

Fecal Calprotectin Responses Following Induction Therapy With Vedolizumab in Moderate to Severe Ulcerative Colitis: A Post Hoc Analysis of GEMINI 1

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Background: In patients with ulcerative colitis (UC), fecal calprotectin (FC) concentrations correlate with endoscopic inflammation evidence. This study investigated the effect of vedolizumab induction on FC concentrations and whether FC concentrations could be a reliable surrogate measure of disease status.

Methods: Data from the placebo-controlled, phase 3 trial GEMINI 1 were used to evaluate week-6 relationships between outcomes (including clinical remission, mucosal healing [MH], and endoscopic remission) and both absolute FC concentration values and relative FC concentration changes from baseline (%FC₀₋₆). Sensitivity and specificity were calculated by cross-tabulation; the value of week-6 FC concentration as surrogate biomarker was measured with Youden J statistic computed for various cut points.

Results: GEMINI 1 induction phase enrolled 895 patients. Fecal calprotectin concentration decreases were deeper in patients with clinical remission, MH, and/or endoscopic remission than in patients without. The best week-6 indicator of clinical or endoscopic remission in this data set was absolute FC concentration ≤ 150 $\mu\text{g/g}$. The surrogate biomarker values (based on areas under the curve) for the best-performing cut points (FC₀₋₆ reduction $>90\%$, FC ≤ 150 $\mu\text{g/g}$) were fair (range, 0.70–0.77, total population). More patients met the ≤ 150 $\mu\text{g/g}$ cut point with vedolizumab than with placebo. Baseline FC concentrations were not correlated with clinical outcomes.

Conclusions: Fecal calprotectin concentration reductions were greater with vedolizumab induction than with placebo. Week-6 FC concentrations had only fair surrogate biomarker value for endoscopic status. Our data suggest that, while FC may reflect inflammatory burden, FC concentration after vedolizumab induction may not be a robust biomarker of mucosal inflammation.

Key Words: colitis, ulcerative, calprotectin, vedolizumab

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized by a relapsing and remitting course. Clinical manifestations of active disease include bloody diarrhea, abdominal cramps, urgency, and fatigue.^{1,2} Traditionally, the main goal of therapy in UC has been clinical remission, defined as the absence of symptoms without corticosteroid therapy. Nonetheless, patients in clinical remission may have a significant inflammatory burden, and so endoscopy is an increasingly used measure of disease severity.³ Accordingly, mucosal healing—as assessed endoscopically—has become an additional therapeutic target for treatment of UC because it is associated with a lower risk of treatment escalation, colectomy, and disease relapse.^{4–7} However, endoscopy is an invasive, time-consuming, and costly procedure, and bowel preparation is uncomfortable for the patient.⁸ Therefore, there is a role for reliable biomarkers to improve the detection of disease activity, predict relapse, and monitor treatment response.

Fecal calprotectin (FC) is a cytosolic protein released by activated neutrophils and macrophages from the inflamed intestinal mucosa.⁹ In IBD patients, increases in FC concentrations have been positively correlated with endoscopic and histologic evidence of mucosal inflammation.^{10, 11} Studies conducted in UC patients who were in clinical remission demonstrated that FC is a strong predictor of clinical relapse and correlates with mucosal disease activity.^{12–16} Although these studies suggest that FC could be used as a surrogate biomarker for mucosal inflammation in UC patients, most were small studies conducted in single institutions.

Vedolizumab is a humanized monoclonal antibody that specifically binds to the $\alpha_4\beta_7$ integrin heterodimer and selectively blocks gut leukocyte trafficking.¹⁷ The safety and efficacy of vedolizumab for the treatment of patients with moderately to severely active UC were demonstrated in the GEMINI 1 trial.¹⁸ The initial GEMINI 1 analysis showed a significantly larger decrease in FC concentrations in patients who received vedolizumab than in those who received placebo at week 6 and

over the course of therapy to week 52.¹⁸ Based upon this finding, we had 3 aims: (1) to assess the value of FC concentrations as a surrogate biomarker of endoscopic outcomes at week 6, (2) to assess the value of baseline FC concentration as predictor of week-6 endoscopic outcomes, and (3) to assess the effect of vedolizumab induction on FC concentration at week 6.

METHODS

Study Design

This post hoc analysis was performed on data from the multicenter, phase 3, randomized, placebo-controlled GEMINI 1 trial of vedolizumab in patients with moderately to severely active UC (ClinicalTrials.gov, NCT00783718). Details of the study design were reported by Feagan et al in 2013.¹⁸ Briefly, eligible patients were 18 to 80 years of age, with moderately to severely active UC, defined as a Mayo Clinic score (MCS) of 6 to 12, with endoscopic subscore of ≥ 2 within 7 days before the first dose of study drug, with disease that extended 15 cm or more from the anal verge. Patients enrolled in the induction portion of the study were assigned to 2 cohorts. In cohort 1, they were randomized in a 3:2 ratio to receive intravenous vedolizumab (300 mg) or placebo in weeks 0 and 2 (double-blind vedolizumab and double-blind placebo, respectively; intent-to-treat [ITT] population) (Supplementary Fig. S1). All patients in the open-label cohort 2 received vedolizumab and used the same induction regimen as in the blinded study (open-label vedolizumab). All patients were permitted use of mesalamine, up to 30 mg of prednisone (or the equivalent) per day, or immunosuppressive agents at stable doses.

Assessments

Stool samples (~ 20 g) were collected in clinical study sites at screening (21 days to 1 day before first day of study, baseline) and at week 6 using standardized instructions. Quantification of FC concentration in stool samples was conducted using the CALPRO Calprotectin ELISA Test (ALP) (distributed by Calpro, Oslo, Norway).¹⁹

Disease outcomes were clinical remission, mucosal healing, and endoscopic remission at week 6 as evaluated by the investigators. Endoscopy was performed at baseline and at week 6 with interpretation by the local investigator. Clinical remission was defined as an MCS of ≤ 2 with no subscore > 1 . Mucosal healing was defined as a Mayo Clinic endoscopic subscore of 0 or 1. Endoscopic remission was defined as a Mayo Clinic endoscopic subscore of 0.

Relationship Between FC Concentrations and Disease Outcomes at Week 6

All patients enrolled in the induction portion of GEMINI 1 who completed FC measurements at week 0 were included in the analysis (baseline evaluable population).

To investigate the relationship between baseline FC concentrations and outcomes, baseline FC concentrations from the evaluable population were grouped by quartiles, and clinical outcome rates (eg, clinical remission, mucosal healing, and endoscopic remission) at week 6 were derived for each quartile.

To evaluate the relationship between week 6 relative FC concentration changes from baseline and outcomes, patients in the total population who completed FC measurements at week 6 or at both week 0 and week 6 (week 6 evaluable populations) were stratified by clinical and endoscopic outcomes at week 6, and the FC concentration changes were reported as percentages ($\%FC_{0-6}$).

To determine the effect of vedolizumab treatment on FC and whether baseline FC could predict response to vedolizumab, the analyses that were performed on the overall population were repeated separately for the vedolizumab and placebo groups.

Sensitivity and Specificity Analysis

Logistic regression analyses were performed with clinical remission, mucosal healing, or endoscopic remission at week 6 as dependent variables and week-6 FC concentration or $\%FC_{0-6}$ as independent predictor variables. Specific cut points for week-6 FC concentration and $\%FC_{0-6}$ were then identified based on receiver operating characteristic (ROC) data. Area under the ROC curve (AUROC) was used to estimate predictive value of the different cut points (0.90–1, excellent; 0.80–0.90, good; 0.70–0.80, fair; 0.60–0.70, poor; 0.50–0.60, fail). For each identified cut point, sensitivity and specificity were calculated by cross-tabulation and plotted as a summary of ROC data. Positive predictive values (PPVs) and negative predictive values (NPVs) for each outcome at each cut point were calculated. The Youden J statistic was computed as (sensitivity + specificity) – 100 for each cut point as a measure of predictive value.²⁰ The best cut points were determined by the optimal balance of sensitivity and specificity, as indicated by the maximal Youden J. The larger the J statistic, the better the predictive value of the cut point. All statistical

analyses were performed using Statistical Analysis Software Version (SAS) 9.0.

Ethical Considerations

The GEMINI 1 study was designed and implemented by the GEMINI 1 Steering Committee in collaboration with Millennium Pharmaceuticals, which held and analyzed the data. The original protocol was approved by an investigational review board at each center, and all patients gave written, informed consent. Authors made the decision to submit the manuscript for publication and approved the submitted manuscript.

RESULTS

Patient Baseline Characteristics

There were 895 patients (of 1406 screened) included in the study: 746 were treated with vedolizumab (double-blind vedolizumab [cohort 1], $n = 225$; and open-label vedolizumab [cohort 2], $n = 521$), and 149 received placebo (double-blind placebo [cohort 1]). Of these patients, 857 represented the baseline evaluable population (with FC measurements at week 0). At baseline, median FC concentrations were similar in both (blinded) cohort 1 placebo (1005.5 $\mu\text{g/g}$; interquartile range [IQR]: 333–2943) and vedolizumab groups (1111.9 $\mu\text{g/g}$; IQR: 449–2931) but were numerically lower in the open-label cohort 2 vedolizumab group (782.3 $\mu\text{g/g}$; IQR: 331–1594 $\mu\text{g/g}$), resulting in an average of 867.9 $\mu\text{g/g}$ (IQR: 344–1915) for vedolizumab combined. Disease activity at baseline, as determined by MCS, was similar in all treatment groups (Supplementary Table S1).

Relationship Between FC Concentrations and Disease Outcomes at Week 6

For the analyses performed at week 6, the evaluable populations were 771 patients with FC evaluations at week 6 (for whom absolute FC concentrations could be calculated) and 743 patients with complete FC evaluations at both baseline and week 6, for whom relative reductions from baseline could be calculated. Of these patients, those who achieved clinical remission, mucosal healing, or endoscopic remission at week 6—regardless of treatment group—had larger decreases from baseline in FC concentration than those who had not achieved response (Fig. 1, Supplementary Table S2).

Specific cut points for FC reduction from baseline to week 6, FC_{0-6} ($>50\%$, $>75\%$, $>90\%$), or for absolute FC concentration at week 6 (≤ 50 , ≤ 150 , ≤ 250 , and ≤ 500 $\mu\text{g/g}$) were determined based on ROC data. Summaries of ROC curve data for the total population are shown in Fig. 2, Supplementary Table S3A, and Supplementary Table S3B. For each cut point and disease outcome sensitivity, the specificity, area under the curve (AUC), and Youden J statistic were examined. The AUC

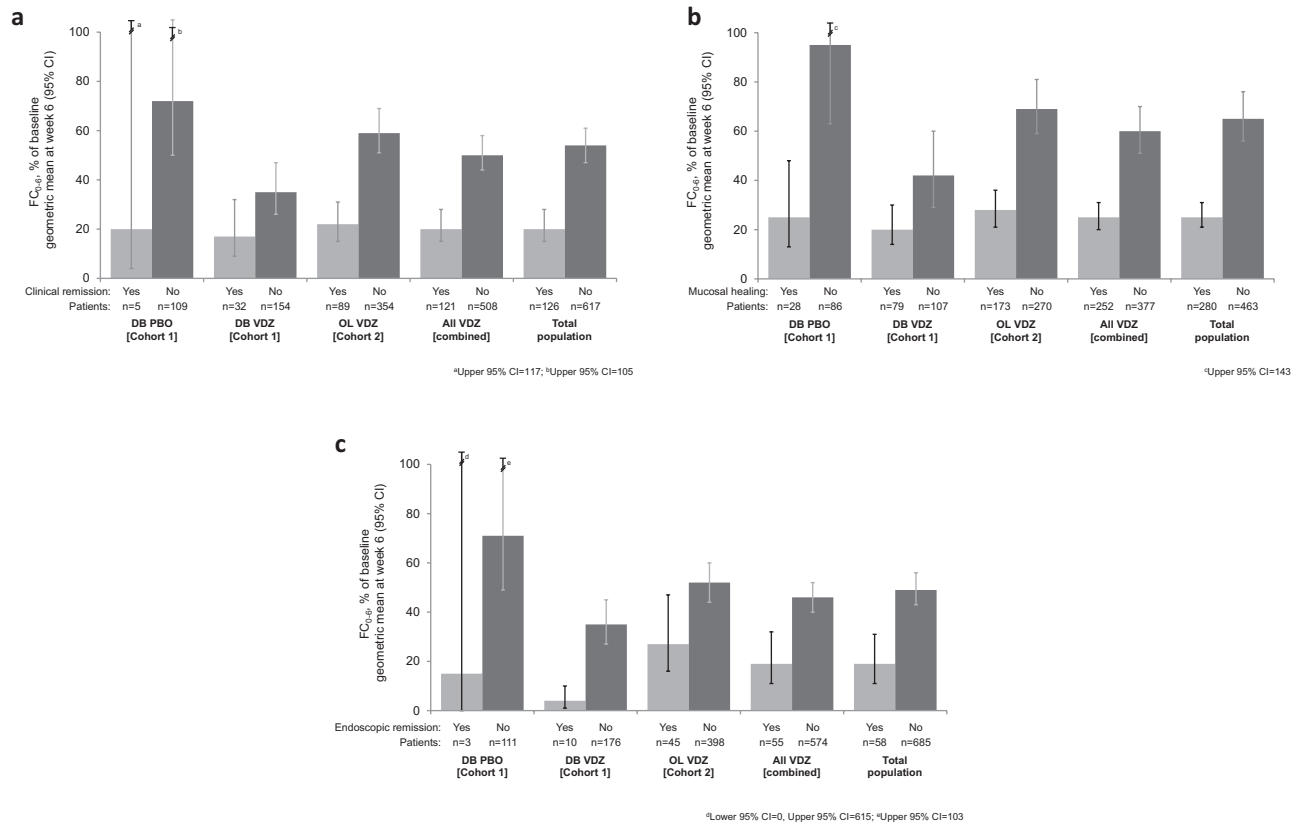


FIGURE 1. Change from baseline in FC concentrations at week 6 (%FC₀₋₆) by clinical outcome status at week 6 and by treatment group: (A) clinical remission, (B) mucosal healing, and (C) endoscopic remission. Error bars represent 95% CIs. ^aUpper 95% CI, 117; ^bUpper 95% CI, 105; ^cUpper 95% CI, 143; ^dLower 95% CI, 0, Upper 95% CI, 615; ^eUpper 95% CI, 103. Abbreviations: CI, confidence interval; DB, double-blind; PBO, placebo; VDZ, vedolizumab.

was fair (range: 0.70–0.77) across all cut points and disease outcomes. In general, the highest sensitivity was associated with the smallest FC₀₋₆ ($\geq 60\%$ at FC₀₋₆ >50%) and the highest FC concentration cutoff at week 6 (>74% for ≤ 500 $\mu\text{g/g}$) across all measures of disease status. Sensitivity declined with increasing change from baseline and with decreasing FC concentration. The opposite trends were observed for specificity: the largest change from baseline (FC₀₋₆ >90%) and the lowest FC concentrations (≤ 50 $\mu\text{g/g}$) at week 6 were associated with the highest specificity for each measure of disease status, with specificity >83% for FC₀₋₆ >90% and >88% for absolute value concentrations ≤ 50 $\mu\text{g/g}$. Limiting the analysis to patients who received vedolizumab yielded similar results. The AUC was fair (AUC range: 0.67–0.75) across cut points and measures of outcomes in patients receiving vedolizumab (Supplementary Table 3B).

Youden J values were generally higher for absolute cut points of FC than for percent reductions. The largest Youden's J for reduction from baseline FC was at FC₀₋₆ >90% (Fig. 2). Overall in this study, the most promising outcome indicators based on Youden's J were absolute FC concentration ≤ 150 $\mu\text{g/g}$ for clinical remission and endoscopic remission, and FC concentration ≤ 500 $\mu\text{g/g}$ for mucosal healing.

These analyses identified multiple values that were informative; however, no robust cut point was identifiable. An FC concentration ≤ 50 $\mu\text{g/g}$ had a PPV of 0.76 for mucosal healing, and an FC concentration >500 $\mu\text{g/g}$ had an NPV of 0.79. The prevalence of these values in the overall population was 12% and 40%, respectively. Similar results were observed when analyses were restricted to vedolizumab-treated patients (Fig. 2 and Supplementary Table 3B).

In the analyses by outcome status and treatment group, there were larger reductions in FC concentrations from baseline among patients who achieved clinical remission, mucosal healing, or endoscopic remission at week 6 than in nonresponders in both treatment groups (Fig. 1). There were similar decreases in FC concentrations from baseline across all 3 outcomes.

Relationship Between Vedolizumab Treatment and FC Concentration

Because a cut point FC concentration of 150 $\mu\text{g/g}$ was identified as the most promising cut point as surrogate biomarker for clinical and endoscopic remission, we compared the proportion of patients achieving this concentration of FC

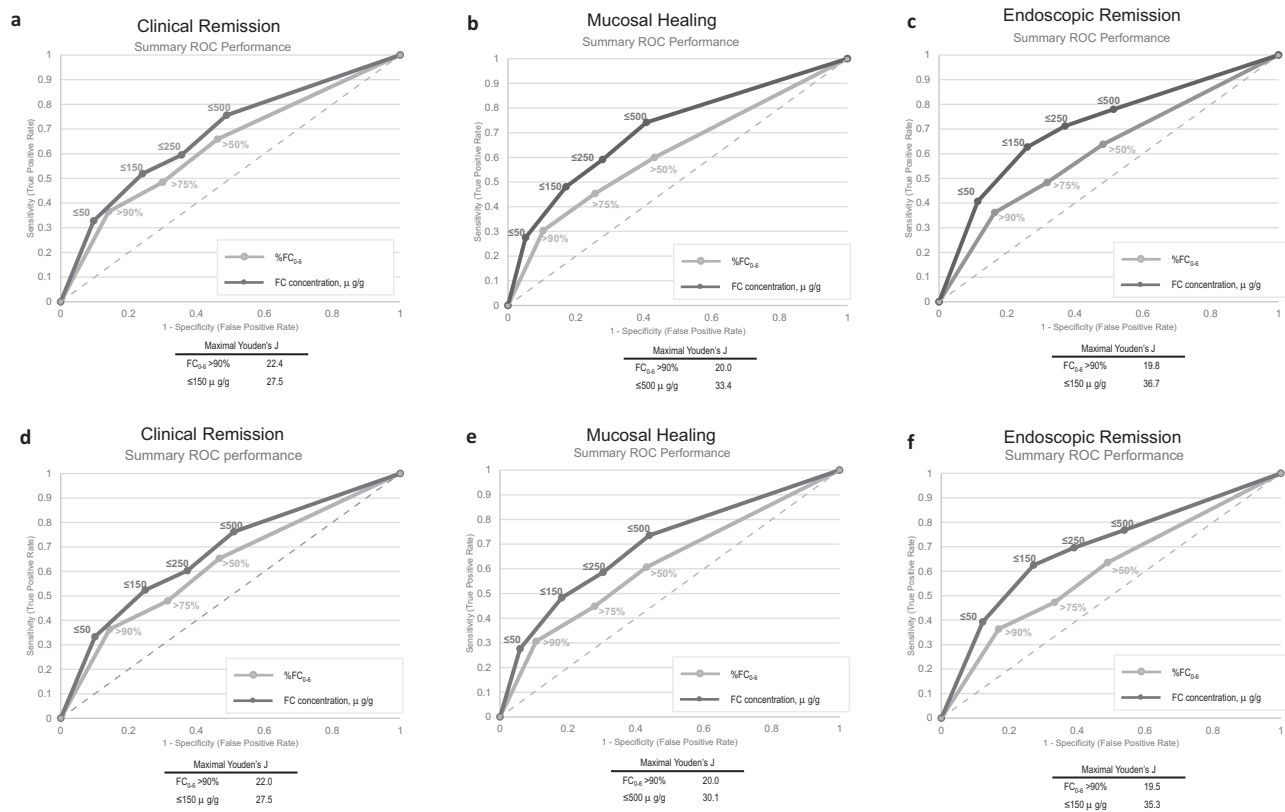


FIGURE 2. Summary of ROC performance at week 6 for clinical remission, mucosal healing, and endoscopic remission: total population (A–C) and vedolizumab-treated population (D–F).

between the treatment groups at week 6. The proportion of patients with $\leq 150 \mu\text{g/g}$ FC at week 6 was higher in the vedolizumab group than in the placebo group (29.3% [95% CI, 23.4–35.3] vs 16.8% [95% CI, 10.8–22.8], respectively).

In general, even among patients who had not achieved clinical remission, mucosal healing, or endoscopic remission at week 6, there was a more pronounced decline in FC concentrations in those receiving vedolizumab than in patients receiving placebo in cohort 1 (Fig. 1A–C).

Relationship Between Baseline Fecal Calprotectin and Disease Status at Week 6

The baseline FC quartiles were calculated to be in the 25th percentile (341 $\mu\text{g/g}$), 50th percentile (898 $\mu\text{g/g}$), and 75th percentile (2126 $\mu\text{g/g}$) in the GEMINI 1 population. As shown in Fig. 3, there were no apparent trends of association between baseline FC quartiles and clinical remission or mucosal healing either in general or among vedolizumab-treated patients. In general, mucosal healing rates were greater in the subgroup of patients with lower baseline FC concentrations (within the 25th percentile, $< 341 \mu\text{g/g}$) than in patients with higher baseline FC concentrations. The potential association with endoscopic remission was not assessed.

DISCUSSION

In this study, we evaluated FC as a biomarker of disease activity and treatment response during induction in a large population of patients with active UC enrolled in a phase 3 study. Fecal calprotectin has generally been considered to be a sensitive, noninvasive method of measuring inflammation in the gastrointestinal (GI) tract.^{13, 14, 21, 22} Although not specific for IBD, FC concentrations can be used as a diagnostic screening assay to rule out UC and Crohn's disease (CD) among patients presenting with GI symptoms (at a threshold $< 50 \mu\text{g/g}$).²¹ In addition, for patients with quiescent UC, it has been reported that FC concentrations $< 150 \mu\text{g/g}$ suggest endoscopic remission, and concentrations $> 150 \mu\text{g/g}$ are associated with an elevated risk of relapse within 2 months.^{12, 23} Whether FC can also be used to monitor treatment response and predict clinical and endoscopic outcomes in induction has not been extensively studied.

In our study, we observed that vedolizumab treatment was associated with a reduction in FC concentrations from baseline, regardless of clinical status at week 6. This finding is consistent with other induction studies that have reported reductions in FC concentrations with vedolizumab or golimumab.^{24, 25} Notably, even patients who did not achieve clinical

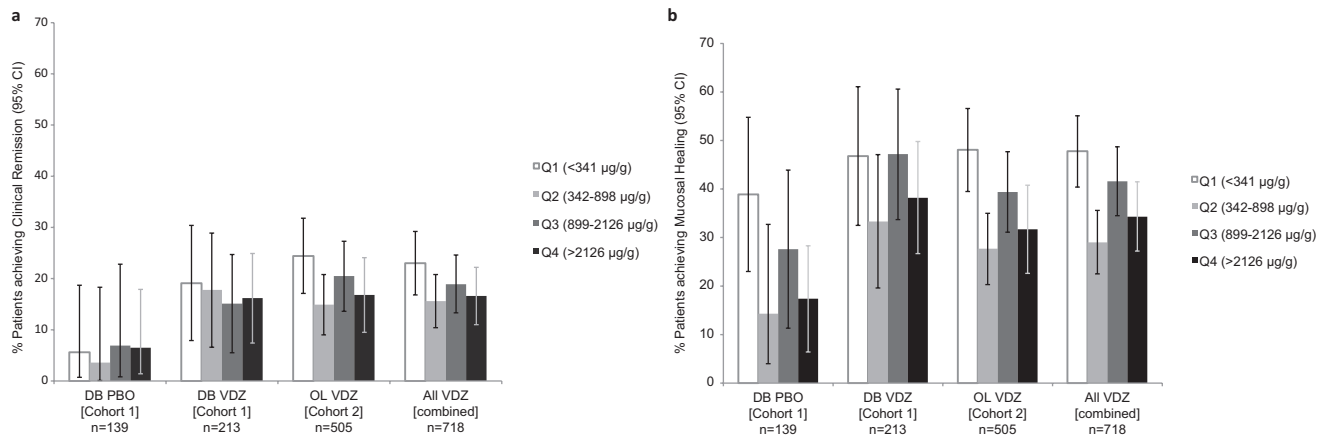


FIGURE 3. Week 6 clinical outcomes by baseline FC concentration quartiles: (A) rates of clinical remission, (B) rates of mucosal healing. Abbreviations: OL, open-label; PBO, placebo; Q, quartile; VDZ, vedolizumab.

response in our study were more likely to have FC <150 µg/g at week 6 if they received vedolizumab than if they received placebo. Indeed in GEMINI 1, the vedolizumab treatment group had both a higher proportion of patients below the threshold FC concentration of 150 µg/g and a larger mean change in FC concentration during the induction period compared with the placebo group. The decrease in FC in vedolizumab treatment groups likely reflects an active anti-inflammatory effect regardless of clinical response, and thus vedolizumab may advance the transition from relapsing to remitting states. The apparent lack of coincidence of FC reductions and endoscopic changes may reflect the different time course that these events follow, resulting in FC reductions in apparent nonresponders. According to this interpretation, FC reductions could be observed first at a given time point in nonresponders, only to be precursors to a potential future response.²⁶

Fecal calprotectin concentration at baseline or week 6 was not strongly associated with clinical and endoscopic outcomes, and the sensitivity and specificity of FC for endoscopically defined mucosal healing was suboptimal. Based on our results, we conclude that, during induction therapy, week-6 FC concentration measurements are not clinically useful indicators of week-6 outcomes such as clinical remission, endoscopic remission, and mucosal healing.

Other studies have also evaluated the relationship between FC and UC disease status during induction therapy, but the studies have varied designs, and results are somewhat conflicting. A recent meta-analysis of 16 trials in UC patients calculated a combined sensitivity and specificity for FC of 88% and 79%, respectively, for the diagnosis of endoscopically active UC in symptomatic patients.²⁷ The authors of the meta-analysis noted that cut points and initial disease status of patients varied from study to study. They concluded that FC could be a useful biomarker but may be specific to clinical context, which is in line with our failure to find a single cut point for FC concentration that could indicate disease state.

We attempted to determine cut points to use FC as a biomarker for clinical remission, mucosal healing status, or endoscopic remission but were unable to locate any that were of great clinical utility. For instance, in our analysis, a 90% reduction in FC concentration had 89% specificity for mucosal healing, but only a few patients (15%) achieved such a substantial percent reduction in FC concentration in GEMINI 1. Cut points for absolute FC concentrations were similarly inappropriate for real-world practice. For example, FC concentrations ≤50 µg/g reliably correlated with the presence of mucosal healing, but this value was observed in only 14% of the patients evaluated. Even for the most promising cut point identified by this study (150 µg/g), the PPV and NPV for mucosal healing in vedolizumab-treated patients were 63% and 72%, respectively, which would leave approximately one third of patients misclassified. Therefore, the results of our analysis did not show that FC concentration would have a high clinical utility potential during induction therapy, which is in line with the interpretation of the meta-analysis previously described.

A few small (n range: 20–53), open-label studies have investigated the biomarker value of FC concentrations for clinical outcomes.^{28–31} In one study with 53 patients treated with 5 mg/kg of infliximab, both the Mayo Clinic score (= 0) and FC (<50 mg/kg) correlated well with endoscopic remission at week 10. An AUC of ROC analyses gave 0.94 for Mayo score and 0.91 for FC.²⁸ However, although patients who achieved endoscopic remission at week 10 showed a significant decrease in FC between baseline and week 2 ($P < 0.001$) compared with patients who did not show a remission, FC concentrations at week 2 had little predictive value (specificity: 67%; sensitivity: 54%, AUC: 0.59) for the protocol-defined remission (endoscopic remission and FC normalization to <50 mg/kg or >80% decrease from baseline) at week 10.²⁸ These results may support our contention that FC values and endoscopic values may be fluctuating at different times within the relapsing and remitting cycle of UC.

One pilot study evaluated FC as an early biomarker of clinical remission at 6 weeks in UC patients receiving infliximab therapy. The study yielded sensitivity and specificity of 90% and 64%, respectively, using a cut point of 10,000 $\mu\text{g}/\text{mL}$ for the FC concentration between days 1 and 3 of therapy (AUC during that period).³¹ Establishing a composite score with calprotectin levels, partial Mayo score, and serum infliximab <120 mg/mL increased the specificity to 79%.³¹ This small study suggested that biomarkers, individual or composite, were potentially useful in predicting outcomes 6 weeks after treatment but would need larger scale confirmation.

Thus, although some studies have yielded what may be promising results for using FC as a biomarker, differences in study design, the selection of cut points, and the time point studied make it difficult to gain clarity or generalize conclusions. Based on our results, we conclude that FC concentration measured at week 6 of vedolizumab induction therapy is not a clinically useful indicator of clinical remission, endoscopic remission, or mucosal healing at this visit, since we could not find a cut point that could be used in general clinical practice. Fecal calprotectin concentration at baseline was not a strong predictor of clinical and endoscopic outcomes at week 6.

The lack of a clinically robust cut point to date is just one barrier to the use of absolute values of FC or change scores as biomarkers of endoscopic healing in induction therapy. Although FC concentrations generally correlate with inflammation, there is substantial inpatient variability.³² Moreover, specific cut points are dependent on consistency in detection methods. At present, multiple manufacturers produce testing kits based on enzyme-linked immunosorbent assay (ELISA) with distinct compositions of detection antibodies, ancillary reagents, and testing protocols.¹⁴

It is also possible that FC concentrations reflect subclinical inflammation or other processes (eg, barrier function, cytokine expression) that may not correlate well with endoscopic measures of disease activity. Knowing that UC is a disease that cycles between relapse and remission, rising FC concentrations may reflect the trafficking of inflammatory cells into the colon, setting the stage for relapse before endoscopically active or symptomatic disease.^{33–35} Our finding that mean FC concentrations decreased in patients receiving vedolizumab treatment, regardless of response, suggests that the $\alpha_4\beta_7$ integrin inhibitor may block the trafficking of memory T cells to the gut, reducing the subsequent inflammation.²⁶

Our study has several strengths including a large sample size, prospective collection of outcome, and the randomized design of GEMINI 1. However, a limitation was the use of the site investigators to assess endoscopic disease status. Although endoscopic assessment of mucosal healing is subjective, use of centralized, blinded readers can minimize bias.³⁶ Baseline assessment of disease severity is often overestimated by local readers in comparison to assessment by central readers.³⁶ Conversely, site readers systematically down code endoscopic scoring in comparison with central

readers following induction therapy.³⁷ Such biases may have contributed to measurement variances and obscured relationships between FC-defined and endoscopic outcomes.

CONCLUSIONS

Although vedolizumab induction was associated with larger reductions in FC concentrations compared with placebo, week-6 FC concentrations had only fair value as indicators of endoscopic status. Our data suggest that, although FC may reflect inflammatory burden, FC concentration measured shortly after vedolizumab induction may not be a clinically useful biomarker of mucosal inflammation or endoscopic outcomes. Additional research could help evaluate whether the surrogate biomarker value of serial FC measurements during the first 12 to 24 weeks of therapy with vedolizumab may help reduce the need for invasive procedures to monitor mucosal healing. Until such data are available, endoscopy will remain the gold standard for assessing mucosal healing in patients treated with vedolizumab.

SUPPLEMENTARY DATA

Supplementary data are available at *Inflammatory Bowel Diseases* online.

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