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Title page

Title: Assessment of serum cytokines predicts clinical and endoscopic outcomes to vedolizumab in ulcerative colitis patients

Running title: Serum cytokines: biomarkers of mucosal healing

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PI Statement: The authors confirm that the Principal Investigator for this paper is Francesco Costa and that he had direct clinical responsibility for patients.

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Abbreviations: Akaike Information Criteria (AIC), Area Under the Curve (AUC), Fecal Calprotectin (FC), Inflammatory Bowel Diseases (IBD), Interleukin (IL), Tumor Necrosis Factor (TNF), Ulcerative Colitis (UC), Vedolizumab (VDZ)

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Abstract

Background and Purpose

Vedolizumab (VDZ) prevents migration of activated leukocytes into inflamed mucosa. This study was aimed at assessing the patterns of serum cytokines in Ulcerative Colitis (UC) patients at baseline and during VDZ treatment, and to investigate their association with mucosal healing and clinical remission.

Experimental Approach

We enrolled consecutive UC patients eligible for treatment with VDZ. A panel of serum cytokines were measured by fluorescence assay at weeks 0, 6 and 22. Colonoscopy was performed at baseline and week 54, to evaluate mucosal healing. The time trends of serum cytokines were analyzed by log-linear mixed effect models, and their prognostic accuracy was evaluated by logistic regression.

Key Results

Out of 27 patients included in the analysis, at week 54 mucosal healing was achieved in 12 (44%) and clinical remission in 17 (63%). Mucosal healing was associated with higher IL-8 values at baseline and with significant decrease in IL-6 and IL-8 levels over the first 6 weeks. A significant reduction of IL-6 and IL-8 levels over the first 6 weeks of treatment was associated also with clinical remission. Logistic models including, among the predictors, IL-6 and IL-8 at baseline and their changes over the first 6 weeks of treatment had 83% sensitivity and 87% specificity to predict mucosal healing, and 82% sensitivity and 90% specificity to predict clinical remission.

Conclusion

In UC patients, the serum patterns of IL-6 and IL-8 at baseline and over the first 6 weeks of treatment with VDZ could be useful to predict therapeutic outcome.

Keywords: Translational Research; cytokines; inflammation

Bullet Point Summary:

- **What is already known**
  - Serum cytokines were used in some studies to predict therapeutic outcome in ulcerative colitis patients treated with Anti-TNF.
  - A reliable biomarker to predict therapeutic efficacy to Vedolizumab is not already available.
- **What this study adds**
  - This real-life study showed that an early assessment (at baseline and after 6 weeks of treatment) of serum IL-6 and IL-8 is useful in predicting mucosal healing and clinical remission after 54 weeks of treatment with Vedolizumab.
  - If these data will be confirmed in larger cohorts, the nomogram that we are proposing should be a useful tool for clinicians in order to predict therapeutic outcome to vedolizumab only after 6 weeks of treatment. This information could lead to significant savings in terms of health care resources.

Introduction

Ulcerative colitis (UC) is a chronic relapsing inflammatory disease, affecting the colon and the rectum, characterized by superficial mucosal ulceration, rectal bleeding, diarrhea and abdominal pain.(1) Vedolizumab (VDZ) is an important therapeutic option for UC patients,(2) due to a different mechanism of action, as compared to non-biological therapeutic approaches or anti-Tumor Necrosis Factor (TNF) agents. This monoclonal antibody, which binds the α4β7-integrin expressed in a subset of T-lymphocytes, prevents their adherence and diapedesis through the mucosal vascular addressing cell adhesion molecule (MAdCAM)-1, expressed only in the gut endothelium.(3)

Despite a satisfactory clinical development program, real-life studies point out that only 40% of patients treated with VDZ achieve clinical remission, and even less mucosal healing.(4, 5) In addition, a non-negligible number of patients experience loss of response during VDZ treatment.(6) In this respect, the identification of biomarkers, useful to characterize or predict the therapeutic response to VDZ, would allow clinicians to optimize the management of UC patients, with a relevant impact also on public health expenditure.

The evaluation of serum cytokine profiles may represent a reliable and non-invasive tool to predict the therapeutic efficacy of biological drugs.(7) Indeed, previous clinical studies in patients treated with infliximab showed that plasma IL-6 levels were significantly lower both at baseline and after 8 weeks of treatment in responders than non-responders, suggesting that the evaluation of serum IL-6 could predict clinical efficacy in response to this treatment.(8, 9) Likewise, a recent study by Soendergaard et al. reported an association between circulating IL-6 levels and clinical response to VDZ.(10) However, these authors did not provide information on the putative correlation between circulating cytokine levels and response to VDZ in terms of mucosal healing. This is a relevant point, since therapeutic

strategies based merely on clinical remission or response have failed to modify the course of Inflammatory Bowel Disease (IBD).(11) By contrast, mucosal healing is associated with lower relapse rates, lower hospitalization rates, reduced need for surgery, and lower risk of colorectal cancer.(12, 13) Nevertheless, to our knowledge, the potential association of circulating cytokine levels and mucosal healing in UC patients treated with VDZ has not been investigated.

Based on the above background, the present study was designed to investigate the patterns of a panel of circulating inflammatory mediators in UC patients, in order to identify one or more cytokines correlated positively with the clinical and endoscopic outcomes of VDZ treatment.

Materials and Methods

Patients and study protocol

All patients with moderate-severe UC, referred consecutively to our unit from June 2016 to June 2017, and eligible to treatment with VDZ, were included in this study, after signing an informed consent. The diagnosis of UC had been confirmed previously by clinical, endoscopic and histological evaluations. Patients treated concomitantly with azathioprine or receiving a non-stable dosage of mesalazine were excluded from the study. Primary non-responders (defined as a decrease in Full Mayo Score ≤2 or a lack of improvement of rectal bleeding at week 14), and patients treated with VDZ for less than 22 weeks for any reason, were excluded from the analysis.

The following data were collected: date of birth, sex, year of diagnosis, therapy with corticosteroids at diagnosis, disease extension at diagnosis, eventual proximal extension of the disease, any previous biological therapy (and its therapeutic outcome), any previous

treatment with azathioprine (and its therapeutic outcome), disease extension at the start of treatment, disease severity, concomitant corticosteroid therapy, value of fecal calprotectin (FC).

This study protocol was conducted in accordance to 1975 Declaration of Helsinki, and was approved by the Ethical Committee of Pisa University Hospital (CEAVNO), with the protocol number 22103. All patients have given their consent to the collection of these data and to their publication

Study design

All patients included in the present study received VDZ 300 mg i.v. at weeks 0, 2, 6, 14, 22, 30, 38 and 46. FC levels were evaluated by an ELISA Kit (Calprest®, Eurospital, Italy) in the week preceding VDZ infusion, at baseline, at week 6, and at week 22. Before drug infusion at the same timepoints (baseline, week 6 and week 22), 9 ml of peripheral venous blood samples were collected in EDTA. Blood samples were 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 Interferon-γ, Interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-22, IL-23 and TNF by a Luminex® fluorescence intensity assay(14). Despite week 22 is not clearly an early time-point, we included it in the analysis since in previous studies VDZ has been reported to induce disease remission slower than anti-TNFs(15); therefore, we reasoned that an evaluation of cytokine levels at this time-point could contribute to highlight clinical relevant differences not detectable at earlier time points. Only IL-6, IL-8 and TNF were then included in the final analysis, since for the other cytokines the detection threshold was not reached in more than 50% of the included patients. The reason for this was dual: 1) in our hands, the fluorescence intensity assay did not allow to achieve the detection threshold for IL-1β, IL-10,

IL-12p70, IL-17A, IL-22 and IL-23 in more than 50% of the included patients; 2) most importantly, previous studies, where serum levels of IL-1β, IL-10, IL-12p70, IL-17A, IL-22 and IL-23 could be detected, found that such cytokines did not correlate significantly with different patterns of clinical response to biologics, and thereby they may not be suitable for predicting the therapeutic responses of UC patients to treatments with biological drugs(9, 10). Serum samples collected at week 6 were employed also to assay VDZ trough levels (VTL), by ELISA (Theradiag®, Marne-la-Valle´e, France).

At each VDZ infusion, patients underwent a clinical examination, to estimate the Partial Mayo Score (PMS) in accordance to current guidelines.(16) All patients enrolled underwent a colonoscopy both at baseline and week 54, or in case of loss of response (after at least 22 weeks of treatment). Mucosal healing was defined as Mayo Endoscopic Score of 0 or 1. Clinical remission was defined as a PMS <2 without concomitant corticosteroid therapy. For patients who discontinued VDZ before the 54-week follow up due to loss of response, the therapeutic outcome was assessed on the basis of endoscopy and clinical examination at discontinuation.

Statistical analysis

Differences among the distribution of age, sex, clinical and serological variables before the beginning of treatment (baseline) were tested with the Mann-Whitney test for the continuous variables and the Fisher's exact test for the categorical variables. Correlations between the serological variables and FC at baseline were tested with the Spearman correlation.

To describe the trend of cytokines and FC over the time since baseline accounting for the correlation of repeated measures within subject, we fitted mixed effect models with the log transformed measures of each biomarker as dependent variable, and age, time, response at

54 weeks (either mucosal healing or clinical remission) and the interaction between time and response as fixed effect and patients as random effect; log-transformation was performed to achieve normality of the residuals of models. The best mixed effect model was that with the lowest Akaike Information Criteria (AIC). Simultaneous tests were conducted on the coefficients from the best model to test for the difference of the biomarkers among responders and non-respondenrs and for their time-dependence.

The ability of each biomarker to predict the response to therapy at week 54 was evaluated through logistic regression models including its baseline level and its change over time. Models were compared with AIC and the Area Under the Curve (AUC). For each model, the optimal cut-off of the linear predictor was determined as the point minimizing the Euclidean distance between the ROC curve and the point (0,1).

All statistical analyses were performed with R software *(R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.)*

Results

Thirty-two UC patients started VDZ treatment during the enrollment period; five patients were defined as primary non-responders and were excluded from the analysis. The demographic and clinical characteristics of the 27 patients included in the analysis are reported in Table 1, upon stratification by response evaluated in terms of mucosal healing; no significant difference was observed between responders and non-responders to VDZ for any variable. Six patients (22%) were naïve to any biologic therapy, and 10 patients (37%) had

been treated previously with azathioprine. After 54 weeks, mucosal healing was achieved in 12 patients (44%), while clinical remission was obtained in 17 patients (63%).

At baseline, the median PMS of all participants was 5 (IQ range, 5 to 6) and their median FC concentration was 314 mg/Kg (IQ range, 249 to 484) without significant difference between responders and non-responders (Table 2). Among the patients with loss of response (22%), four dropped off at week 30 and two at week 38. Clinical relapse was confirmed by means of endoscopic evaluation. No adverse events were recorded throughout the study period.

Serum concentrations of cytokines and fecal calprotectin at baseline

At baseline, the serum concentrations of IL-6 and IL-8 correlated positively (Spearman correlation coefficient, 0.41, p-value=0.03), as did the concentrations of IL-8 and TNF (Spearman correlation coefficient, 0.55, p-value=0.003). Serum levels of IL-6, IL-8 and TNF were higher in patients achieving mucosal healing than in those who failed this goal, although statistical significance was reached only for IL-8 (p-values of Mann-Whitney test, 0.15, 0.01 and 0.11 for IL-6, IL-8 and TNF respectively). None of the demographic or clinical variables was associated with the baseline concentration of any cytokine.

Baseline levels of FC were similar in responders and non-responders (p-values, 0.39) and correlated inversely with baseline TNF levels (Spearman correlation coefficient, rho=-0.41, p-value=0. 03), but not to IL-6 (rho=0.03 and p-value=0.90) nor IL-8 levels (rho=-0.16 and p-value=0.41).

Serum concentration of cytokines and fecal calprotectin during treatment

Serum levels of IL-6, IL-8 and TNF as well as FC concentrations over the first 22 weeks are reported in Table 2. The results of the mixed effect models, as described in the method section, are reported in Figure 1. For each cytokine, when the response to treatment was evaluated in terms of mucosal healing, the best mixed effect model included age, response and the interaction between response and time; the effect of time alone was not significant (p-value>0.2), suggesting that non-responders did not present significant changes in serum cytokine levels over the time. Cytokine levels in patients with mucosal healing decreased significantly over the first 6 weeks (Figure 1): the percent changes at week 6, as compared to baseline, were 34% (95% CI, 4% to 54%; p-value, 0.024) for IL-6, 49% (95% CI, 17% to 68%; p-value, 0.003) for IL-8 and 61% (-2% to 85%; p-value, 0.06) for TNF. No further significant decrease in any cytokine was observed from week 6 to week 22.

At week 6, VTL were 28.9µg/ml in patients who achieved mucosal healing at one year, and 14.8µg/ml in non-responders (Mann-Whitney's test, p-value=0.06).

With regard for FC, the best mixed effect models included age, response and time; the interaction between time and mucosal healing was not significant (p-value=0.26), suggesting that the trend of FC over the time did not differ between responders and non-responders in terms of mucosal healing. FC values at week 6 were significantly lower in responders than non-responders (p-value=0.03); over the first 6 weeks of treatment FC decreased significantly in all patients irrespective by their mucosal healing status at week 54 (percent change, 63%; 95% CI, 26% to 81%; p-value, 0.002). No further significant decrease in FC was observed from week 6 to week 22.

Similar trends were observed for IL-6, IL-8, TNF and FC when the response was evaluated in terms of clinical remission (Supplemental Figure 1).

Diagnostic accuracy of cytokines and fecal calprotectin to predict the response to treatment

The logistic model including both IL-8 and IL-6 at baseline and their change over the first 6 weeks was the most accurate to predict mucosal healing at week 54, immediately followed by the one including IL-8 at baseline and its change over the first 6 weeks (Table 3). The AUC for the model including both IL-6 and IL-8 was 0.93 (95%CI: 0.85 to 1.00); for this model a cut-off of -0.40 in the linear predictor corresponded to 83% sensitivity and 87% specificity (Figure 2). The upper panel of figure 2 shows the nomogram for the best diagnostic model and the lower panel the density plot of the linear predictor of the best model for responders and non-responders.

With regard for clinical remission, the model including both IL-6 and IL-8 and their change over the first 6 weeks presented also the highest accuracy (AUC, 0.95, 95% CI, 0.87 to 1.00); a cut-off of 0.53 in the linear predictor corresponded to a sensitivity of 0.82 and a specificity of 0.9 (Supplemental Table 1 and Supplemental Figure 2).

Discussion

The present study was designed to identify serum markers of mucosal healing and clinical remission in patients treated with VDZ. We observed that baseline serum levels of IL-6, IL-8 and TNF were higher in responders than non-responders, and that the serum levels of these cytokines decreased during the first 6 weeks of treatment in responders, but not in non-

responders. The patterns of IL-6 and IL-8 within the first 6 weeks of treatment well predicted the outcome of therapy.

During the first 54 weeks of treatment with VDZ, 6 out of 27 (22%) patients included in the analysis experienced loss of response, as compared to 39% reported in the largest real-life study.(5) After 54 weeks of treatment, 44% of patients included in the analysis achieved mucosal healing, this being a higher proportion than that reported in the GEMINI I trial,(2) but lower when compared with other real life studies, such as 54% reported in a French cohort(5) and 69% in a US prospective study.(17) The proportion of patients achieving clinical remission at week 54 (63%) was consistent with that reported in a Swedish real-life cohort(18), and higher than that observed in German(19) and French(5) real life studies. No adverse events were recorded in our cohort, in line with previous studies(5, 17, 19) reporting that treatment with VDZ was not associated with an increased risk of serious or opportunistic infections, incidence of malignancies, and infusion-related reactions.(4)

Several attempts, by means of clinical trials and real-life studies, have been made to identify at an early stage UC patients with a higher chance of responding to pharmacological treatment, but current data are scarce and often inconsistent. At present, the most acknowledged clinical indicator of worse response to VDZ is a previous treatment with anti-TNF.(2, 4, 20) In parallel, an early decrease in PMS has been considered a marker of subsequent clinical response: in the French real-life cohort,(5) patients who achieved a clinical response with VDZ at week 6 were more likely to achieve steroid-free clinical remission after one year. Consistently, Shelton et al.(21) reported that an early clinical response to VDZ at week 6 was a significant predictor of clinical remission at week 14 in patients with UC. In addition, in a post-hoc analysis of the GEMINI I trial,(22) clinical remission at week 14 was suggested as a predictor for sustained clinical remission at one year. In this regard, the role

played by C-Reactive Protein has been investigated by various authors with inconsistent findings.(20) Conversely, in a prospective real-life study,(23) a decrease in FC at week 14 was associated with clinical remission at one year. However, a post-hoc analysis of the GEMINI I trial(24) showed that FC levels, when measured shortly after VDZ induction, may not be a useful biomarker of mucosal inflammation or endoscopic outcomes. In parallel, Boden et al.(25) have proposed additional candidate biomarkers, including VTL, α4β7 baseline expression and its receptor. The role of an early measurement of VTL was recently demonstrated also in predicting treatment persistency.(26) Recently, Zwicker et al.(27) observed higher serum levels of CCL20, CCL23, and CXCL1 at week 10 in non-responders as compared to responders, and suggested the potential prognostic value of circulating chemokines in predicting the response to VDZ.

The present study suggests a role of IL-6 and IL-8 in predicting the therapeutic outcome to VDZ in UC patients. In particular, high IL-8 levels at baseline, as well as the decrease in IL-6 and IL-8 over the first 6 weeks of treatment, correlate with mucosal healing at week 54. Previous studies evaluated also possible correlations of circulating proinflammatory cytokines at baseline with therapeutic outcome in IBD patients treated with biological therapy, including VDZ.(8-10) IL-8 is a pro-inflammatory cytokine released by macrophages and epithelial cells, and involved in the chemiotactic attraction of neutrophils into the inflammatory site.(28) In our study, the association of higher baseline serum IL-8 levels and their decrease over the time with the response to VDZ might be explained with an IL-8-dependent increased expression of the drug molecular target (i.e. α4β7 integrin). However, data supporting this contention are conflicting: a study by Fuchs et al.(29) showed that a lower baseline frequency of α4β7-positive CD4 T cells in patients with IBD was associated with clinical response to VDZ; by contrast, Boden et al.(25) found higher basal α4β7 expression on

CD4 and CD8 T cells as well as NK cells in responders as compared with non-responders. A concomitant evaluation of α4β7 expression and IL-8 serum levels would likely better substantiate this hypothesis. Moreover, since higher IL-8 tissue levels have been found in mucosal ulcerations of UC patients, as compared to areas of non-inflamed mucosa,(30) the evaluation of IL-8 levels in colonic in mucosa might strengthen further its predictive value in UC patients under treatment with VDZ.

Despite the molecular mechanisms underlying the anti-inflammatory effect of VDZ in IBD remain scarcely understood, recent evidence suggests that an effect on mucosal innate immunity could contribute to its therapeutic efficacy.(31) This is an intriguing point, since IL-8 is a pro-inflammatory cytokine produced mainly by innate immune cells, such as macrophages and epithelial cells. Therefore, it is conceivable that the increased efficacy of VDZ in patients displaying higher circulating levels of IL-8 could be related to a greater innate immune cellularity, targeted by the drug, in responders. Clearly, additional investigations are needed to verify this hypothesis.

IL-6 plays a critical role in the regulation of immune responses, not only facilitating the communication among cells within the innate and adaptive immune arms, but allowing also for interactions between them.(32) In particular, IL-6, produced by innate immune and supporting stromal cells, is known to activate adaptive T and B effector cells.(32) In our patient cohort, baseline serum IL-6 levels where higher in responders as compared to non-responders, even if statistical significance was not achieved. However, a recent paper by Soendergaard et al.(10) reported that patients with lower IL-6 levels at baseline displayed a clinical response to VDZ at weeks 14-20. Although additional evidence from independent studies is needed to reach a reliable conclusion on the prognostic value of IL-6, it cannot be excluded that current discrepancies are due to heterogeneity of the respective patient

populations, since both UC and Crohn's disease patients were included in the study by Soendergaard et al.(10) In this regard, it is noteworthy recognized that IL-6 was found to be significantly higher in Crohn's Disease, as compared to UC patients.(33) By contrast, we included only UC patients, and this likely reduced the bias related to the different cytokine paradigms involved in the onset and progression of UC (Th_2)(34) and Crohn's disease (Th_1/Th_{17}).(35) Moreover, another important difference between of our study and that by Soendergaard et al.(10), is that the latter did not perform endoscopic evaluations, even at baseline, in all their patients. Interestingly, we observed a significant correlation between the decrease in serum IL-6 levels over the first 6 weeks of treatment and mucosal healing or clinical remission at week 54. This finding is in line with previous studies showing a correlation between the decrease in IL-6 levels and the clinical response to anti-TNF therapy in UC patients.(9, 36)

The present results show also that serum TNF levels declined more rapidly in endoscopic responders than non-responders, even if data were only near to statistical significance ($p=0.06$). This finding is in line with a recent study on UC patients treated with VDZ,(37) where the authors observed a decline of TNF in responders, although not significant. Of note, the most interesting data on TNF have been obtained at tissue level, where there appear to be good evidence that its concentration decreases in the colonic mucosa of UC patients during anti-TNF therapy and could predict therapeutic response.(38)

FC is recognized as a helpful marker to evaluate disease activity in UC.(39) It has been proposed as a predictive marker of mucosal healing in UC patients treated with anti-TNF,(40) and of clinical remission in patients treated with VDZ(19). Of note, both clinical trials(41) and real life studies(18) highlighted that VDZ promoted a significant reduction of FC in UC patients. However, the evaluation of FC, as a predictive marker of mucosal healing in VDZ-treated

patients, has never been investigated. In this respect, our study showed that FC levels at week 6 and 22 were significantly lower in responders than in non-responders, consistently with previous findings in UC patients treated with anti-TNF(40).

Of interest, in the present study an inverse correlation was found between TNF and FC at baseline. This is an interesting and intriguing finding, which remains unexplained and likely deserves more specific investigations. However, it is worthy to mention that a very recent paper by Smillie et al.(42), investigating the relationship between the tissue immune cell composition and the clinical response to anti-TNF biodrugs in UC patients, highlighted an increased infiltration of TNF-secreting Treg cells (TNF^+Tregs) in non-responders. Since Treg cells are known to display a heterogeneous expression of α/β integrins, it can be tentatively speculated that, in our study, the inverse correlation between TNF and FC in non-responder patients could depend on two parallel processes: 1) a decrease in the colonic density of VDZ-sensitive $\alpha4\beta7^+$ immune cells, with a consequent reduction of FC at week 6 and 22; 2) an increase in the tissue density of VDZ-insensitive TNF^+Treg cells, responsible for a sustained release of TNF, despite the decrease in FC production. In support of this hypothesis, it is noteworthy that the putative presence of TNF^+Treg cells in the inflamed colon of our non-responder patients would be justified by the circumstance that 16 over 17 of these subjects were non-responder to anti-TNF (described by Smillie et al.(42) as patients bearing a peculiar colonic cellularity, with a preponderant presence of TNF+Treg cells).

The statistical analysis of our data showed that a combined assessment of IL-8 and IL-6 (evaluating both the decrease over the first 6 weeks of treatment and values at baseline) identified the best model for predicting the therapeutic outcome, in terms of both mucosal healing and clinical remission. Clearly, these findings need to be confirmed in studies on larger patient populations. Nevertheless, our analysis is of clinical interest, as it suggests that an

early assessment of IL-6 and IL-8 serum levels at week 0 and 6 could be useful to better manage VDZ therapy.

The major strengths of our study are: (1) the completeness of clinical and serological data collected in the included patients; (2) the inclusion of consecutive patients eligible to treatment with VDZ at our unit, thus reflecting a real-life practice; (3) having adopted mucosal healing as primary outcome of VDZ treatment, this being currently recognized as a parameter of crucial relevance in the outcome of UC. The major limitations of our study include the relatively small sample size and the lack of colonic bioptic specimens, to evaluate tissue cytokine levels and perform a histological evaluation of the healing process. However, it is noteworthy that this study was conceived as a pilot, explorative, monocentric study, aimed at evaluating patients enrolled during the first year of real-life prescription of VDZ, and that the small sample size was not a drawback to the performance of a reliable statistical analysis.

Overall, the results obtained in the present study highlight an interesting association of serum cytokine levels and FC with mucosal healing and clinical remission, thus paving the way to further studies, based on a larger number of patients, aimed at corroborating the suitability of such parameters as biomarkers of therapeutic response to VDZ. In the near future, the use of biomarkers in IBD will increase, in order to identify early patients responding to a biological treatment. If our data will be confirmed, an early assessment of IL-6, IL-8 and FC in UC patients treated with VDZ could lead to significant savings in terms of health care resources.

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Table 1. Demografic and clinical characteristics of the study patients stratified by mucosal healing at week 54 since baseline

		Mucosal Healing		
		No, N (%)	**Yes, N (%)**	***p.value (*)***
age (years), median (IQ range)		45.75 (41.24-60.5)	53.2 (32.74-67.54)	*0,64*
sex	Females	5 (33)	2 (17)	*0,41*
	Males	10 (67)	10 (83)	
disease extension at diagnosis	pancolitis	7 (47)	5 (42)	*1,00*
	left colitis	4 (27)	3 (25)	
	proctitis	4 (27)	4 (33)	
disease extension at baseline	pancolitis	9 (60)	6 (50)	*0,87*
	left colitis	4 (27)	3 (25)	
	proctitis	2 (13)	3 (25)	
disease progression until baseline	No	11 (73)	10 (83)	*0,66*
	Yes	4 (27)	2 (17)	
previous AZA therapy	No	9 (60)	8 (67)	*1,00*
	Yes	6 (40)	4 (33)	
previous biological therapy	No	1 (7)	5 (42)	*0,06*
	Yes	14 (93)	7 (58)	
concomitant CS therapy at baseline	No	6 (40)	2 (17)	*0,24*
	Yes	9 (60)	10 (83)	

(*) Mann-Whitney's test for age and Fisher's exact test for categorical variables

CS: Corticosteroid; AZA: Azathioprine

Table 2. Serological and fecal markers in patients stratified by mucosal healing at week 54 since baseline.

		Mucosal Healing		
		No (N=17)	**Yes (N=12)**	
		median (IQ range)	**median (IQ range)**	***p.value (*)***
IL-6 (pg/mL)	baseline	1.49 (1.22, 2.96)	3.37 (1.83, 4.13)	*0.15*
	6 weeks	1.57 (1.34, 2.00)	1.75 (1.23, 2.91)	*0.94*
	22 weeks	1.34 (1.03, 1.86)	1.96 (1.34, 2.60)	*0.32*
	6 weeks-baseline	0.21 (-0.70, 0.50)	-1.11 (-2.46, -0.26)	*0.03*
	22 weeks-baseline	-0.10 (-1.17, 0.25)	-1.21 (-2.45, -0.38)	*0.07*
IL-8 (pg/mL)	baseline	8.16 (5.73, 19.27)	22.07 (17.99, 33.75)	*0.01*
	6 weeks	9.95 (7.39, 14.16)	12.05 (10.10, 15.14)	*0.53*
	22 weeks	9.90 (6.70, 14.62)	7.43 (5.17, 12.2)	*0.34*
	6 weeks-baseline	0.94 (-1.70, 3.14)	-6.17 (-19.44, -5.47)	*<0.001*
	22 weeks-baseline	1.79 (-1.55, 6.95)	-14.62 (-24.09, -10.43)	*0.001*
TNF (pg/mL)	baseline	0.13 (0.00, 2.69)	2.05 (1.33, 2.6)	*0.11*
	6 weeks	0.70 (0.00, 3.17)	1.44 (0.29, 3.27)	*0.49*
	22 weeks	0.55 (0.00,3.46)	0.76 (0.32, 1.54)	*0.98*
	6 weeks-baseline	0.00 (-0.21, 0.22)	-0.34 (-1.7, 0.03)	0.22
	22 weeks-baseline	0.00 (0, 0.65)	-1.12 (-1.77, -0.16)	0.007
FC (mg/Kg)	baseline	314 (256, 538)	306 (228, 402)	*0.39*
	6 weeks	254 (200, 500)	81 (27, 127)	*0.02*
	22 weeks	191 (114, 394)	44.5 (15, 115)	*0.04*
	6 weeks-baseline	-51 (-263, 0)	-283.15 (-331, -107)	0.15
	22 weeks-baseline	-289 (-448, 4)	-164.2 (-306, -34)	0.64

(*) Mann-Whitney's test

IL-6: Interleukin-6; IL-8: Interleukin-8; TNF: Tumor Necrosis Factor; FC: Fecal Calprotectin

Table 3. Diagnostic accuracy of IL-6, IL-8, TNF and FC to predict mucosal healing at week 54

	Model 1	Model 2	Model 3	Model 4	Model 5
coeff ± SE (p-value)					
Intercept	-0.80 ± 0.81 (0.321)	-1.36 ± 0.86 (0.114)	-0.36 ± 0.48 (0.459)	1.35 ± 0.77 (0.081)	-2.08 ± 1.48 (0.162)
IL-6 baseline (pg/mL)	0.09 ± 0.29 (0.772)				0.24 ± 0.61 (0.695)
IL-6 (6 weeks - baseline)	-0.62 ± 0.39 (0.107)				-1.00 ± 0.88 (0.254)
IL-8 baseline (pg/mL)		0.00 ± 0.03 (0.915)			-0.03 ± 0.05 (0.486)
IL-8 (6 weeks - baseline)		-0.22 ± 0.1 (0.023)			-0.26 ± 0.11 (0.017)
TNF baseline (pg/mL)			0.02 ± 0.12 (0.882)		
TNF (6 weeks - baseline)			-0.14 ± 0.24 (0.551)		
FC baseline (mg/Kg)				-0.01 ± 0.00 (0.055)	
FC (6 weeks - baseline)				-0.01 ± 0.00 (0.084)	
AIC	37.33	29.87	39.72	34.47	28.26
AUC (95% CI)	0.74 (0.54 to 0.95)	0.90 (0.78 to 1.00)	0.66 (0.44 to 0.88)	0.83 (0.65 to 1.00)	0.93 (0.85 to 1.00)
cut-off of p	0.389	0.348	0.413	0.516	0.402
cut-off of the linear predictor	-0.45	-0.63	-0.35	0.06	-0.40
Sensitivity	0.75	1	0.75	0.83	0.83
Specificity	0.73	0.8	0.67	0.87	0.87

Model 1: logit(p)~IL6.0+delta.IL6.6
Model 2: logit(p)~IL8.0+delta.IL8.6

Model 3: logit(p)~TNF.0+delta.TNF.6
Model 4: logit(p)~calprotectin.0+delta. calprotectin
Model 5: logit(p)~IL6.0+delta.IL6.6+ IL8.0+delta.IL8
IL-6: Interleukin-6; IL-8: Interleukin-8; TNF: Tumor Necrosis Factor; FC: Fecal Calprotectin; AIC: Akaike Information Criteria; AUC: Area Under the Curve

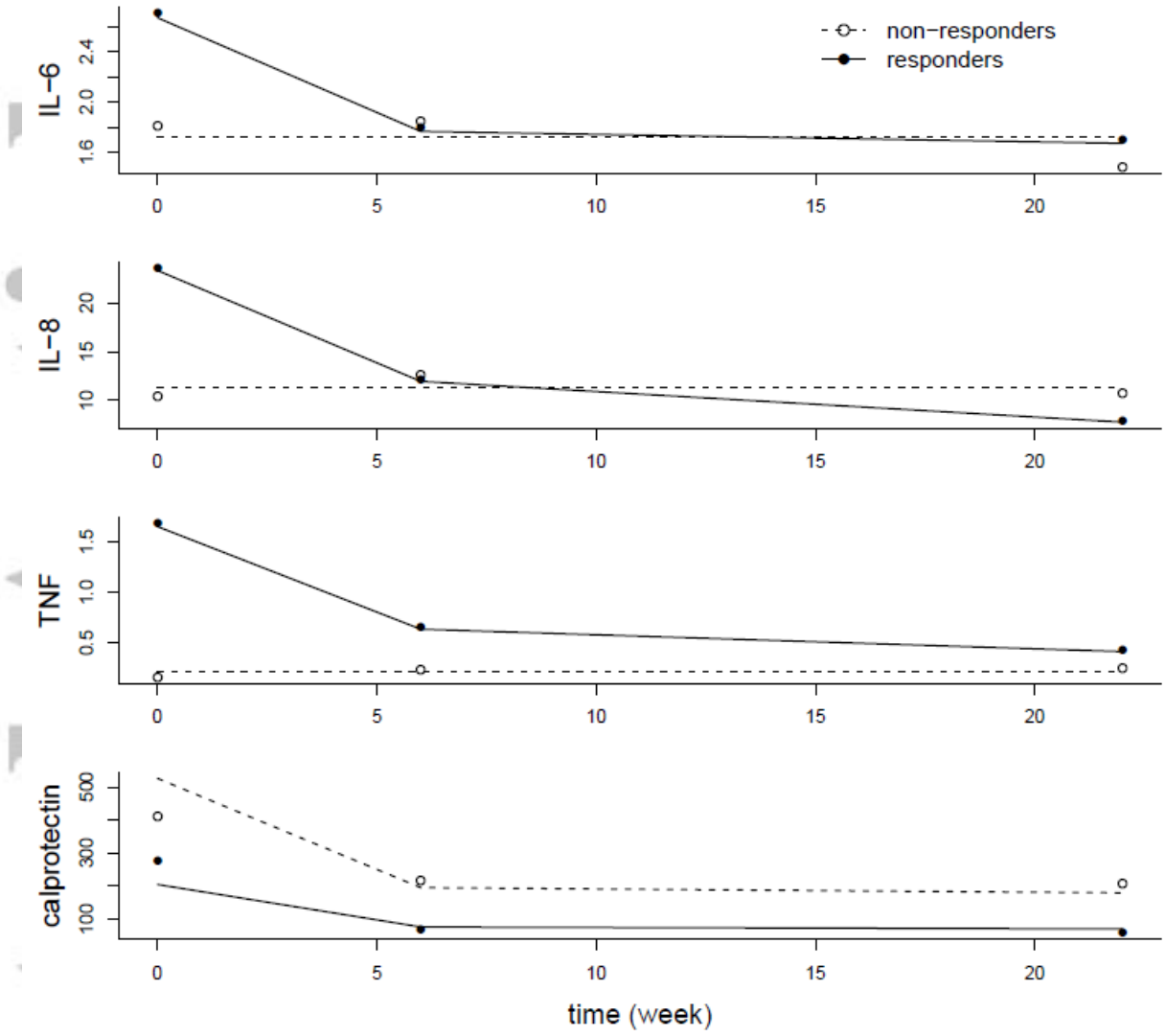

Figure 1. Trend of IL-6, IL-8, TNF and FC during the first 22 weeks of treatment with vedoluzimab in patients stratified by response evaluated as mucosal healing at week 54. Points represent the geometric means and lines the estimated marginal means of each factor predicted by the mixed effect model.

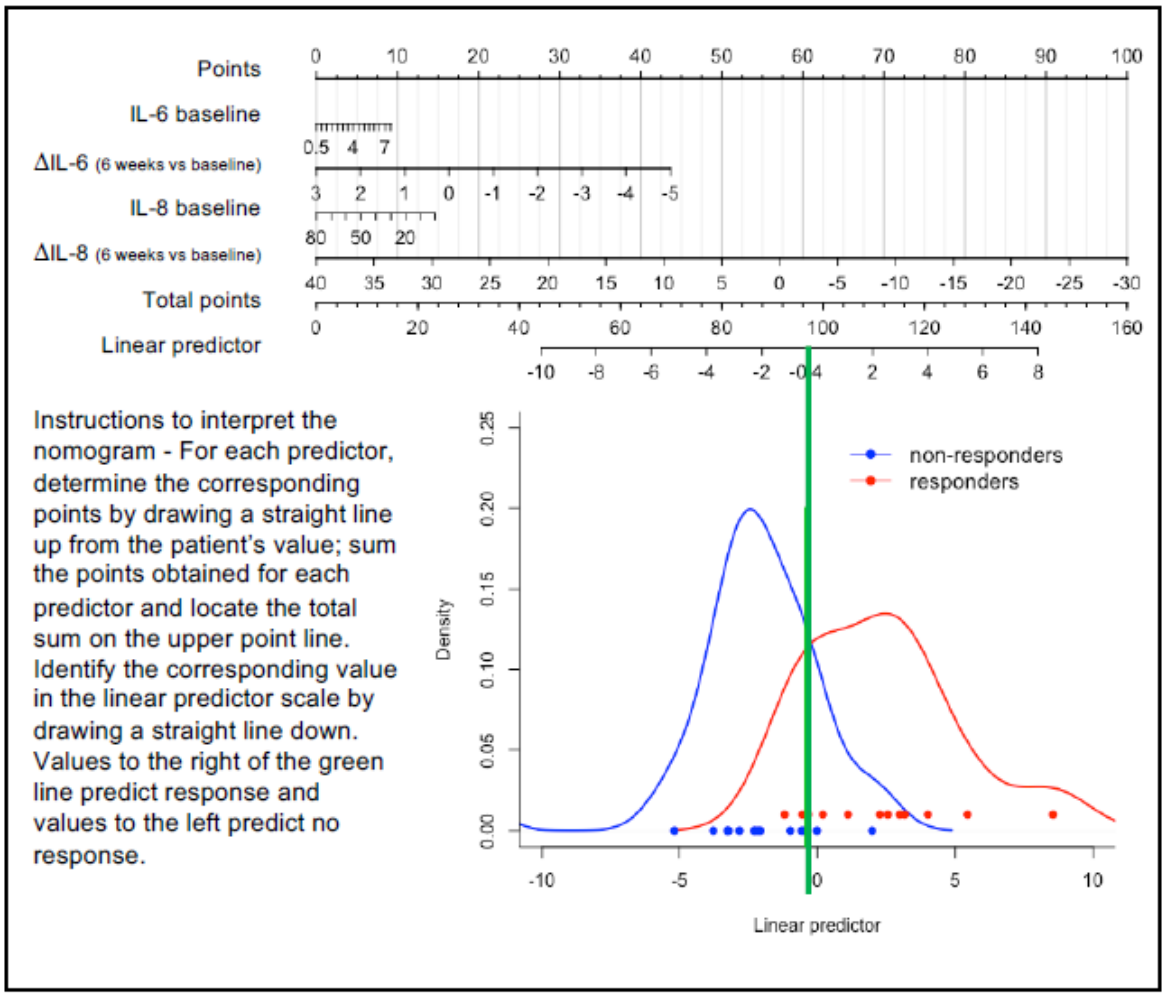

Figure 2. Prognostic nomogram of mucosal healing for patients with UC treated with vedoluzimab.