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Soluble human Suppression of Tumorigenicity 2 is associated with endoscopic activity in patients with moderate-to-severe ulcerative colitis treated with golimumab

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Abstract

Background: Suppressor of Tumorigenicity 2 (ST2) is an IL33 receptor detected in the mucosa and serum of ulcerative colitis (UC) patients. We evaluated soluble ST2 (sST2) as a surrogate biomarker of disease outcome and therapeutic response, in moderate-to-severe UC patients treated with golimumab.

Methods: We conducted an open-label single-arm multicentre prospective study. At screening/baseline, week 6 (W6) and week 16 (W16), clinical and endoscopic activity (total Mayo score), histologic activity (Geboes index) and biomarkers were evaluated.

Results: From 38 patients, 34 (89.5%) completed W6 and 29 (76.3%) completed W16. Mean age (±SD) was 34.6 ± 12.6 years; 55.9% were female. At W16, 62.1% achieved clinical response. Patients with endoscopic activity at W6 ($n = 20$) had higher baseline sST2 (median, 24.5 *versus* 18.7 ng/ml, $p = 0.026$) and no decrease from baseline (median change, 0.8 *versus* −2.7, $p = 0.029$). At W6, sST2 levels correlated with endoscopic activity ($rs = 0.45$, $p = 0.007$) but not with histological activity ($rs = 0.25$, $p = 0.151$). The best cut-offs for endoscopic activity were sST2 = 16.9 ng/ml (sensitivity = 85%; specificity = 71%) and faecal calprotectin (FC) = 353 µg/g (sensitivity = 90%, specificity = 67%). Patients with histological activity at W6 ($n = 27$) had higher baseline ST2 levels (median, 23.0 versus 13.7 ng/ml, $p = 0.035$). sST2 did not correlate with FC or serum C-reactive protein. FC levels correlated with histological activity and baseline FC were higher when Geboes ⩾3.1 at W6.

Conclusions: sST2 may be a surrogate biomarker of UC activity and therapeutic response as it correlates with endoscopic and clinical activity at W6 of golimumab treatment, and subjects with endoscopic and histological activity at W6 had higher baseline ST2 levels.

Keywords: endoscopic activity, golimumab, histological activity, serum soluble ST2, ulcerative colitis

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Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) causing continuous mucosal inflammation of the colon without granulomas, and with a relapsing and remitting course.[1] Current IBD biomarkers include serological levels of specific antibodies (e.g. ASCA, ANCA, anti-OmpC, anti-Cbir, and antiglycans), serum C-reactive protein (CRP), cytokines and faecal proteins (calprotectin and lactoferrin).[2] Nevertheless, most of these markers show low sensitivity and specificity and do not adequately reflect intestinal damage.[2,3]

Mucosal healing has become a major objective of IBD treatment but endoscopy is still the 'gold standard' for assessing inflammation of intestinal mucosa.[4] Faecal calprotectin (FC) levels significantly correlate with endoscopic activity in IBD and are currently one of the best surrogate markers of intestinal inflammation.[5,6] A meta-analysis reported FC pooled sensitivity and specificity estimates of 0.88 and 0.79, respectively, regarding UC endoscopic activity.[7] CRP is an acute-phase marker produced in the liver and in the mesenteric adipocytes, and is a potential IBD biomarker.[8] FC seems to be more sensitive in UC than in Crohn's disease, while CRP sensitivity in UC (50–60%) seems to be lower than in Crohn's disease (70–100%).[7,8] Other simple, rapid, sensitive, specific, inexpensive and noninvasive markers are still needed to detect and monitor intestinal inflammation, especially during early treatment.[9,10]

IL-33 is a member of the IL-1 family that binds to its receptor, ST2 (human Suppression of Tumorigenicity 2), with the complex IL-33/ST2 leading to cytokine inactivation.[11] However, the IL-33/ST2 axis seems to have a dual and dichotomous role in the pathogenesis of IBD. On the one hand, proinflammatory cytokine stimuli (e.g. TNF) result in an increase of IL-33 in epithelial cells. On the other hand, IL-33 may be released by injured epithelial cells to induce production of proinflammatory cytokines through activation of ST2 in mast cells, macrophages, eosinophils and neutrophils.[12] In mucosal tissue of mouse models, IL-33 levels correlate positively with the severity of gut inflammation.[12] Similarly, the expression of ST2 seems to be increased in both colonic wall and serum of UC patients, and levels of serum soluble ST2 (sST2) also correlate positively with the severity of colonic mucosal disease and inflammatory cytokines.[3,11,12]

Treatment with TNFα inhibitors modulates IL-33 and sST2 levels. In a small cohort of IBD patients, Pastorelli and colleagues evaluated infliximab's effects in a group of 9 UC patients and 11 subjects with Crohn's disease, observing a decrease of IL-33 and of the IL-33/sST2 ratio, especially in UC patients.[12] However, few studies have correlated the levels of sST2 with endoscopic and histological activity in UC patients receiving anti-TNFα therapy.

Golimumab is a fully human anti-TNFα monoclonal antibody that binds with high affinity to human TNFα. It has been shown to induce a clinical response in 51% of patients with moderate-to-severe UC at week 6, with remission and mucosal healing being achieved by 18% and 42% of patients, respectively.[13,14]

The present work was an exploratory study aiming to evaluate sST2 as a surrogate biomarker of disease outcomes and therapeutic response in subjects with moderately-to-severely active UC who had started treatment with golimumab. The correlation of sST2 levels with clinical activity (total Mayo score), endoscopic activity (Mayo endoscopic subscore) and histological activity (Geboes index) were evaluated in this population. In addition, the association between sST2, FC and CRP levels was studied, as well as the performance of these biomarkers in predicting endoscopic and histological activity in UC patients treated with golimumab.

Methods

This was an exploratory, multicentre, open-label, prospective, interventional, single-arm study, conducted in nine reference sites in Portugal (Clinical Trial Identification: EudraCT 2014–003262–25). All procedures complied with the ethical principles of the Declaration of Helsinki and Good Clinical Practice requirements. The protocol and informed consent procedures were approved by the National Ethics Committee for Clinical Research and the Data Protection Authority.

Eligible subjects were aged ⩾18 and ⩽65 years old, with a previous diagnosis of moderate-to-severely active UC, who have had an inadequate response to conventional therapy (including corticosteroids and 6-mercaptopurine or azathioprine) or were intolerant to, or had medical contraindications for,

such therapies. Subjects were naïve to TNFα inhibitors, and eligible to start golimumab according to its approved indication and dosing regimen. Subjects who had extensive severe colitis, symptomatic colonic or small bowel obstruction, history of colonic mucosal dysplasia, presence of adenomatous polyps, who had used apheresis or had rectal corticosteroids or 5-ASA compounds within 2 weeks prior to the study were excluded. In addition, subjects who received cyclosporine, tacrolimus, sirolimus, or mycophenolate mofetil within 8 weeks prior to the study, who received natalizumab or any agent that depletes B or T cells within 12 months prior to the study, with history of, or ongoing, chronic infectious disease, immune deficiency, malignancy, chronic heart failure or other severe, progressive or uncontrolled disease, were also excluded.

Study treatment

Golimumab treatment followed the summary of product characteristics. The first dose of subcutaneous golimumab (200 mg) was administered at the trial site at baseline. Subsequent dosing was done by the subject (i.e. unsupervised at their home) at week 2 (100 mg) and every 4 weeks thereafter (50 mg if weight <80 kg or 100 mg if ≥ 80 kg).

Procedures and definitions

After signing informed consent, subjects entered a screening period of up to 42 days, for confirmation of eligibility criteria. Eligible subjects had initiated treatment with golimumab at the baseline visit. Subjects were followed for 16 weeks for clinical assessments and data collection, including stool frequency, amount of blood in stool, the physician's global assessment and clinical classifications. Blood and stool samples were collected at baseline and at subsequent visits at week 6 (end of induction) and week 16 (short-term maintenance).

Soluble ST2 levels (expressed in ng/ml) were measured using an ELISA kit for human ST2 (DuoSet, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Blood samples were centrifuged, and serum was stored at −80°C. FC was measured using the Quantum Blue® Calprotectin test (Bühlmann, Schönenbuch, Switzerland). Stool samples were kept at 4°C for a maximum of 48 h before being sent to the Central Laboratory (Department of Biomedicine, Unity of Pharmacology and Therapeutics, Faculty of Medicine of University of Porto, Portugal). FC was extracted from stools within 7 days after collection using a specific preparation kit (Roche Diagnostics, Mannheim, Germany), and stored at −80°C until quantification.

At screening, week 6 (W6) and week 16 (W16), all subjects were evaluated for endoscopic activity (through fibrosigmoidoscopy) and rectum and sigmoid biopsies (two samples of each) were taken to be evaluated by two independent pathologists. The samples were formalin fixed and sent to the Central Laboratory. Histological activity was graded using the Geboes index, and blinded for patient's disease status and endoscopic score.[15] The biopsy with most severe inflammatory activity (according to the Geboes index) was selected for analysis of histological score. The Geboes index is a validated score for evaluating histologic disease activity in UC. Active histological disease was defined as a Geboes score >3.0 (presence of epithelial neutrophils with or without crypt destruction or erosions).[15] The Geboes score was also converted into the Robarts histopathology index (RHI).[16] First, we treated the Geboes index as a continuous scale, as previous established in the literature.[17,18] Then we extrapolated the RHI values by applying a simple rule of three to the continuous version of the Geboes index. Active histological disease was defined as RHI > 6.[16]

The total Mayo score is a scale for assessing UC activity that results from the sum of four subscores and ranges from 0 to 12 points.[19,20] 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, in addition to a decrease of at least 1 point in the rectal bleeding scale or a rectal bleeding score of 0 or 1.[21] Clinical remission was defined as Mayo score ≤ 2, with no individual subscore >1.[19] The partial Mayo score results from the exclusion of the endoscopy subscore of the Mayo score and its values range from 0 to 9 points.[22] Endoscopic response (i.e. mucosal healing) at W6 and W16 was defined as Mayo endoscopic subscore of 0 or 1. Endoscopic active disease was defined as endoscopic subscore >1.[23]

Statistical analysis

This was an exploratory study, with no formal hypothesis testing. It was planned to include 37 subjects based on the recruitment capacity of the

centres. Statistical analyses were based on the full analysis set (FAS), defined as all subjects who received study medication and had at least one valid postbaseline assessment for the primary endpoint, that is, the correlation between sST2 and endoscopic/histological activity at W6 of golimumab treatment. The statistical significance of the correlation between sST2 levels and other markers of UC activity at W6 and W16 was assessed through Spearman's correlation coefficient (r_s) with the correspondent 95% two-sided confidence intervals (CI). Receiver operating characteristic (ROC) curve analysis was performed, and sensitivity, specificity, and positive and negative predictive values were estimated. The comparison of sST2, FC and CRP levels between groups of UC activity at W6 and W16 were performed by t-test or the Mann–Whitney nonparametric U test. Within-group changes were compared by paired t-test or equivalent nonparametric tests (signed rank and Wilcoxon signed-rank test). Statistical analysis assumed a significance level of 0.05, and was performed with SAS® (version 9.4, SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

A total of 45 participants were screened and 38 were included. All enrolled participants received at least one dose of golimumab, and 29 (76.3%) subjects completed follow up until W16 (Figure S1); 34 subjects had data at W6 (FAS population). Subjects had a mean age (± SD) of 34.6 ± 12.6 years and 55.9% were female. The median time since UC diagnosis was 5.0 years. At baseline, 52.9% had extensive colitis and 44.1% presented left-sided colitis; 79.4% were receiving immunosuppressants. Additional information about included patients was described previously.[24]

Clinical, endoscopic, histological and biomarker evolution of UC

At W16, the clinical response rate was 62.1% (95% CI: 42.3–79.3%) and clinical remission 37.9% (95% CI: 20.7–57.7%). Total Mayo score decreased significantly over time, from a median score of 8 points before golimumab administration to 4 points at W16. The dimensions of Mayo score also improved during the study, as well as the rate of endoscopic healing (Figure 1, Table S1). Regarding histological activity, 97.0%, 79.4% and 75.9% subjects had histologically active UC (Geboes index >3.0) at screening, W6 and W16, respectively (Table S2). Changes in biomarker (sST2, FC and CRP) levels during the study were not statistically significant (Figure 2).

Baseline levels of biomarkers by clinical response, endoscopic and histological activity at week 6 and week 16

Subjects with endoscopic activity at W6 had higher baseline levels of sST2 (median, 24.5 *versus* 18.5 ng/ml, $p = 0.026$) (Figure 3), and a different change of sST2 levels from baseline [median change (interquartile range), 0.8 (−3.2–7.8) *versus* −2.7 (−9.2 to −0.3) ng/ml, $p = 0.029$]. In addition, baseline sST2 levels were higher among subjects with endoscopic activity at W16 (median, 11.3 *versus* 6.0 ng/ml, $p = 0.025$) (Table S3). With regards to histological results, baseline sST2 levels were higher among subjects with histological activity (assessed by Geboes index) at W6 (median, 23.0 *versus* 13.7 ng/ml, $p = 0.035$) (Figure 3) and W16 (median, 21.5 *versus* 11.7 ng/ml, $p = 0.016$) (Table S3). Baseline sST2 levels were also higher among subjects with histological activity at week 16 as assessed by RHI: median, 20.8 *versus* 12.7 ng/ml, $p = 0.038$). No statistically significant differences were observed regarding baseline sST2 levels by clinical response at W6 and W16.

Subjects with histological activity (as assessed by Geboes index) at W6 had higher FC baseline levels (median, 884 *versus* 414 µg/g, $p = 0.010$) but no statistically significant different CRP baseline levels (Figure 3). Similar results were observed when classifying histological activity at W6 through the RHI (Table S3). Subjects with histological activity (as assessed by Geboes index) at W16 had higher FC baseline levels (median, 831 *versus* 300 µg/g, $p = 0.006$) and higher CRP baseline levels (median, 4.2 *versus* 0.8 ng/ml, $p = 0.033$) (Table S3). When considering the RHI classification, FC baseline levels were higher among subjects with histological activity at W16 (median, 3.1 *versus* 0.3 ng/ml, $p = 0.003$) but no statistically significant differences were observed on CRP baseline levels (Table S3). Baseline levels of FC and CRP were not statistically significant different when comparing subjects by clinical response or endoscopic activity, at W6 (Figure 3) and W16 (Table S3).

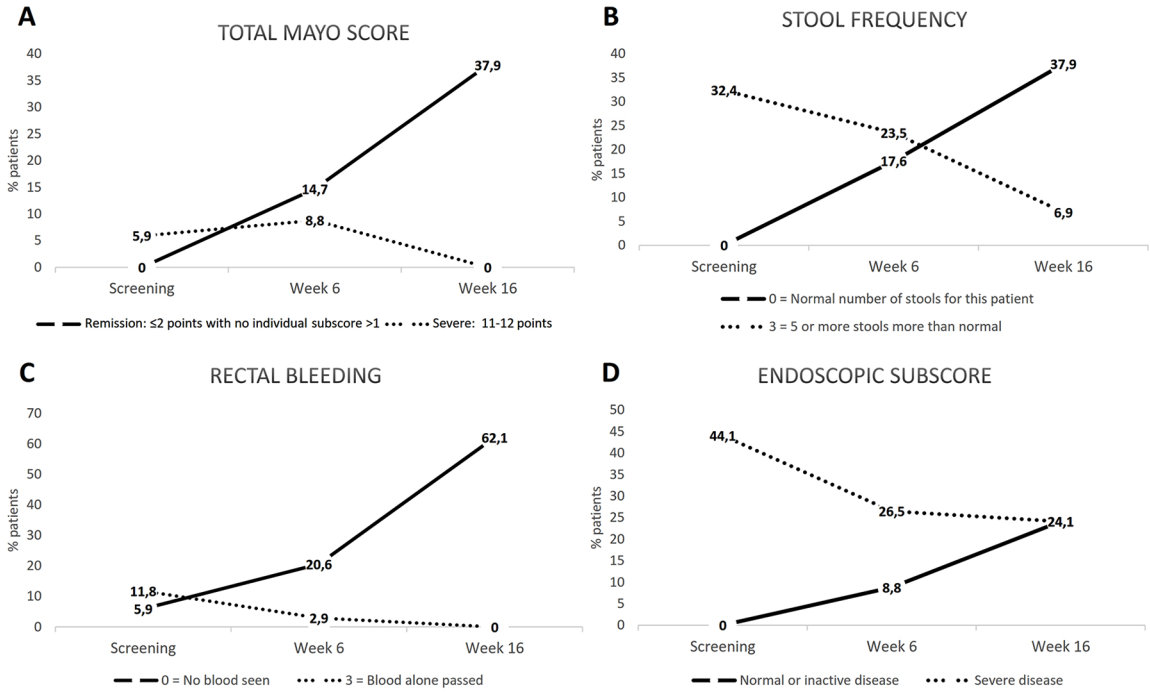

Figure 1. Proportion (%) of patients by total Mayo score (A), stool frequency (B), rectal bleeding (C) and endoscopic Mayo subscore (D) during the study.

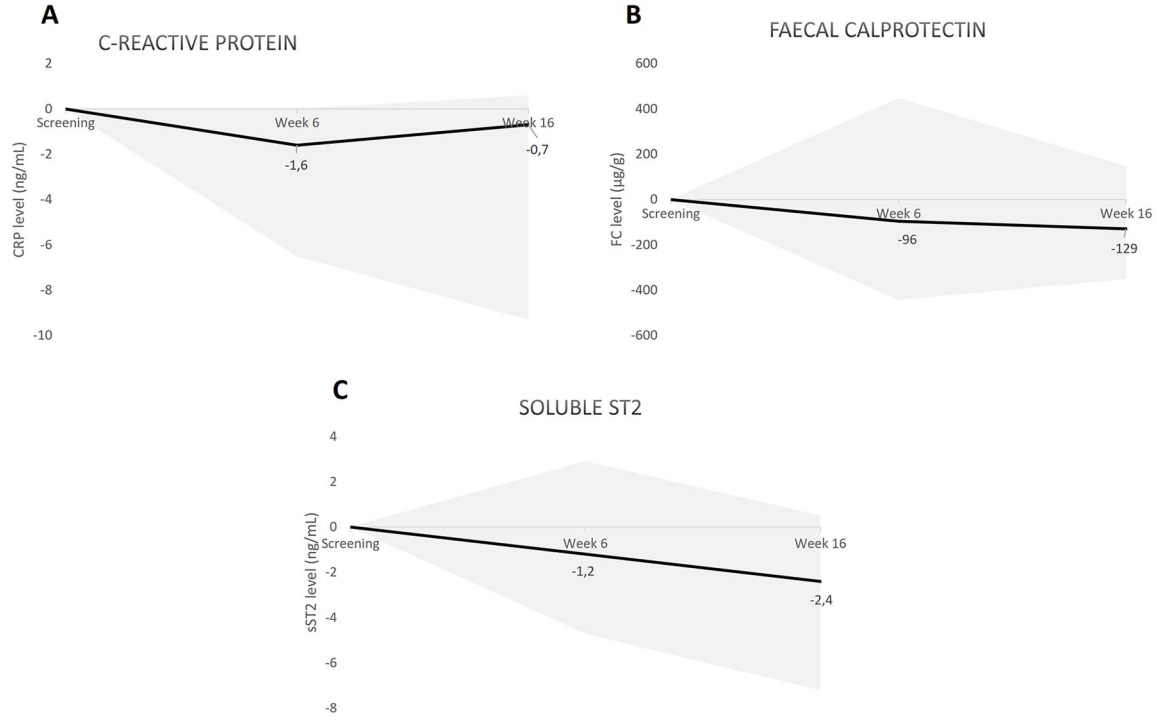

Figure 2. Evolution of CRP (A), FC (B) and sST2 (C) levels during the study: median and interquartile range (grey area).
CRP, serum C-reactive protein; FC, faecal calprotectin; sST2, serum soluble Suppression of Tumorigenicity 2.

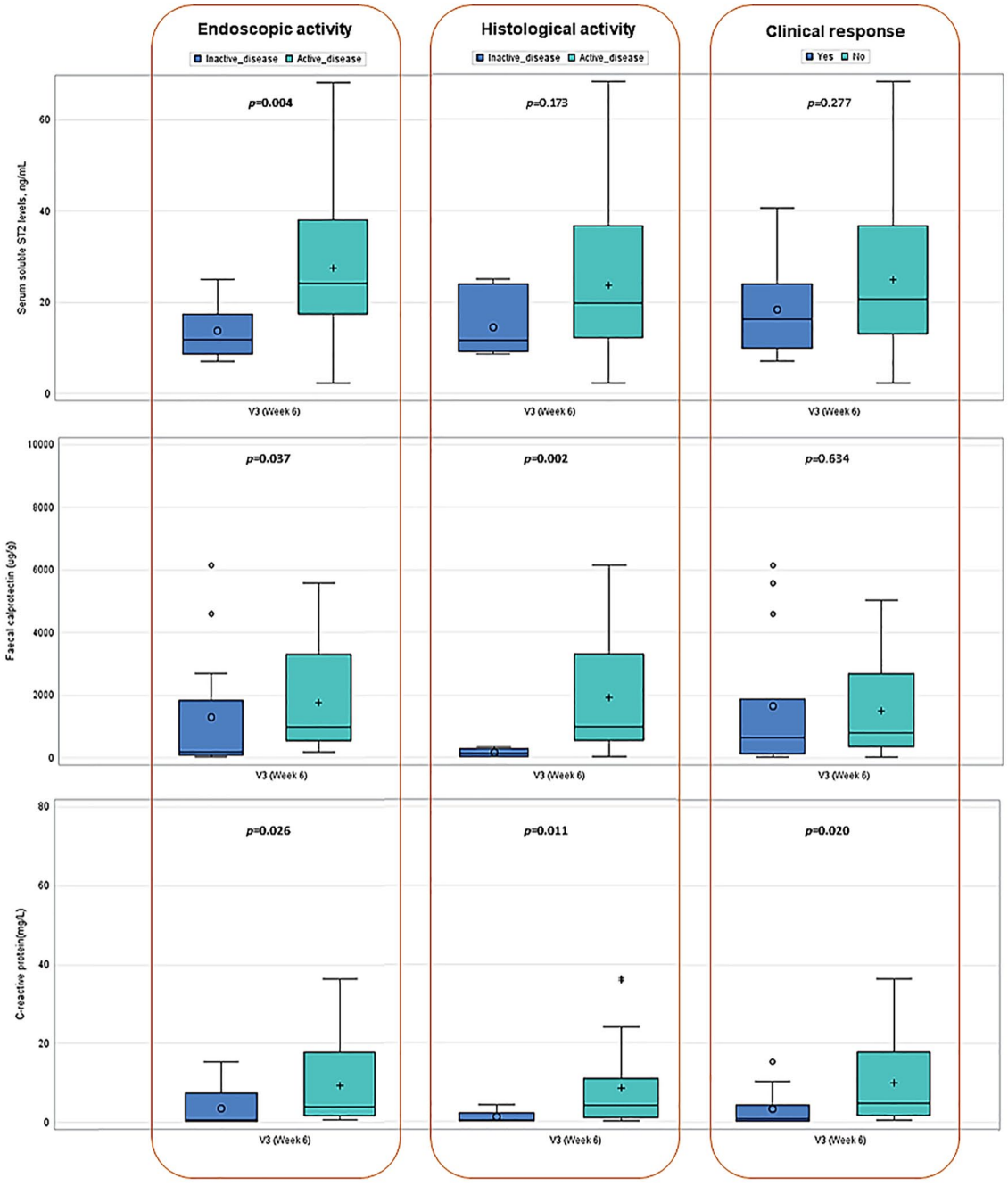

Figure 3. Levels of sST2, FC and CRP at baseline, by endoscopic and histological activity and clinical response at week 6.
CRP, serum C-reactive protein; FC, faecal calprotectin; sST2, serum soluble Suppression of Tumorigenicity 2.

Comparison of biomarkers' levels at week 6 and week 16, by endoscopic and histological activity and clinical response

At W6, subjects with endoscopic findings had significantly higher levels of sST2 than subjects without endoscopic activity (median, 24.1 *versus* 11.9 ng/ml, $p = 0.004$) (Figure 4, Table S3). The optimal sST2 cut-off to discriminate endoscopic activity at W6 was 16.9 ng/ml (AUC = 0.80, $p < 0.001$), with a sensitivity of 85%, specificity of 71% and a positive predictive value (PPV) of 81% (Figure S2 and Table 1). At W16, no statistically

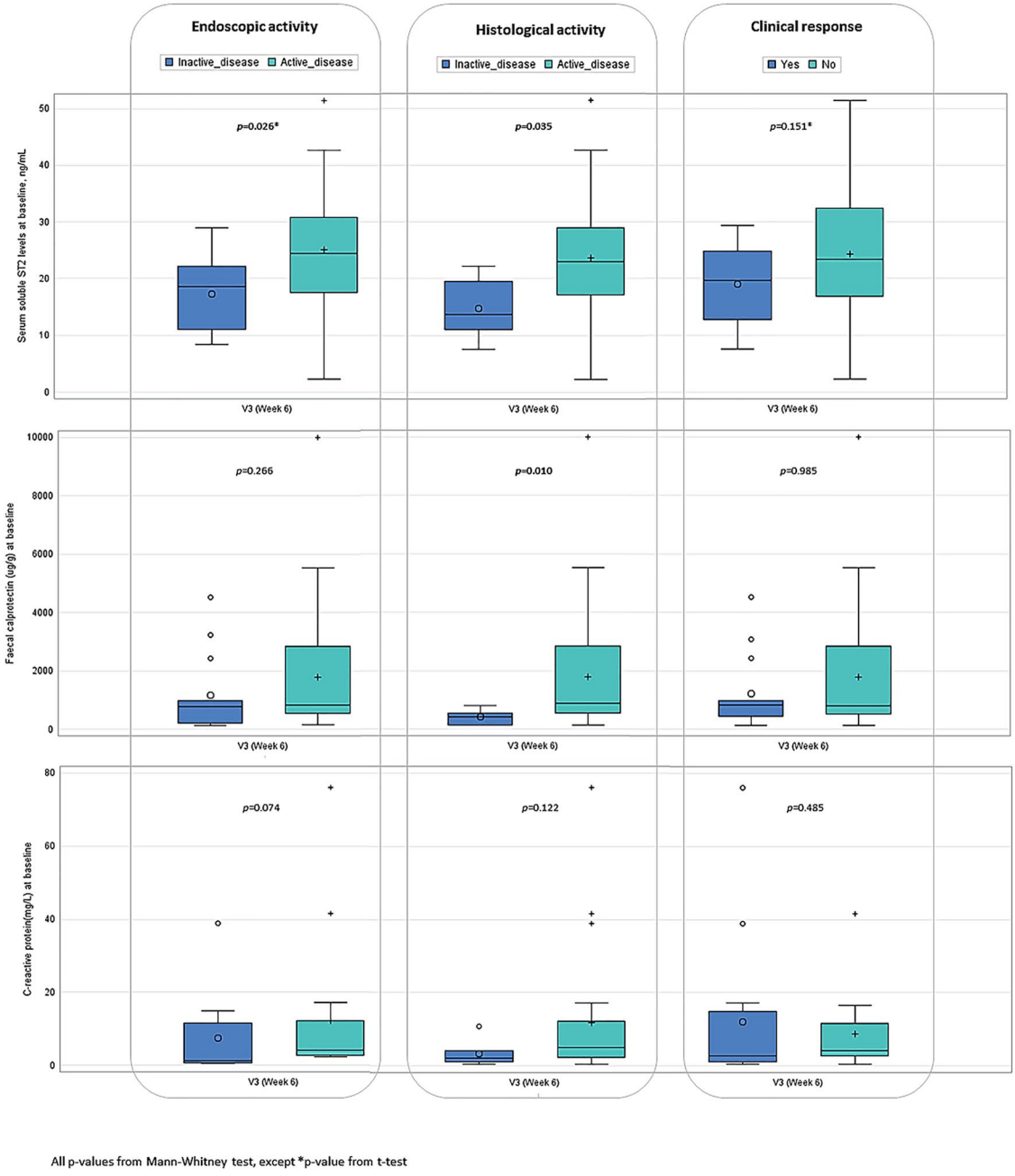

Figure 4. Levels of sST2, FC and CRP at week 6, by endoscopic and histological activity and clinical response.
CRP, serum C-reactive protein; FC, faecal calprotectin; sST2, serum soluble Suppression of Tumorigenicity 2.

significant differences were observed regarding sST2 levels by endoscopic activity and, at W6 and W16, no statistically significant differences were observed by clinical response or histological activity (Table S3).

No statistically significant differences were observed regarding FC levels at W6, for subjects with clinical response at the same moment (Figure 4, Table S3). Subjects with endoscopic activity at W6 had higher FC levels (median, 983 *versus* 180 µg/g, $p = 0.037$) (Figure 4, Table S3). The optimal cut-off for endoscopic activity was 353 µg/g (AUC = 0.73, $p = 0.049$), with a sensitivity of 90%, specificity of 67% and a PPV of 81% (Figure S2 and Table 1). Subjects with histological activity at

Table 1. Accuracy of sST2, FC and CRP measurement in predicting endoscopic and histological activity at week 6.

	AUC	95% CI	*p* value	SEN	SPE	PPV	NPV
Serum soluble ST2							
endoscopic activity (cut-off value ⩾16.9 ng/ml)	0.80	0.65–0.95	<0.001	85%	71%	81%	77%
histological activity (cut-off value ⩾15.5 ng/ml)	0.67	0.46–0.88	0.111	74%	71%	91%	42%
Faecal calprotectin							
endoscopic activity (*cut-off value ⩾353* µg/g)	0.73	0.50–0.96	0.049	90%	67%	81%	80%
histological activity (*cut-off value ⩾353* µg/g)	0.92	0.82–1.00	<0.001	84%	100%	100%	60%
C-reactive protein							
endoscopic activity (cut-off value ⩾0.7 mg/l)	0.73	0.54–0.92	0.016	95%	50%	72%	88%
histological activity (cut-off value ⩾4.4 mg/l)	0.82	0.65–0.99	<0.001	50%	100%	100%	35%

95% CI, 95% (two-sided) Confidence Interval; AUC, area under the curve; NVP, negative predictive value; PPV, positive predictive value; SEN, sensibility; SPE, specificity; ST2, Suppression of Tumorigenicity 2.

W6 had higher FC levels than subjects without active UC (median, 983 *versus* 132 µg/g, $p=0.002$) (Figure 4, Table S3). The optimal FC cut-off for histological activity was 353 µg/g (AUC = 0.92, $p<0.001$), with a sensitivity of 84%, specificity of 100% and a PPV of 72% (Figure S2 and Table 1). At W16, subjects with histological activity also had higher FC levels at the same timepoint (median, 771 *versus* 46 µg/g, $p=0.001$); no statistically significant differences were observed by clinical response or endoscopic activity (Table S3).

CRP levels at W6 were lower among subjects with clinical response (0.9 *versus* 4.8 mg/l, $p=0.020$), no endoscopic activity (0.8 *versus* 4.0 mg/l, $p=0.026$) and no histological activity (0.5 *versus* 4.2 mg/l, $p=0.011$) (Figure 4). The optimal cut-off for endoscopic activity was 0.7 mg/l (AUC = 0.73, $p=0.016$), with a sensitivity of 95%, specificity of 50% and a PPV of 72% (Figure S2 and Table 1). Regarding discrimination of histological activity, the optimal cut-off was 4.4 mg/l (AUC = 0.82, $p<0.001$), with a sensitivity of 50%, specificity of 100% and a PPV of 100% (Figure S2 and Table 1). At W16, subjects with histological activity had higher CRP levels (median, 3.5 *versus* 0.4 mg/l, $p<0.001$); no statistically significant differences were observed by clinical response or endoscopic activity at the same moment (Table S3).

Correlation of biomarkers levels and clinical, endoscopic and histological activity

At W6, the correlations between sST2, FC and CRP levels were poor and not statistically significant (Table 2). At W16, the correlation between FC and CRP levels was positive ($r_s=0.60$, $p<0.001$). No statistically significant correlations were observed between sST2 and FC or CRP levels at W16.

At W6, a positive correlation between sST2 levels and total Mayo score ($r_s=0.40$, $p=0.018$) and endoscopic activity ($r_s=0.45$, $p=0.007$) was observed (Table 2). The correlation of sST2 with histological activity (both Geboes index and RHI classifications), partial Mayo score and rectal bleeding subscore were poor and not statistically significant. FC levels at W6 correlated with statistical significance with histological activity assessed by Geboes index only ($r_s=0.47$, $p=0.008$), but not with histological activity assessed by RHI,

Table 2. Correlation between biomarker levels and clinical, endoscopic and histological activity, at week 6 and week 16.

	Week 6	*p* value	Week 16	*p* value
	r_s (95% CI)		r_s (95% CI)	
Serum soluble ST2 levels versus				
Total Mayo score	**0.40 (0.08; 0.65)**	**0.018**	0.10 (–0.28; 0.45)	0.615
Endoscopic activity	**0.45 (0.13; 0.69)**	**0.007**	0.27 (–0.11; 0.58)	0.159
Histological activity (Geboes)	0.25 (–0.09; 0.54)	0.151	0.18 (–0.20; 0.51)	0.358
Histological activity (RHI)	0.33 (–0.01; 0.60)	0.059	0.29 (–0.09; 0.59)	0.128
Partial Mayo score	0.33 (–0.01; 0.60)	0.058	–0.02 (–0.38; 0.35)	0.922
Rectal bleeding Mayo subscore	0.23 (–0.12; 0.53)	0.187	–0.17 (–0.50; 0.21)	0.392
Faecal calprotectin	–0.02 (–0.37; 0.34)	0.906	–0.14 (–0.49; 0.25)	0.478
C-reactive protein	0.20 (–0.15; 0.51)	0.264	0.02 (–0.35; 0.38)	0.936
Faecal calprotectin levels versus				
Total Mayo score	0.20 (–0.16; 0.52)	0.275	0.27 (–0.11; 0.59)	0.163
Endoscopic activity	0.32 (–0.04; 0.61)	0.077	0.31 (–0.08; 0.61)	0.112
Histological activity (Geboes)	**0.47 (0.14; 0.71)**	**0.008**	**0.65 (0.36; 0.82)**	**<0.001**
Histological activity (RHI)	0.28 (–0.08; 0.58)	0.126	**0.47 (0.11; 0.72)**	**0.012**
Partial Mayo score	0.13 (–0.24; 0.46)	0.501	0.19 (–0.20; 0.53)	0.330
Rectal bleeding Mayo subscore	0.02 (–0.34; 0.37)	0.916	0.27 (–0.12; 0.58)	0.172
C-reactive protein	0.26 (–0.12; 0.56)	0.174	**0.60 (0.29; 0.80)**	**<0.001**
C-reactive protein results versus				
Total Mayo score	**0.55 (0.25; 0.75)**	**0.001**	0.37 (0.00; 0.65)	0.052
Endoscopic activity	**0.40 (0.07; 0.65)**	**0.021**	0.34 (–0.03; 0.63)	0.070
Histological activity (Geboes)	**0.38 (0.04; 0.64)**	**0.031**	**0.64 (0.35; 0.81)**	**<0.001**
Histological activity (RHI)	**0.48 (0.17; 0.71)**	**0.005**	**0.59 (0.28; 0.78)**	**0.001**
Partial Mayo score	**0.52 (0.22; 0.73)**	**0.002**	0.31 (–0.07; 0.61)	0.103
Rectal bleeding Mayo subscore	**0.39 (0.05; 0.65)**	**0.026**	0.14 (–0.24; 0.49)	0.456

95% CI, 95% confidence interval; RHI, Robarts Histopathology Index; r_s, Spearman's correlation coefficient.
Note: correlations with statistical significance are shown in bold.

endoscopic activity, total/partial Mayo score or rectal bleeding. CRP levels correlated with total Mayo score ($r_s = 0.55$, $p = 0.001$) and with endoscopic ($r_s = 0.40$, $p = 0.021$) and histological (Geboes: $r_s = 0.38$, $p = 0.031$; RHI: $r_s = 0.48$, $p = 0.005$) activity, as well as with partial Mayo score ($r_s = 0.52$, $p = 0.002$) and rectal bleeding Mayo subscore ($r_s = 0.39$, $p = 0.026$).

At W16, FC and CRP levels were significantly correlated with histological activity assessed by Geboes index ($r_s = 0.65$ and $r_s = 0.64$, $p < 0.001$,

respectively) and by RHI ($r_s = 0.47$, $p = 0.012$ and $r_s = 0.59$, $p = 0.001$, respectively) (Table 2). At the same time point, the correlations between these biomarkers *versus* total Mayo score and endoscopic activity, as well as the correlations between sST2 and disease activity outcomes, were poor and without statistical significance.

Discussion

Previous studies have shown that sST2 levels correlate positively with the severity of colonic mucosal disease and inflammatory cytokines,[3,11,12] but few have evaluated the potential of biomarkers in predicting response to biological therapy.[25] We observed that sST2 correlates moderately, although with statistical significance, with clinical and endoscopic activity at W6 of golimumab treatment, and that higher baseline levels of sST2 were associated with endoscopic and histological activity at W6 and W16. Furthermore, subjects without endoscopic activity at W6 had a decrease in sST2 levels from baseline, while subjects who maintained endoscopic findings at W6 showed almost no change. Together, these findings suggest that sST2 levels at baseline can predict endoscopic response and histological remission after induction and at an early phase of maintenance treatment with golimumab.

After 6 weeks of treatment with golimumab, all biomarkers were significantly higher for subjects with endoscopic activity, but no statistically significant differences were observed in sST2 levels by clinical response or histological activity. In addition, FC levels were correlated only with histological activity assessed by Geboes index, in contrast with other studies.[3,5,26,27] CRP levels were significantly correlated with all UC activity outcomes at W6, as described by others.[28,29]

At W6, sST2 levels showed a good performance for discriminating endoscopic activity with a cut-off value of 16.9 ng/ml (sensitivity = 85%; specificity = 71%; PPV = 81%), with a higher discriminating capacity (AUC = 0.80) than that observed for FC (AUC = 0.73) or CRP (AUC = 0.73). Díaz-Jiménez and colleagues estimated a sST2 cut-off of 74.87 pg/ml (sensitivity = 83%, specificity = 83%) to discriminate endoscopic activity in UC patients.[3] This lower sST2 cut-off could be due to the inclusion of UC patients irrespective of disease activity or treatment.[3] The FC cut-off (353 μg/g) resulted in a PPV of 81% for endoscopic activity and, for histological activity (Geboes index), a specificity and PPV of 100%. This high specificity for histological activity was reported previously for a lower cut-off of 100 μg/g.[30] However, few studies have evaluated the correlation between FC and endoscopy at 6 weeks, that is, during induction, and the higher cut-off of 353 μg/g is probably more adequate for this treatment phase since the 100 μg/g was determined for asymptomatic patients in remission. Magro and colleagues reported median FC levels of 230 (40–425) μg/g at 8 weeks after induction with infliximab in UC patients.[30] Hence, we hypothesize that different cut-offs should be used according to induction and maintenance treatment phases.

CRP showed a high specificity and PPV for histological outcome (Geboes index), suggesting that all subjects with CRP $\geq$ 4.4 mg/l present histological activity. However, this cut-off had low sensitivity (50%) and poor NPV (35%) for histological activity and the cut-off of 0.7 mg/l had inadequate specificity (50%) for endoscopic activity. Consequently, CRP levels seem to have poor performance and utility for predicting endoscopic and histological activity at W6.

Subjects with histological activity at W6 had higher baseline levels of sST2 and FC, and those with histological activity at W16 had higher baseline levels of sST2 (when classified by both Geboes and RHI) and CRP (Geboes only). These results suggest that sST2, FC and CRP are biomarkers of different manifestations of UC inflammatory process during early treatment with golimumab. Although the mechanism is still to be clarified, sST2 expression seems to be upregulated in IBD patients and might reduce the protective effect of IL-33 when combined to it, by reducing macrophage modulation, and consequently wound healing, in UC patients.[31–33] For that reason and based on our results, we hypothesize that sST2 may be useful as a surrogate biomarker, both in terms of assessing endoscopic activity and when predicting early treatment response to golimumab treatment.

Of note, we did not observe any statistical correlation between FC and sST2 levels. FC is recognized as a useful marker of mucosal damage as its levels seem to be increased when neutrophils are present in the epithelium, the main marker of histological activity.[6] Infiltration of neutrophils

through the inflamed mucosa occurs after local release of cytokines and compromises mucosal architecture, epithelial barrier and production of inflammatory mediators.[34] Histological healing is frequently incomplete and, compared with clinical and endoscopic response, takes a longer time to be observed after treatment initiation.[35–37] In our study, FC levels showed a stronger correlation with histological activity, which some consider the ultimate goal of UC treatment, as subclinical inflammation is predictor of UC relapses.[38,39]

With regards to CRP, its short half-life ensures that serum concentrations quickly decrease once the acute-phase stimulus is removed.[40] CRP levels correlated with all disease activity markers at W6 and were statistically higher among subjects with histological activity at W16. Therefore, CRP can provide additional support when investigating subclinical inflammation, namely during maintenance treatment with golimumab, even though it is less specific to the intestinal inflammatory process and inadequate for predicting endoscopic and histological findings.[8,29]

Even though clinical improvement (based on total Mayo score and its dimensions) was noticeable at W6, the follow-up period was probably insufficient to clearly observe the cellular inflammatory response.[6] In fact, more than two thirds of the patients had histological active UC (Geboes >3.0, i.e. with presence of neutrophils) during the study period.

The study presents other limitations. Due to the exploratory nature of the study, no formal sample size was determined. Hence, the small sample size may have also limited the comparison between subgroups of disease activity. We cannot exclude that, due to the number of comparisons made, some results could have resulted from statistical chance. Larger studies should include sST2 assessment to clarify its role as a predictor of endoscopic activity. Finally, we should be cautious with generalisation of results as the eligibility criteria, aiming at a more homogeneous sample and the safety of the participants, may have compromised the external validity of the study.

The use of well-defined indexes of endoscopic, clinical and histological activity, which are commonly used in the clinical practice, and the correlation between several biomarkers, are major strengths of this study. This is particularly relevant with regards to the use of the Geboes index and RHI to assess histological activity, both of which have shown good reproducibility and are the most commonly used indices in UC.[6,15,16]

Recent research has identified histological healing as an important predictor of long-term benefit.[36–38,41] Still, the STRIDE consensus does not consider histological healing as a treatment target; probably due to the cost and workload of adding pathological assessment.[42] Therefore, a biomarker that would be correlated with histological activity would be useful in clinical practice. Further research should be conducted with a longer follow-up period, control groups and larger samples to confirm the role of sST2 levels among other biomarkers, when treating moderately-to-severely active UC with golimumab.

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Author contributions

Fernando Magro was involved in the conception and design of the study, interpretation of data, and drafting and revising the manuscript. All other authors were responsible for data acquisition. All authors read and approved the final manuscript.

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Conflict of interest statement

Fernando Magro received a fee for presenting from: AbbVie, Ferring, Falk, Hospira, PharmaKern, MSD, Schering, Lab. Vitoria, Vifor, OmPharma. Helena Tavares de Sousa reports expert fees and nonfinancial support from MSD, Abbvie, Ferring, Dr Falk Pharma, PharmaKern, Janssen and Takeda, outside the

submitted work. Patrícia Machado is employee of MSD Portugal; Philip G is employee of Merck and Co., USA; and Isabel Redondo was an employee of MSD Portugal at the time the study was conducted. All other authors: none to declare.

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Supplemental material

Supplemental material for this article is available online.

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