation tissue	1 [5%]	7 [35%]	7 [35%]	9 [45%]
5.1 Recovering epithelium + adjacent inflammation	0 [0%]	1 [5%]	1 [5%]	1 [5%]
5.2 Probable erosion: focally stripped	6 [30%]	3 [15%]	2 [10%]	1 [5%]
5.3 Unequivocal erosion	3 [15%]	3 [15%]	3 [15%]	1 [5%]
5.4 Ulcer or granulation tissue	10 [50%]	6 [30%]	7 [35%]	8 [40%]

Data are expressed as numbers and percentages. For each grade of Geboes index, the most severe histological finding was considered.

Table 3. Evolution of histological, clinical, endoscopic, and biomarker outcomes over 52 weeks.

	Baseline	Week 8	Week 30	Week 52
Histological remission [Geboes ≤ 3.0], *n* [%]	1 [5]	3 [15]	6 [30]	7 [35]
Persistence of histological remission among patients with Geboes ≤ 3.0 at Week 8 [*n* = 3], *n* [%]	NA	NA	2 [67]	2 [67]
Persistence of histological remission among patients with Geboes ≤ 3.0 at Week 30 [*n* = 6], *n* [%]	NA	NA	NA	5 [83]
Clinical remission, *n* [%]	0 [0]	8 [40]	9 [45]	10 [50]
Clinical response, *n* [%]	0 [0]	14 [70]	16 [80]	18 [90]
Mucosal healing, *n* [%]	0 [0]	9 [45]	11 [55]	11 [55]
Clinical + endoscopic + histological remission, *n* [%]	0 [0]	2 [10]	5 [25]	5 [25]
Calprotectin levels [µg/g], median [IQR]	624.1 [417.1–1403.8]	230.0 [40.6–425.4]	162.1 [68.3–986.2]	125.1 [41.7–379.4]
Calprotectin < 100 µg/g, n [%]	1 [5]	7 [35]	7 [35]	8 [40]
Lactoferrin levels [µg/g], median [IQR]	271.9 [88.3–674.3]	53.2 [14.8–175.0]	77.3 [7.3–159.8]	33.3 [4.5–157.0]
Lactoferrin ≤ 7.25 µg/g, n [%]	0 [0]	3 [15]	5 [25]	7 [35]
Clinical + histological + calprotectin remission, *n* [%]	0 [0]	2 [10]	4 [20]	2 [10]
Clinical + histological + calprotectin + lactoferrin remission, *n* [%]	0 [0]	2 [10]	4 [20]	2 [10]
Clinical + histological + calprotectin + lactoferrin remission + mucosal healing, *n* [%]	0 [0]	2 [10]	4 [20]	2 [10]
CRP [mg/l], median [IQR]	5.38 [2.40–13.00]	2.10 [0.50–7.40]	2.15 [0.65–3.78]	2.45 [0.95–4.30]
CRP < 3 mg/l, *n* [%]	8 [40]	11 [58]	15 [75]	11 [55]

Data are expressed as numbers and percentages or median and interquartile range [IQR]. Unless otherwise indicated, *N* = 20. Clinical remission defined as Mayo score of 2 points or lower, with no individual subscore exceeding 1 point. Clinical response defined as reduction in the Mayo score ≥ 3 points and a decrease of at least 30% from the baseline score, accompanied by a decrease of at least 1 point in the rectal bleeding scale or an absolute rectal bleeding score of 0 or 1. Mucosal healing defined as Mayo endoscopic subscore ≤ 1. Calprotectin remission defined as levels < 100 µg/g; lactoferrin remission defined as levels ≤ 7.25 µg/g.

NA, not available; CRP, C-reactive protein.

At every follow-up visit, all patients with histological remission also had clinical response. At Weeks 8 and 30, all patients who achieved histological remission [three and six patients, respectively] also had mucosal healing. The highest rate of agreement between mucosal healing and histological remission was observed at Week 30 [74%].

At Week 8, all patients who were in histological remission showed calprotectin levels < 100 µg/g and 67% had lactoferrin levels <= 7.25 µg/g. The highest rate of agreement between histology and calprotectin was achieved at Week 30 [85%], and for lactoferrin the highest rate was observed at Week 8 [90%].

At each time point, median calprotectin levels were significantly lower among patients with histological remission compared with patients without remission. Similar results were found for lactoferrin, although the difference was not significant at Week 30 [p = 0.187].

Table 4. Evolution of clinical, endoscopic, and biomarker outcomes over 52 weeks, according to histological activity.

	Week 8		Week 30		Week 52	
Outcomes	Geboes ≤ 3.0	Geboes > 3.0	Geboes ≤ 3.0	Geboes > 3.0	Geboes ≤ 3.0	Geboes > 3.0
N	3	17	6	14	7	13
Clinical remission, *n* [%]	2 [67]	6 [35]	5 [83]	4 [29]	5 [71]	5 [38]
Agreement, %	65		75		65	
Clinical response, *n* [%]	3 [100]	11 [65]	6 [100]	10 [71]	7 [100]	11 [85]
Agreement, %	45		50		45	
Mucosal healing, *n* [%]	3 [100]	6 [35]	6 [100]	5 [38]	6 [86]	5 [38]
Agreement, %	70		74		70	
Calprotectin levels [µg/g], median [IQR]	25.0 [6.2–37.6]	242.4 [154.8–455.6]	63.8 [53.4–83.3]	622.8 [130.8–1019.4]	27.5 [22.4–105.6]	195.7 [64.8–439.0]
p-Value		0.017		0.032		0.029
Calprotectin remission, *n* [%]	3 [100]	4 [24]	5 [83]	2 [14]	4 [57]	4 [31]
Agreement, %	80		85		65	
Lactoferrin levels, [µg/g], median [IQR]	6.8 [5.7–7.3]	73.1 [31.0–182.4]	6.8 [2.1–93.2]	86.1 [22.4–211.0]	1.5 [0.2–26.0]	106.7 [27.7–181.8]
p-Value	0.013		0.187		0.024	
Lactoferrin remission, *n* [%]	2 [67]	1 [6]	3 [50]	2 [14]	5 [71]	2 [15]
Agreement, %	90		75		80	
CRP levels, [mg/l], median [IQR]	2.90 [0.60–63.00]	1.70 [0.38–6.15]	0.95 [0.50–2.90]	2.85 [0.80–4.60]	1.90 [0.60–4.30]	2.90 [1.30–5.60]
p-Value	0.467		0.265		0.781	
CRP remission, *n* [%]	2 [67]	9 [56]	5 [83]	10 [71]	4 [57]	7 [54]
Agreement, %	47		45		50	

Data are expressed as numbers and percentages or median and interquartile range [IQR]. Mann-Whitney test was used to compare subgroups regarding calprotectin, lactoferrin and C-reactive protein [CRP] levels. Statistical significance was set at $p < 0.05$. Geboes ≤ 3.0 corresponds to histological remission. Clinical remission defined as Mayo score of 2 points or lower, with no individual subscore exceeding 1 point. Clinical response defined as reduction in the Mayo score ≥ 3 points and a decrease of at least 30% from the baseline score, accompanied by a decrease of at least 1 point in the rectal bleeding scale or an absolute rectal bleeding score of 0 or 1. Mucosal healing defined as Mayo endoscopic subscore ≤ 1. Calprotectin remission defined as levels < 100 µg/g. Lactoferrin remission defined as levels ≤ 7.25 µg/g. CRP remission defined as levels ≤ 3.0 mg/l.

CRP levels had no statistical significance and the rate of agreement with histological remission was poor. Figure 2 illustrates the individual patient's evolution of faecal calprotectin and lactoferrin levels according to histological outcome [remission vs activity].

3.4. Biomarkers' sensitivity and specificity for histological and endoscopic activity

Calprotectin showed an AUC of 0.94 at Week 8 [cut-off of 100 µg/g], with a sensitivity of 76% to detect histological activity and a specificity of 100% [$p = 0.017$]. The sensitivity increased to 86% at Week 30 [specificity of 83%; $p = 0.032$] but decreased to 69% at Week 52 [specificity 57%; $p = 0.029$] with AUC remaining at 0.8 [Table 5]. Lactoferrin showed an AUC of 0.96 at Week 8 [cut-off of 7.25 µg/g], with a sensitivity of 94% and a specificity of 66% [$p = 0.013$]. The sensitivity decreased to 85% at Weeks 30 and 52 [specificity of 50% and 71%, respectively]. At Week 30, the AUC for lactoferrin was 0.69 [$p = 0.187$]. The highest positive predictive value for calprotectin and lactoferrin regarding histological remission was observed at Week 8 [100% and 94%, respectively].

At Week 8, calprotectin showed an AUC of 0.92, with a sensitivity of 90% to predict endoscopic activity and a specificity of 66% [$p = 0.002$]. The predictive value of this marker became weaker over time, with no statistical significance at Week 30 [$p = 0.083$]. Likewise, lactoferrin showed excellent predictive value of endoscopic activity at Week 8 [AUC = 0.92, a sensitivity of 100% and a specificity of 33%; $p = 0.002$]. The highest negative predictive value for calprotectin [100%] and lactoferrin [100%] was achieved at Week 30 and Week 8, respectively.

4. Discussion

Histological remission is becoming a relevant therapeutic endpoint in IBD, as evidence suggests that microscopic inflammation may persist in endoscopically quiescent mucosa.[31] This relevance relies on the premise that persistent inflammation in IBD leads to earlier relapse, progressive damage, and cumulative disability, and increases the risk of hospitalisation, colectomy, and colorectal cancer.[32] At present, there is no standard definition for histological remission.[8] We used a Geboes index ≤ 3.0 to express remission, which corresponds to the absence of neutrophils in the epithelium, as crypt abscesses and a mucosal breach have been linked with relapse.[8,11] The Geboes score has been widely used in clinical studies and clinical practice.[25]

To our knowledge, this was the first study to investigate histological activity as primary outcome in UC patients treated with infliximab. We found that the proportion of patients with histological remission increased gradually over time. After 8 weeks of treatment with infliximab, 15% of patients had histological remission, this rate increasing to 35% at Week 52. Furthermore, improvement in histological outcomes was accompanied by improvement in clinical outcomes over time. At Week 52, only 2 out of 20 [10%] patients had no clinical response and half of the patients achieved clinical remission. Regarding the endoscopic endpoint, nearly half of the patients achieved mucosal healing at Week 8, this proportion maintaining relatively steady until the end of follow-up. The highest rate of agreement of histological remission with either clinical remission or mucosal healing was observed at Week 30 [75%]. At Week 30, one-quarter of the patients achieved clinical, endoscopic remission and histological remission, which was sustained until Week 52.

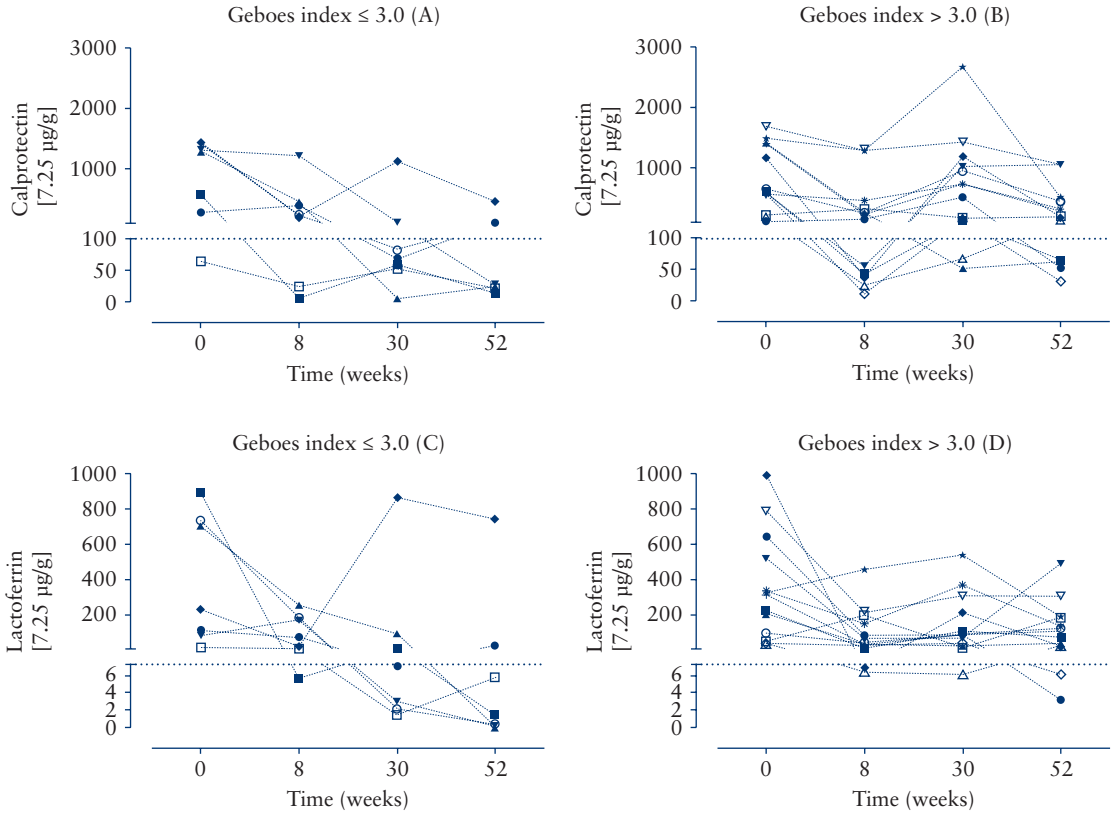

Figure 2. Individual evolution of faecal calprotectin [A and B] and lactoferrin [C and D] levels according to histological outcome [remission vs activity] over 52 weeks. The majority of patients with histological remission [Geboes ≤ 3.0] also had biomarkers levels below the predefined cut-offs. The criteria for Geboes score were taken by the histological evaluation of each patient at Week 52.

Table 5. Accuracy of faecal calprotectin and lactoferrin measurement in predicting histological and endoscopic activity among ulcerative colitis patients.

	Histological activity [Geboes > 3.0]							Endoscopic activity [Mayo subscore > 1]						
	AUC	95%CI	*p*	SEN	SPE	PPV	NPV	AUC	95%CI	*p*	SEN	SPE	PPV	NPV
Calprotectin [cut-off value ≥ 100 µg/g]														
Week 8	0.94	0.84–1.0	0.017	76%	100%	100%	42%	0.92	0.80–1.0	0.002	90%	66%	77%	85%
Week 30	0.81	0.57–1.0	0.032	86%	83%	92%	71%	0.74	0.51–0.97	0.083	100%	55%	62%	100%
Week 52	0.80	0.58–1.0	0.029	69%	57%	75%	50%	0.79	0.59–1.0	0.025	77%	55%	54%	75%
Lactoferrin [cut-off value > 7.25 µg/g]														
Week 8	0.96	0.88–1.0	0.013	94%	66%	94%	66%	0.92	0.79–1.0	0.002	100%	33%	65%	100%
Week 30	0.69	0.34–1.0	0.187	85%	50%	80%	60%	0.67	0.42–0.92	0.215	88%	57%	50%	80%
Week 52	0.81	0.55–1.0	0.024	85%	71%	85%	71%	0.76	0.52–0.99	0.053	88%	55%	62%	86%

AUC, area under the curve; SEN, sensitivity; SPE, specificity; PPV, positive predictive value; NVP, negative predictive value.
Statistical significance was set at $p < 0.05$.

Other trials have reported histological remission or improvement following therapy in UC. Histological improvement was observed in UC patients treated with oral budesonide and aminosalicylates, sometimes reaching histological remission.[33,34,35,36] Fewer studies have assessed histological remission to immunomodulators or biological therapies.[37,38,39] One retrospective study with 61 UC patients treated with infliximab for at least 11 months showed that 59% of patients achieved clinical, endoscopic and histological remission.[38] However, the results from that study may be overestimated, as it only included primary responders to induction therapy. In addition, the authors used a different histological scoring. More recently, a study evaluated mucosal healing and histological remission as co-primary endpoints in 34 biological-naïve patients with active UC treated with adalimumab.[40] At Week 8, 26% of patients achieved histological remission, this rate increasing to 52% at Week 52, higher than the rates observed in our study. In addition, mucosal healing was observed in 50% [Week 8] and 60% of patients [Week 52]. Still, this was a retrospective study and findings were only published as an abstract.

Histological remission should be distinguished from endoscopic mucosal healing in UC. In our study, we found a moderate agreement between histological and endoscopic remission. Bryant *et al*. showed that histological remission was more valuable as a marker of 'complete' remission than endoscopic remission in predicting the need for corticosteroids or hospitalisation due to acute severe colitis over a 6-year follow-up.[41] Other studies highlighted the value of histology in predicting the risk of colorectal cancer,[42,43] clinical relapse,[9,44] and decreased hospitalisation or colectomy rates in UC.[13,45,46,47] Recently, a 12-month prospective study with UC patients in clinical remission showed that Geboes histology grade was strongly associated with the risk of clinical relapse, independently of the Mayo endoscopy score.[48]

In general, there was a remarkable decrease in the rates of most severe subgrades of the Geboes index over time. The most striking reduction was related with unequivocal crypt destruction [subgrade 4.3], which decreased from 80% at baseline to 25% at Week 52. On the other hand, the proportion of patients with no erosion, ulceration, or granulation tissue [subgrade 5.0] increased markedly between baseline and Week 52 [from 5% to 45%]. We could theoretically admit that the histological modifications observed over time in our study were due to concomitant treatments. Still, we found that the proportion of patients on corticosteroids and azathioprine decreased with time. Thus, it is plausible to assume that histological alterations were due to infliximab.

Faecal calprotectin and lactoferrin are well-established markers of mucosal inflammation in UC, showing good correlation with both clinical and endoscopic activity.[19] Several studies have demonstrated that calprotectin concentrations correlate well with endoscopic activity.[20,49,50] The evidence is more limited regarding the utility of faecal lactoferrin as a surrogate marker in UC. In the study of Masoodi *et al*., the decline of lactoferrin correlated with a decrease in Mayo Score.[51] The data are even scarcer on how these biomarkers correlate with histological inflammation.[20]

In our study, the rates of calprotectin remission [< 100 µg/g] and lactoferrin remission [≤ 7.25 µg/g] increased over time, following the same trend as clinical, endoscopic, and histological remission rates. At each follow-up time point, patients with histological remission had lower median calprotectin and lactoferrin levels compared with patients without histological remission. Calprotectin and lactoferrin showed the highest predictive value for histological activity at Week 8. Furthermore, lactoferrin showed higher sensitivity than calprotectin for histological activity at Week 8 whereas calprotectin had higher specificity. Of note, both biomarkers showed very high positive predicitive value [PPV] regarding histological outcome at Week 8 [100% and 94%, respectively], indicating that levels above the biomarker's cut-off are highly suggestive of histological activity. Following the same trend as for histological activity, the highest ability of calprotectin and lactoferrin to predict endoscopic activity was found at Week 8.

One of the major strengths of this study was the use of the Geboes index, which is the best-validated instrument to measure histological activity and more reproducible for clinical practice.[8] Recently, two additional scores for ulcerative colitis have been validated and published (Nancy index and Robarts histopathology index [RHI]).[24,31] Both scores can be considered modifications of the Geboes score. The correlation between the Nancy index and the Geboes score was very good, and the RHI uses similar features as the Geboes score.

The small sample size is the major limitation of this study. There was one patient in our sample with Geboes < 3.1 at baseline. Although this patient met eligibility criteria, with an endoscopic Mayo score = 2, a global Mayo score = 6 and high levels of faecal biomarkers [calprotectin = 64.7 µg/g and lactoferrin = 14.8 µg/g], the inclusion of one individual with histological remission at baseline introduces bias in our sample. Therefore, one should be cautious when translating these findings to broader populations.

Some authors have suggested that the presence of basal plasmacytosis and a Geboes score ≥ 3.1 are predictive of disease relapse.[44] Since the Geboes score does not assesses basal plasmacytosis separately, which is included in the 'chronic inflammation' item, this histological feature was not analysed, constituting a limitation of our study.

In conclusion, this was the first study to investigate histological activity as primary outcome in UC patients treated with infliximab. We found that infliximab is able to induce histological remission. Of clinical relevance is the strong positive predictive value revealed by faecal calprotectin at Week 8. The growing interest among clinicians for the use of histological remission as a treatment target in IBD is supported by our findings showing that infliximab is able to improve histological outcomes in UC patients. Further research should be carried out with larger samples and control groups to clearly establish the impact of infliximab on histological outcome.

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Conflicts of Interest

FM served as speaker and received honoraria from Merck Sharp & Dohme, Abbvie, Vifor, Falk, Laboratorios Vitoria, Ferring, Hospira, and Biogen. The other authors have no competing interests.

Author Contributions

Study concept, design, and trial responsibility: FM; histological assessments: FC, KG, JL, MRS, CL, PM, and LM; pathologist: KG; faecal markers assessment: JA; data acquisition: FM, SIL, FP, JC, MM, PL, PP, AA, SR, and GM; statistical analysis and data interpretation: CC, LV, FM, FC, and KG. All authors revised the manuscript critically for important intellectual content and approved its final version.

Supplementary Data

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

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