Title page

Title: Serum oncostatin M at baseline predicts mucosal healing in Crohn's disease patients treated with infliximab

Running title: Oncostatin M: a new biomarker of mucosal healing

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Abbreviations: Crohn's Disease (CD), Tumor Necrosis Factor (TNF), Infliximab (IFX), Faecal Calprotectin (FC), Harvey Bradshaw Index (HBI), Simple Endoscopic Index for CD (SES-CD), Area Under the Curve (AUC), confidence intervals (CI)

Summary

Background

Oncostatin M is upregulated in Crohn's disease inflamed intestinal mucosa, and has been suggested as a promising biomarker to predict responsiveness to anti-TNF therapy in patients with inflammatory bowel diseases.

Aim

The primary aim was to evaluate the suitability of serum oncostatin M as a predictive marker of response to infliximab in Crohn's disease.

Methods

We included patients treated with infliximab in monotherapy. All patients underwent colonoscopy at week 54 to evaluate mucosal healing. Serum oncostatin M and faecal calprotectin were measured at baseline and after 14 weeks of treatment. Mann-Whitney test was used to evaluate correlation of oncostatin M and faecal calprotectin at baseline and week 14 with mucosal healing at week 54. Their accuracy in predicting mucosal healing was assessed by area under the curve (AUC).

Results

In a cohort of 45 included patients, 27 displayed mucosal healing. At both baseline and week 14, oncostatin M levels were significantly lower in patients with mucosal healing than in patients not achieving this endpoint ($p<0.001$). Faecal calprotectin levels at week 14 were lower also in responders than non-responders ($p<0.001$). Oncostatin M values at baseline and week 14 were significantly associated (Spearman correlation=0.92, $p<0.001$). The diagnostic accuracy of oncostatin M at baseline in predicting mucosal healing (AUC=0.91) was greater than faecal calprotectin (AUC=0.51, $p<0.001$).

Conclusion

The present results suggest that oncostatin M can predict the outcome of infliximab treatment. At variance with faecal calprotectin, the predictive capability of oncostatin M was appreciable at baseline, thus indicating oncostatin M as a promising biomarker for driving therapeutic choices in Crohn's disease.

Keywords: Crohn's Disease; Inflammation; Inflammatory Bowel Disease; Biologics (IBD)

Introduction

Crohn's disease (CD) is a chronic relapsing disease, caused mainly by an imbalance between pro- and anti-inflammatory cytokines produced by effector T cells versus naturally regulatory T cells, respectively[1]. Tumor necrosis factor (TNF) plays a pivotal role in CD pathogenesis. Indeed, different cell populations produce high levels of TNF, and this cytokine is known to mediate several pro-inflammatory functions in the inflamed mucosa, where it promotes also tissue injury[2]. Based on this knowledge, the monoclonal antibody anti-TNF, infliximab (IFX), was the first developed biological drug for treatment of CD.

CD course and response to treatment are characterized by high variability among patiens[3]. In this context, IFX is able to induce and maintain clinical remission and promote mucosal healing[4], even though a small proportion of patients achieves a complete response[5]. Similar situations are being experienced with other biologics. Owing to such a variability, the possibility of predicting therapeutic outcomes is one of the most important challenge for clinicians. Indeed, a personalized approach would be expected to improve the rates of therapeutic response. In particular, the identification and clinical validation of biomarkers would allow an optimization of the therapeutic management of patients with CD, with a favorable impact also on public health expenditure[6].

The evaluation of serum cytokine profiles may represent a reliable and non-invasive tool to predict therapeutic efficacy of biological drugs[7]. West et al.[8] showed that oncostatin M, a cytokine of the IL-6 family, is emerging as one of the most highly and consistently expressed cytokine in the inflamed tissues of patients with inflammatory bowel diseases. Interestingly, high tissue levels of oncostatin M have been associated with

refractoriness to anti-TNF therapy[8]. Along the same line, a recent study by Minar et al.[9] showed that baseline plasma oncostatin M was higher in children with CD defined as non-responders to IFX treatment in terms of decrease in faecal calprotectin (FC) concentrations. However, to the best of our knowledge, oncostatin M has been never evaluated as a possible early biomarker for the prediction of endoscopic response to biological therapy. In this regard, the high relevance of mucosal healing in inflammatory bowel diseases is currently well recognized, since it is expected to have a more solid impact on the clinical course than therapeutic strategies pursuing merely clinical remission or clinical response[10].

Based on the above background, the primary aim of the present study was to evaluate the putative role of serum oncostatin M in the prediction of clinical and endoscopic response to IFX treatment in adult patients with CD.

Methods

Patients and study protocol

In the present study, we included all consecutive biological-naïve patients with moderate to severe CD, who started IFX therapy at our Unit from January 2017 to June 2018. The diagnosis of CD had been confirmed previously by clinical, radiological, endoscopic and histological evaluations. Patients treated concomitantly with azathioprine and patients with other immune-mediated diseases (such as psoriasis, arthritis, uveitis…) were excluded from the study. Primary non-responders [defined as a decrease in Harvey Bradshaw Index (HBI) <3 at week 10] were excluded from the analysis.

The following data were collected at baseline: age, sex, year of diagnosis, disease severity according to HBI and to Simple Endoscopic Index for CD (SES-CD), concomitant corticosteroid therapy, value of CRP and FC levels.

This study protocol was conducted in full compliance with the Declaration of Helsinki, and was approved by the Ethical Committee of Pisa University Hospital (CEAVNO). All patients gave their informed consent to the collection and publication of the data.

Study design

Patients were treated with IFX according to current guidelines, with an intravenous drug infusion of 5 mg/Kg at weeks 0, 2, 6, and then every 8 weeks. At each IFX infusion, patients underwent a clinical examination, to estimate HBI, in accordance with guidelines. Clinicians could escalate therapy to a 4-week regimen in case of loss-of-response, defined as an increase of 3 points in HBI.

All the enrolled patients underwent a colonoscopy both at baseline and at week 54, when mucosal healing (defined as the disappearance of ulcers) was assessed. All colonoscopies were performed prospectively by three operators, who are expert in the evaluation of SES-CD and had attended "IG-IBDEndo" courses[11]. Clinical remission was defined as a HBI <5 without concomitant corticosteroid therapy. Clinical response was defined as a decrease in HBI ≥3.

Before starting drug infusions at baseline and week 14, 9 ml of peripheral venous blood were collected in EDTA, and centrifuged at 3000 rpm for 10 minutes. Serum was aliquoted, frozen and stored at -20 °C. Subsequently, serum samples were used to assess the levels of oncostatin M by an ELISA assay (Life Technologies, Monza, Italy). Likewise, at the same time-points, FC levels were evaluated by an ELISA Kit (Calprest®, Eurospital, Italy) in the week preceding IFX infusion.

Statistical analysis

The primary endpoint of the present study was mucosal healing at week 54, whereas clinical response at week 14 and clinical remission at week 54 were assessed as secondary endpoints.

The associations of age, sex, baseline HBI and SES-CD, FC and oncostatin M, as determined at baseline and week 14, with the primary endpoint and the secondary outcomes were assessed by the Mann-Whitney test for the continuous variables and the Fisher's exact test for the categorical variables. Spearman correlations between the values at baseline and week 14 were computed for both FC and oncostatin M, as well as between oncostatin M and FC values at these time-points. The accuracy of oncostatin M and FC in predicting mucosal healing at week 54 was

estimated by the area under the receiver operating characteristic (ROC) curve (AUC). The AUCs for oncostatin M and FC were compared by the DeLong test both at baseline and week 14. FC and oncostatin M were dichotomized with the thresholds corresponding to the minimum distance between the ROC curve and the point with both specificity and sensitivity 1. The 95% confidence intervals (CI) for AUC were computed with the DeLong method, and those for sensitivity and specificity by the Wilson method.

All statistical analyses were performed with R version 3.6.2 (R Core Team (2019). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (URL: https://www.R-project.org/.)*

Results

Fifty-two patients met the inclusion and exclusion criteria. Seven patients were identified as primary non-responders and were excluded from the analysis. The characteristics of the 45 included patients are reported in Table 1. The study population included 21 females and 24 males; the mean age was 34 years, the youngest patient was 18 and the eldest 68 years old. All patients displayed ulcers at the baseline colonoscopy.

Thirty-two patients displayed clinical remission at week 54, and 27 of them achieved concomitant mucosal healing. Out of 34 patients with clinical response at week 14, 30 achieved clinical remission at week 54; conversely, two patients without clinical response at week 14 reached clinical remission at week 54.

With regard for CRP at baseline, normal levels were observed in 22% of the patients with mucosal healing at week 54 and 6% of the patients without mucosal healing ($p = 0.22$). Likewise, when evaluating CRP levels at week 14, normal levels were observed in 56% of the patients with mucosal healing and 50% of the patients without mucosal healing ($p = 0.77$).

Association of oncostatin M and FC with clinical and demographic characteristics

Oncostatin M values at baseline and week 14 were significantly associated (Spearman correlation coefficient = 0.92, $p < 0.001$), as were those for FC at baseline and week 14 (Spearman correlation coefficient = 0.34, $p = 0.02$).

At baseline, none of the demographic or clinical variables were associated with oncostatin M or FC levels (not shown), with exception for an association between oncostatin M and SES-CD (Spearman correlation coefficient = 0.36, $p = 0.02$) and an association between FC and HBI (Spearman

correlation coefficient = 0.41, p = 0.004). There was no evidence of association between baseline oncostatin M and FC levels (Spearman correlation coefficient = 0.09, p = 0.55).

Prediction of therapeutic response by oncostatin M and FC

With regard for oncostatin M, responders, according to all the three outcomes, displayed significantly lower levels than non-responders at both baseline and week 14. When considering FC, responders had significantly lower levels than non-responders only at week 14 (Table 1 and Figure 1).

Oncostatin M levels at both baseline and week 14 predicted mucosal healing at week 54 (AUC = 0.91, 95% CI 0.81 to 1.00, and 0.83, 95% CI 0.70 to 0.95, respectively). On the other hand, FC levels at baseline did not discriminate between responders and non-responders (AUC = 0.51, 95% CI 0.32 to 0.69), while at week 14 a significant difference was observed (AUC = 0.91, 95% CI 0.81 to 1.00) (Figure 2). The difference in AUC between oncostatin M and FC was significant at baseline, but not at week 14 (DeLong test $p < 0.001$ and $p = 0.39$ respectively). The best cut-off for baseline oncostatin M was 14 pg/ml, with a sensitivity of 0.96 (95% CI 0.82 to 1.00) and a specificity of 0.89 (95% CI 0.67 to 0.97). For FC at week 14 the threshold was 147 mg/Kg, with a sensitivity of 0.89 (95% CI 0.82 to 1.00) and a specificity of 0.89 (95% CI 0.82 to 1.00).

Discussion

The present study was designed primarily to evaluate the putative role of oncostatin M as an early predictor of clinical and endoscopic response in patients with CD treated with IFX. As a secondary purpose, the predictive capability of FC was evaluated as well. Our results showed that both biomarkers are reliable when the evaluation was performed after 14 weeks of treatment, while oncostatin M was able to predict the therapeutic outcome even at baseline.

In recent years, the therapeutic target in CD has been raised up to mucosal healing, defined as the disappearance of ulcers[12]. This choice is supported by a better expectancy of outcome in terms of hospitalizations and surgery rates[12]. Moreover, a substantial proportion of patients achieving clinical remission have been found to retain persistent endoscopic inflammation[13]. In the majority of studies on CD, mucosal healing rate with IFX ranged from 30 to 40%[14-16]. Our cohort displayed a higher rate (60%), which depends likely on the inclusion of only naïve patients, who are known to present frequently a more favourable response to all biological therapies[17,18]. Moreover, in some studies mucosal healing was defined as a CD Endoscopic Index of Severity <3 or as a Simple Endoscopic Index Score for CD <2[19], which are very difficult goals to achieve with anti-TNF mono-therapy. Last, but not the least, we performed the endoscopy to assess mucosal healing after 54 weeks of treatment, while several studies had evaluated the endoscopic response at week 26 or even earlier[14]; these time-points might not be appropriate since, in the perspective of a treat-to-target strategy, we should allow enough time to a drug therapy of expressing its full potential. Indeed, consistently with our results, a mucosal healing rate of 60% was obtained even in a prospective study evaluating a cohort of biologic-naïve patients with CD treated with IFX in monotherapy[20].

In the era of a rapidly expanding therapeutic armamentarium, the possibility of predicting response to IFX before treatment initiation would be a major clinical advance. In this context, few studies in CD have evaluated mucosal healing as an outcome and limited data are presently available to drive therapeutic choices based on the achievement of this endpoint[17]. Currently, CRP is the most widely used biomarker in clinical practice for CD. High baseline CRP is associated with an increased likelihood of response to IFX therapy both in clinical trials[21] and in real-life studies[22]. This is acknowledged as a well established evidence, even though CRP cannot certainly be regarded as a specific biomarker.

Faecal biomarkers are more specific than CRP, and FC is the most used one in clinical practice[23]. In previous studies, early anti-TNF response was associated with post-induction reductions of FC in the pediatric setting[24], while less evidence is currently available for adults. For instance, Roblin et al.[25] proposed a model, which includes FC, for the prediction of loss of response in patients with CD in remission during IFX maintenance therapy. Moreover, a recent study by Beltran et al.[26] showed that baseline levels of FC could predict primary non-response to IFX therapy. In this context, our study highlights a significant correlation between FC levels after the induction of IFX treatment and mucosal healing in patients with CD. To the best of our knowledge, these data are lacking in the current literature[27], and should encourage the use of this biomarker in the clinical practice even in this perspective.

High serum drug levels have been associated also with clinical and endoscopic remission[28,29]. In particular, Dreesen and colleagues[30] proposed IFX trough levels as a predictive biomarker, showing that a threshold of 23.1 mg/L at week 2 and 10.0 mg/L at week 6 was associated with mucosal healing at week 12. However, the overall data on therapeutic drug monitoring are controversial and the routine use of therapeutic drug monitoring for this purpose is not being encouraged[31].

With regard for serum cytokines, some studies suggested a role of IL-6 in predicting the therapeutic response to biological drugs in ulcerative colitis[32,33]. Moreover, a study by Billiet et al.[34] on patients with CD showed that IL-6 concentrations decreased significantly at week 2 and week 6 in responders, as compared to primary non-responders to IFX therapy.

Recently, an interesting study by West et al.[8] showed a strong correlation between high oncostatin M expression in inflamed bowel tissues and anti-TNF refractoriness. oncostatin M is a cytokine belonging to the IL-6 family, which shares the gp130 as receptor subunit. This cytokine is likely to play a significant role in inflammatory bowel diseases, since high levels of oncostatin M and its receptors have been found to be expressed in intestinal biopsies from patients with ulcerative colitis and CD[8]. Interestingly, oncostatin M appears to promote intestinal inflammation by stimulating the expression of chemokine, cytokines and adhesion factors in gut resident stromal cells. Of note, the expression levels of both oncostatin M and its receptors correlate closely with histopathological disease severity[8]. However, oncostatin M expression did not correlate with traditional clinical or laboratory markers of disease severity[8]. Minar et al.[9] evaluated plasma oncostatin M in a cohort of CD children treated with IFX in monotherapy, showing that its levels were significantly higher in non-responders as compared to responders. An important limitation of that study was represented by the selected primary endpoint, since the authors defined as responders patients with >50% reduction of FC from baseline at week 12. Nevertheless, their results suggested a possible role of oncostatin M in predicting the therapeutic response to IFX in CD. Conversely, the only available study in the setting of ulcerative colitis, presented as a congress abstract, reported that mucosal oncostatin M was not associated with IFX response in a small cohort of patients with acute severe disease[35]. Our study evaluated the therapeutic outcome in terms of clinical response to the induction of IFX therapy (week 14), as well as clinical remission and, above all, mucosal healing after 54 weeks of treatment. We found that

oncostatin M is reliable, both at baseline and week 14 in predicting all of the three therapeutic outcomes, and the ROC curve analysis showed how the correlation with the primary endpoint (mucosal healing) was quite strong. Of note, these findings are original and pave the way for a possible use of oncostatin M as a biomarker in the clinical practice.

This study has some limitations. 1) A combined evaluation of oncostatin M levels both in serum and in bowel biopsies could improve the significance of the results, even though the study by West et al.[8] had already suggested the possible relevance of evaluating tissue oncostatin M. 2) The analysis of the putative role of serum oncostatin M in primary non-responders could be also of interest, but we decided of excluding these patients owing to their low number; clearly, future studies, aimed at investigating the predictive value of serum oncostatin M in primary non-responders to IFX therapy, should be implemented. 3) A control group of patients with CD treated with other biological therapies should be considered for future studies as well, in order to clarify whether oncostatin M is suitable for predicting the predictive value of oncostatin M in a disease-specific or a drug-specific manner. In our opinion, our inclusion and exclusion criteria allowed a reliable characterization of the predictive value of oncostatin M in a specific cohort of patients, thus avoiding possible selection bias, and this has to be regarded more as an added value than as a limitation of the study. 4) The number of patients included in the present analysis is relatively small, and therefore our results require further validation in future studies on larger cohorts of patients with CD. It is worthy to mention also that recent studies have been conducted to investigate the role of transmural healing, evaluated as cross-sectional imaging, in comparison with mucosal healing. In particular, the study by Castiglione et al.[36] showed that transmural healing is associated with longer intervals until clinical relapse, hospitalization and surgery than mucosal healing. However, current guidelines suggest that the resolution of the signs of inflammation on cross-sectional imaging should be defined as a therapeutic

goal only in patients who cannot be assessed adequately with ileocolonoscopy[12]. Probably, at present, we can consider mucosal healing an indispensable outcome, while transmural healing should be intended as an adjunctive goal. Based on this reasoning, we decided to evaluate therapeutic effectiveness in terms of mucosal healing only.

The major point of strength of the present study is the strong correlation between oncostatin M levels at baseline and mucosal healing after 54 weeks of treatment, which could suggest the use of this biomarker before starting IFX therapy. Moreover, it is noteworthy that our study showed also a possible role of FC assessment after the induction of IFX therapy in the perspective of an early prediction of the therapeutic outcome in terms of mucosal healing. If confirmed in larger studies, both biomarkers could be used to drive treatment decisions, thus supporting the clinicians in their efforts for a personalization of biological therapy, which could improve the therapeutic outcomes. Furthermore, the assessment of oncostatin M could be helpful even before starting the administration of IFX therapy, thus avoiding possible adverse effects and allowing significant savings for healthcare.

In conclusion, the present study encourages the use of serum oncostatin M at baseline and FC levels after the induction of IFX therapy, based on their strong correlation with mucosal healing after 54 weeks of treatment. Of note, both biomarkers are cheap, easy to perform and reliable in predicting therapeutic outcomes to IFX in patients with CD.

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References

1. Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet.* 2012;380(9853):1590-1605.
2. Neurath MF. Cytokines in inflammatory bowel disease. *Nature reviews Immunology.* 2014;14(5):329-342.
3. Burisch J, Jess T, Martinato M, Lakatos PL, EpiCom E. The burden of inflammatory bowel disease in Europe. *Journal of Crohn's & colitis.* 2013;7(4):322-337.
4. Rutgeerts P, Diamond RH, Bala M, et al. Scheduled maintenance treatment with infliximab is superior to episodic treatment for the healing of mucosal ulceration associated with Crohn's disease. *Gastrointestinal endoscopy.* 2006;63(3):433-442; quiz 464.
5. Vermeire S, Louis E, Carbonez A, et al. Demographic and clinical parameters influencing the short-term outcome of anti-tumor necrosis factor (infliximab) treatment in Crohn's disease. *The American journal of gastroenterology.* 2002;97(9):2357-2363.
6. Guidi L, Pugliese D, Tonucci TP, et al. Therapeutic Drug Monitoring is More Cost-Effective than a Clinically Based Approach in the Management of Loss of Response to Infliximab in Inflammatory Bowel Disease: An Observational Multicentre Study. *Journal of Crohn's & colitis.* 2018;12(9):1079-1088.
7. Bertani L, Antonioli L, Fornai M, et al. Evaluation of cytokine levels as putative biomarkers to predict the pharmacological response to biologic therapy in inflammatory bowel diseases. *Minerva gastroenterologica e dietologica.* 2019;65(4):298-308.
8. West NR, Hegazy AN, Owens BMJ, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. *Nature medicine.* 2017;23(5):579-589.
9. Minar P, Lehn C, Tsai YT, Jackson K, Rosen MJ, Denson LA. Elevated Pretreatment Plasma Oncostatin M Is Associated With Poor Biochemical Response to Infliximab. *Crohn's & colitis 360.* 2019;1(3):otz026.

10. Zallot C, Peyrin-Biroulet L. Deep remission in inflammatory bowel disease: looking beyond symptoms. *Current gastroenterology reports.* 2013;15(3):315.

11. Daperno M, Comberlato M, Bossa F, et al. Training Programs on Endoscopic Scoring Systems for Inflammatory Bowel Disease Lead to a Significant Increase in Interobserver Agreement Among Community Gastroenterologists. *Journal of Crohn's & colitis.* 2017;11(5):556-561.

12. Peyrin-Biroulet L, Sandborn W, Sands BE, et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. *The American journal of gastroenterology.* 2015;110(9):1324-1338.

13. Peyrin-Biroulet L, Reinisch W, Colombel JF, et al. Clinical disease activity, C-reactive protein normalisation and mucosal healing in Crohn's disease in the SONIC trial. *Gut.* 2014;63(1):88-95.

14. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *The New England journal of medicine.* 2010;362(15):1383-1395.

15. Colombel JF, Panaccione R, Bossuyt P, et al. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. *Lancet.* 2018;390(10114):2779-2789.

16. D'Haens G, Vermeire S, Lambrecht G, et al. Increasing Infliximab Dose Based on Symptoms, Biomarkers, and Serum Drug Concentrations Does Not Increase Clinical, Endoscopic, and Corticosteroid-Free Remission in Patients With Active Luminal Crohn's Disease. *Gastroenterology.* 2018;154(5):1343-1351 e1341.

17. Cholapranee A, Hazlewood GS, Kaplan GG, Peyrin-Biroulet L, Ananthakrishnan AN. Systematic review with meta-analysis: comparative efficacy of biologics for induction and maintenance of mucosal healing in Crohn's disease and ulcerative colitis controlled trials. *Alimentary pharmacology & therapeutics.* 2017;45(10):1291-1302.

18. Singh S, Garg SK, Pardi DS, Wang Z, Murad MH, Loftus EV, Jr. Comparative efficacy of biologic therapy in biologic-naive patients with Crohn disease: a systematic review and network meta-analysis. *Mayo Clinic proceedings.* 2014;89(12):1621-1635.

19. Khanna R, Nelson SA, Feagan BG, et al. Endoscopic scoring indices for evaluation of disease activity in Crohn's disease. *The Cochrane database of systematic reviews.* 2016(8):CD010642.

20. Laharie D, Reffet A, Belleannee G, et al. Mucosal healing with methotrexate in Crohn's disease: a prospective comparative study with azathioprine and infliximab. *Alimentary pharmacology & therapeutics.* 2011;33(6):714-721.

21. Reinisch W, Wang Y, Oddens BJ, Link R. C-reactive protein, an indicator for maintained response or remission to infliximab in patients with Crohn's disease: a post-hoc analysis from ACCENT I. *Alimentary pharmacology & therapeutics.* 2012;35(5):568-576.

22. Louis E, Vermeire S, Rutgeerts P, et al. A positive response to infliximab in Crohn disease: association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. *Scandinavian journal of gastroenterology.* 2002;37(7):818-824.

23. Mumolo MG, Bertani L, Ceccarelli L, et al. From bench to bedside: Fecal calprotectin in inflammatory bowel diseases clinical setting. *World journal of gastroenterology.* 2018;24(33):3681-3694.

24. Zubin G, Peter L. Predicting Endoscopic Crohn's Disease Activity Before and After Induction Therapy in Children: A Comprehensive Assessment of PCDAI, CRP, and Fecal Calprotectin. *Inflammatory bowel diseases.* 2015;21(6):1386-1391.

25. Roblin X, Duru G, Williet N, et al. Development and Internal Validation of a Model Using Fecal Calprotectin in Combination with Infliximab Trough Levels to Predict Clinical Relapse in Crohn's Disease. *Inflammatory bowel diseases.* 2017;23(1):126-132.

26. Beltran B, Iborra M, Saez-Gonzalez E, et al. Fecal Calprotectin Pretreatment and Induction Infliximab Levels for Prediction of Primary Nonresponse to Infliximab Therapy in Crohn's Disease. *Digestive diseases (Basel, Switzerland).* 2019;37(2):108-115.

27. Bertani L, Mumolo MG, Tapete G, et al. Fecal calprotectin: current and future perspectives for inflammatory bowel disease treatment. *European journal of gastroenterology & hepatology.* 2020.

28. Maser EA, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2006;4(10):1248-1254.

29. Paul S, Del Tedesco E, Marotte H, et al. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. *Inflammatory bowel diseases.* 2013;19(12):2568-2576.

30. Dreesen E, Baert F, Laharie D, et al. Monitoring a Combination of Calprotectin and Infliximab Identifies Patients With Mucosal Healing of Crohn's Disease. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2019; 18(3):637-646.

31. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology.* 2015;148(7):1320-1329 e1323.

32. Nishida Y, Hosomi S, Watanabe K, et al. Serum interleukin-6 level is associated with response to infliximab in ulcerative colitis. *Scandinavian journal of gastroenterology.* 2018;53(5):579-585.

33. Bertani L, Baglietto L, Antonioli L, et al. Assessment of serum cytokines predicts clinical and endoscopic outcomes to vedolizumab in ulcerative colitis patients. *British journal of clinical pharmacology.* 2020.

34. Billiet T, Cleynen I, Ballet V, et al. Evolution of cytokines and inflammatory biomarkers during infliximab induction therapy and the impact of inflammatory burden on primary response in patients with Crohn's disease. *Scandinavian journal of gastroenterology.* 2017;52(10):1086-1092.

35. O'Connell J, Mc Donagh P, Clarke N, et al. Association between tissue oncostatin M expression and infliximab response in corticosteroid refractory acute severe ulcerative colitis. *Journal of Crohn's & colitis.* 2019;13(Supplement 1):S133-S134.

36. Castiglione F, Imperatore N, Testa A, et al. One-year clinical outcomes with biologics in Crohn's disease: transmural healing compared with mucosal or no healing. *Alimentary pharmacology & therapeutics.* 2019;49(8):1026-1039.

Table 1. Demographic and clinical characteristics of the patients, overall and by the three outcomes: clinical response at week 14, clinical remission at week 54, and mucosal healing at week 54. IQR: interquartile range; HBI: Harvey-Bradshaw Index; SES-CD: Simple endoscopic score for Crohn's Disease; FC: faecal calprotectin

	Clinical response (week 14)			**Clinical remission (week 54)**			**Mucosal healing (week 54)**		
	No	Yes	*P-value**	No	Yes	*P-value**	No	Yes	*P-value**
	n=11 (24%)	n=34 (76%)		n=13 (29%)	n=32 (71%)		n=18 (40%)	n=27 (60%)	
	Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)		Median (IQR)	Median (IQR)	
Age, years	28 (22, 44)	31 (24, 45)	*0.87*	36 (19, 48)	29 (25, 43)	*0.98*	28 (23, 45)	32 (24, 44)	*0.98*
Disease duration, years	2 (1, 6)	4 (2, 10)	*0.33*	3 (1, 11)	3 (2, 8)	*0.79*	3 (1, 10)	3 (2, 8)	*0.61*
Sex†			*0.01*			*0.10*			*0.04*
Female	9 (82)	12 (35)		9 (69)	12 (38)		12 (67)	9 (33)	
Male	2 (18)	22 (65)		4 (31)	20 (62)		6 (33)	18 (67)	
Smoking†			*0.85*			*0.73*			*1.00*
Never smoker	9(82)	24(71)		10(77)	23(72)		13(72)	20(74)	

Ex-smoker	2(18)	7(21)		3(23)	6(19)	4(22)	5(19)	
Current smoker	0(0)	3(9)		0(0)	3(9)	1(6)	2(7)	
Family history of IBD†			*1.00*		*1.00*			*1.00*
No	9 (82)	26 (76)		10 (77)	25 (78)	14 (78)	21 (78)	
Yes	2 (18)	8 (24)		3 (23)	7 (22)	4 (22)	6 (22)	
Location†			*0.23*		*0.92*			*0.58*
Ileal	4 (36)	12 (35)		4 (31)	12 (38)	5 (28)	11 (41)	
Colonic	4 (36)	5 (15)		3 (23)	6 (19)	5 (28)	4 (15)	
Ileocolonic	3 (27)	17 (50)		6 (46)	14 (44)	8 (44)	12 (44)	
Behavior†			*0.15*		*0.05*			*<0.001*
Non -stricturing, non-penetrating	6 (55)	12 (35)		8 (62)	10 (31)	12 (67)	6 (22)	
Stricturing	2(18)	17 (50)		2 (15)	17 (53)	2 (11)	17 (63)	
Penetrating	3 (27)	5 (15)		3 (23)	5 (16)	4 (22)	4 (15)	
Previous azathioprine†			*0.04*		*1.00*			*1.00*

No	9 (82)	15 (44)		7 (54)	17 (53)		10 (56)	14 (52)	
Yes	2 (18)	19 (56)		6 (46)	15 (47)		8 (44)	13 (48)	
HBI at baseline	7 (6, 8)	7 (6, 8)	*0.88*	7 (7, 8)	7 (6, 8)	*0.41*	7 (6, 8)	7 (6, 8)	*0.57*
SES-CD at baseline	10 (8, 14)	10 (8, 13)	*0.81*	12 (8, 14)	10 (8, 13)	*0.48*	12 (8, 16)	9 (8, 12)	*0.15*
Normal CRP at baseline†			*0.17*			*0.09*			*0.22*
No	11 (100)	27 (79)		13 (100)	25 (78)		17 (94)	21 (78)	
Yes	0 (0)	7 (21)		0 (0)	7 (22)		1 (6)	6 (22)	
Normal CRP at week 14†			*1.00*			*0.74*			*0.77*
No	5 (45)	16 (47)		7 (54)	14 (44)		9 (50)	12 (44)	
Yes	6 (55)	18 (53)		6 (46)	18 (56)		9 (50)	15 (56)	
Oncostatin M at baseline	26 (16, 191)	0 (0, 10)	*0.004*	175 (25, 203)	0 (0, 0)	*<0.001*	89 (20, 199)	0 (0, 0)	*<0.001*
Oncostatin M at 14 weeks	114 (0, 174)	0 (0, 0)	*0.03*	174 (121, 336)	0 (0, 0)	*<0.001*	139 (5, 265)	0 (0, 0)	*<0.001*
FC at baseline	500 (382, 580)	408 (297, 643)	*0.66*	500 (380, 670)	408 (297, 571)	*0.35*	460 (299, 660)	423 (304, 581)	*0.94*
FC at 14 weeks	500 (377, 584)	32 (15, 140)	*<0.001*	366 (199, 500)	30 (15, 133)	*<0.001*	353 (185, 521)	15 (15, 55)	*<0.001*

*Mann-Whitney's test for the continuous variables and Fisher's exact test for the categorical variables; †frequencies (percentages). IQR = interquartile range, HBI = Harvey Bradshaw Index, SES-CD = Simple Endoscopic Index for CD, CRP = C-reactive protein, FC = faecal calprotectin.

Figure legends

Figure 1. Boxplots in logarithmic scale of each biomarker at baseline and week 14, by mucosal healing (MH) status at week 54: faecal calprotectin (FC) at baseline and week 14 (panels A and B); oncostatin M (OSM) at baseline and week 14 (panels C and D). The horizontal dotted lines in panel B and C show the best threshold levels of biomarkers.

Figure 2. Receiver operating characteristic (ROC) curves for oncostatin M (OSM) and faecal calprotectin (FC) at baseline and week 14 as predictors of mucosal healing at week 54.

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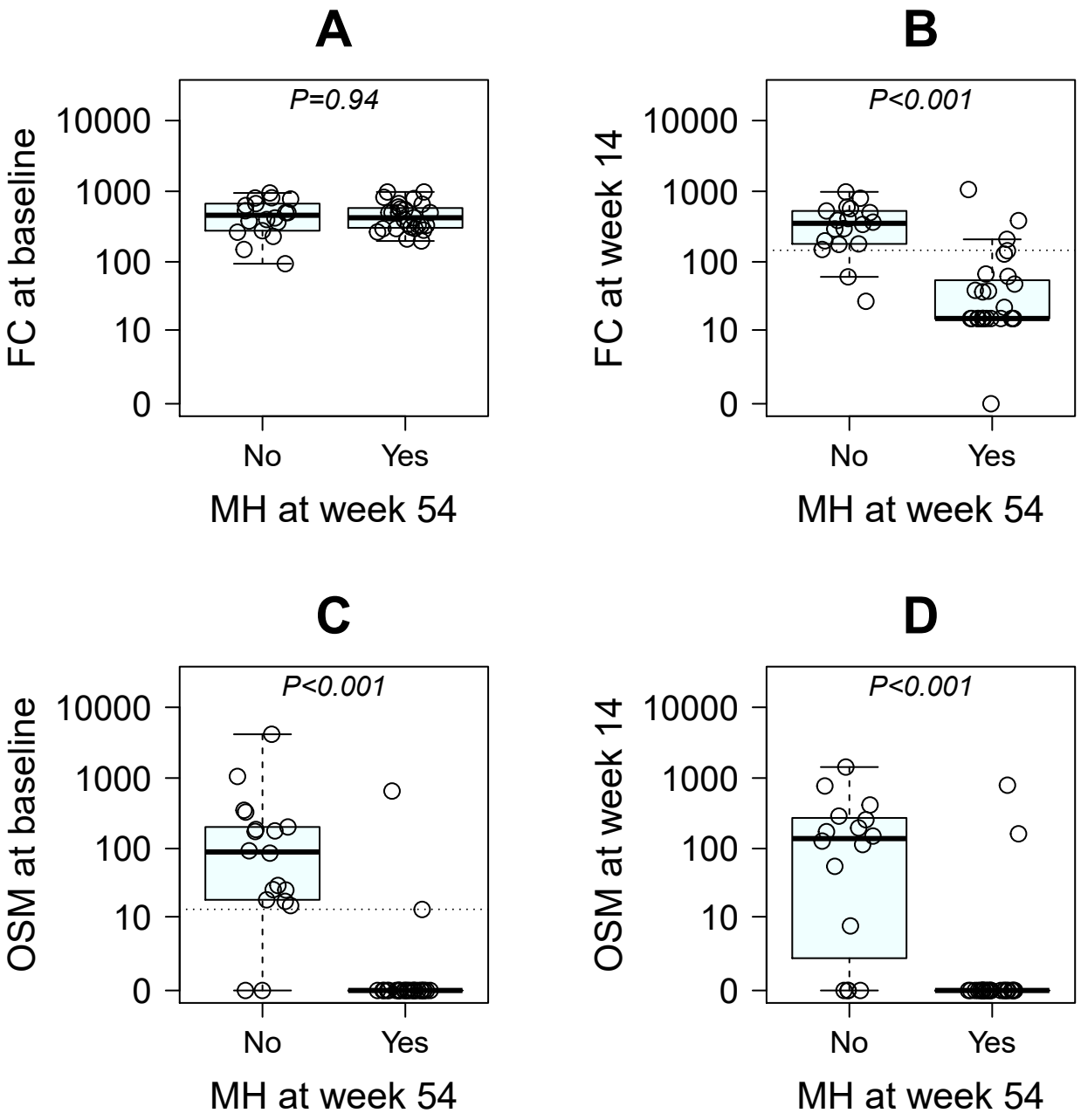
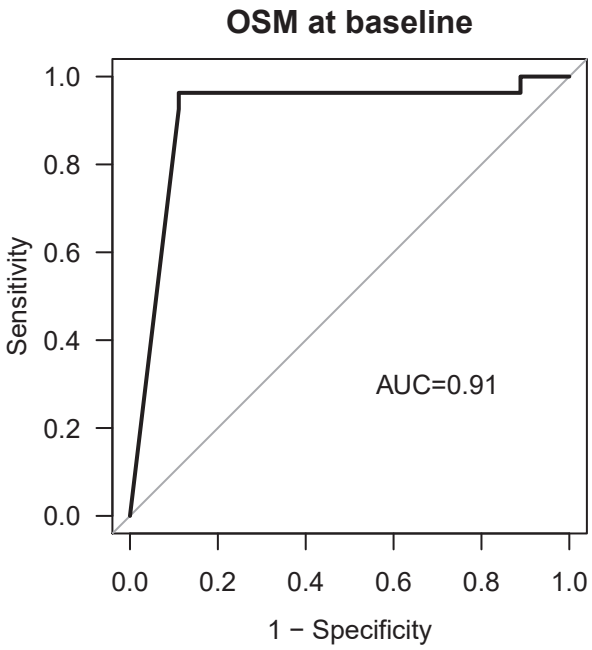
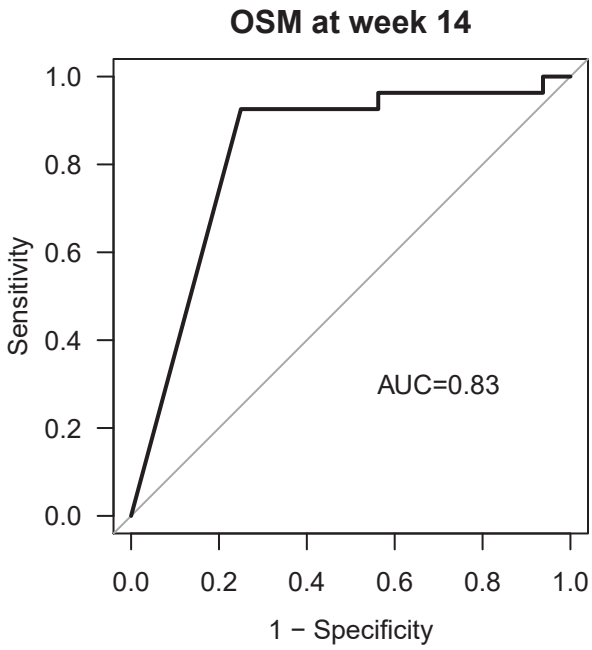
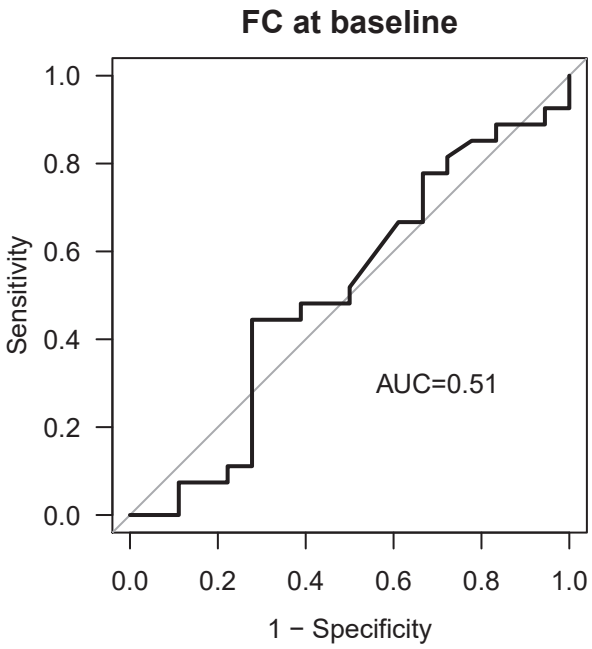
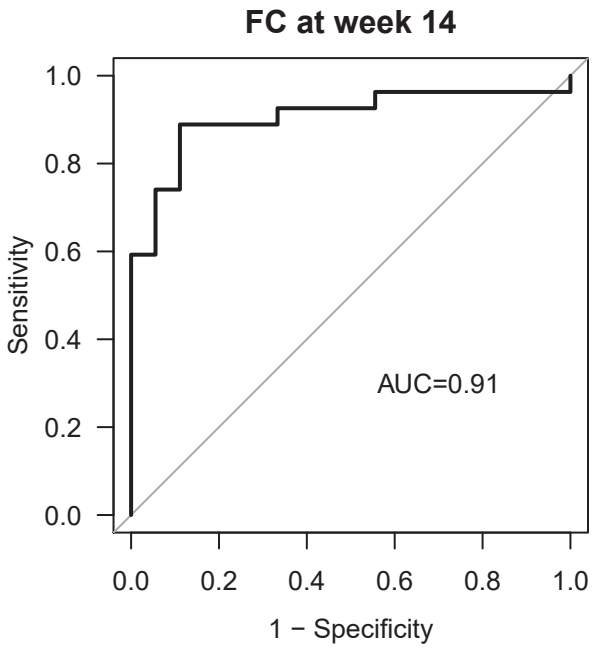

A
P=0.94
FC at baseline
10000
1000
100
10
0
No
Yes
MH at week 54
B
P<0.001
FC at week 14
10000
1000
100
10
0
No
Yes
MH at week 54
C
P<0.001
OSM at baseline
10000
1000
100
10
0
No
Yes
MH at week 54
D
P<0.001
OSM at week 14
10000
1000
100
10
0
No
Yes
MH at week 54


OSM at baseline
Sensitivity
1 − Specificity
AUC=0.91
0.0
0.2
0.4
0.6
0.8
1.0


OSM at week 14
Sensitivity
1 − Specificity
AUC=0.83
0.0
0.2
0.4
0.6
0.8
1.0


FC at baseline
Sensitivity
1 − Specificity
AUC=0.51
0.0
0.2
0.4
0.6
0.8
1.0


FC at week 14
Sensitivity
1 − Specificity
AUC=0.91
0.0
0.2
0.4
0.6
0.8
1.0