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Research Article

Trajectory of fecal lactoferrin for predicting prognosis in ulcerative colitis

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Abstract

Objectives: To investigate the characteristics and prognostic value of fecal lactoferrin trajectories in ulcerative colitis (UC).

Methods: This study used data from the UNIFI trial (ClinicalTrials.gov, NCT02407236) and included patients who received ustekinumab during induction for trajectory modeling ($n = 637$). Patients who received ustekinumab during maintenance therapy were used for 1-year outcome analyses ($n = 403$). The levels of fecal lactoferrin, fecal calprotectin, and serum C-reactive protein were measured at weeks 0, 2, 4, and 8. The trajectories of these biomarkers were developed using a latent class growth mixed model.

Results: The trajectories of fecal lactoferrin, fecal calprotectin, and serum C-reactive protein were distinct, but all were associated with prior exposure to anti-tumor necrosis factor agents and vedolizumab. Furthermore, the fecal lactoferrin trajectory was the most valuable predictor of endoscopic, clinical, and histological remission. Compared to the high/moderate-rapid decrease trajectory group, the moderate-slow decrease, high-slow decrease, and high-stable groups had adjusted odds ratios (95% confidence interval) of 0.38 (0.18, 0.78; $P = 0.010$), 0.47 (0.23, 0.93; $P = 0.032$), and 0.33 (0.17, 0.63; $P = 0.001$), respectively, of 1-year endoscopic remission. Patients with high/moderate-rapid decrease trajectories also had the highest likelihood of achieving clinical and histological remission. Finally, we developed a patient-stratification scheme based on fecal lactoferrin trajectories and concentrations. Patients with good, moderate, and poor prognoses in the scheme had a distinct probability of achieving 1-year endoscopic remission (52.7%, 30.9%, and 12.8%, respectively).

Conclusions: The trajectory of fecal lactoferrin is a valuable prognostic factor for 1-year remission in UC.

Keywords: ulcerative colitis, fecal lactoferrin, trajectory

Introduction

Ulcerative colitis (UC) is a chronic and disabling inflammatory bowel disease that affects ~0.2%–0.5% of the population in Europe and North America.[1] Moreover, the incidence and prevalence rates of UC are rapidly increasing in newly industrialized countries.[2] UC causes a huge and gradually increasing burden worldwide; however, the patient management strategies are far from optimal.

Predicting prognosis is one of the most important aspects of patient management. Inflammatory biomarkers, particularly fecal calprotectin (FC), fecal lactoferrin (FL), and serum C-reactive protein (CRP), have been shown to provide prognostic information in patients with UC.[3,4] For example, Dulai *et al*. revealed that the concentration of FC at the end of induction therapy could predict 1-year endoscopic and histological remission. FC is also associated with hospitalization and colectomy.[5] Furthermore, FL, which is primarily secreted by neutrophils at the site of intestinal inflammation, could reflect disease severity in patients with UC.[6] Our recent research has suggested that FL concentration could be an early predictor of long-term disease remission, as well as risk of colectomy in UC.[7] Nevertheless, previous studies have focused on a single measurement of these inflammatory biomarkers, overlooking the prognostic value of longitudinal trajectories. Trajectories can capture the evolving patterns of biomarkers over time and hold great promise for predicting the prognosis of various diseases, such as colorectal cancer and hepatocellular carcinoma.[8,9] Therefore, we hypothesize that the trajectory of these inflammatory biomarkers is a critical prognostic factor in UC.

This study aimed to (1) demonstrate the trajectories of FL, FC, and CRP during induction therapy, (2) investigate the prognostic value of biomarker trajectories, and (3) ascertain whether these trajectories provide additional predictive value beyond a single biomarker measurement. We anticipate that the results of this study will assist clinicians in improving the prognosis of patients with UC.

Methods

Study population

This study used data from the UNIFI trial (ClinicalTrials.gov, NCT02407236).[10] The UNIFI trial is a randomized, double-blind, placebo-controlled, phase-three trial that recruited adult patients with moderate-to-severe UC and aimed to assess the efficacy of ustekinumab.[10] Other information regarding the UNIFI trial can be found in the original article published by Sands *et al*.[10] Our study recruited patients who received ustekinumab during the induction phase of the UNIFI trial. Patients with fewer than two measurements of FL, FC, and CRP were excluded from this study. Eligible patients were included to develop trajectory models

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and assess the associations between trajectories and week-8 outcomes. Patients who entered the maintenance phase and received ustekinumab were utilized to assess the prognostic value of the biomarker trajectories for 1-year outcomes. Patients who did not undergo efficacy assessment at the end of the maintenance phase were excluded. Supplementary Fig. 1, see online supplementary material, presents a patient flow diagram.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines. The data for this study were obtained from the Yale University Open Data Access Project (No. 2022–5104), which is in agreement with JANSSEN RESEARCH & DEVELOPMENT, L.L.C. Additional ethical approval and informed consent were not required for this study because the data were collected previously and presented anonymously.

Inflammatory biomarkers

Inflammatory biomarkers, including FL, FC, and CRP, were detected at weeks 0, 2, 4, and 8, if available. The lowest detectable levels of FL, FC, and CRP were 0.82 μg/ml, 30 μg/g, and 0.2 mg/l, respectively. If the concentrations of these biomarkers were lower than their lowest detectable level, we set the concentration to 0.82 μg/ml for FL, 30 μg/g for FC, and 0.2 mg/l for CRP. Owing to the skewed distribution of inflammatory biomarkers, we performed log-transformation of FL, FC, and CRP for trajectory modeling.

Covariates

In this study, we collected various covariates, including sex, age, disease duration, body mass index (BMI), smoking history, baseline concomitant medications, history of antitumor necrosis factor (TNF) agent exposure, history of vedolizumab exposure, baseline partial Mayo score, endoscopic Mayo score (EMS), and baseline concentrations of FL, FC, and CRP. For patients who failed to provide the exact date of disease diagnosis (e.g. only reported the year or year and month of diagnosis), we imputed the median date of the reported year (July 1) or month (15th day) to calculate the disease duration.

Outcomes

Week-8 and 1-year (at the end of the maintenance phase) outcomes were assessed. The primary outcomes were 1-year endoscopic remission. Endoscopic remission was defined as an EMS of zero. Secondary outcomes included histological and clinical remission at 1 year. Histological remission was defined as a highest Geboes score of < 2.0. Patients with a partial Mayo score ≤ 2 and all subscores ≤ 1 were considered to have clinical remission. Additional secondary outcomes included week-8 endoscopic response. Endoscopic response was defined as a reduction of ≥ 1 point in the EMS score from baseline. Patients who exited the clinical trial early were considered unable to achieve the desired outcome.

Statistical analyses

A latent class growth mixed model (LCGMM) was employed to develop the FL, FC, and CRP trajectories. LCGMM is a validated approach used to analyze longitudinal data and identify subgroups with distinct trajectories. It combines two powerful techniques: latent class analysis and growth mixture modelling. We developed the LCGMM using the *lcmm* package in R software.[11] To determine the optimal trajectory, we performed LCGMM using a linear, quadratic, or cubic polynomial function with different class numbers ranging from 2 to 5.[12] The optimal trajectory was selected based on (1) the lowest Bayesian information criterion, (2) a minimum of 5% of patients in each class, and (3) the posterior probability of assignments being >0.7 in each class.[8,12,13] Ultimately, a quadratic function with three classes, a cubic function with five classes, and a cubic function with three classes fit the optimal trajectories of FL, FC, and CRP, respectively (supplementary Table 1, see online supplementary material).

Table 1. Baseline characteristics.

Variable[a]	N = 637
Male	384 (60.3%)
Age, years	30.00 (30.00, 45.00)
Disease duration, years	6.01 (2.78, 11.02)
BMI, kg/m^2	24.40 (21.21, 27.55)
Smoking history	208 (32.7%)
Concomitant medications	
Corticosteroids	352 (55.3%)
5-Aminosalicylic acid	456 (71.6%)
Immunomodulators	192 (30.1%)
History of anti-TNF exposure	345 (54.2%)
History of vedolizumab exposure	121 (19.0%)
Partial Mayo score	6.00 (5.00, 7.00)
Endoscopic Mayo score	3.00 (2.00, 3.00)
CRP, mg/l	4.69 (1.62, 12.40)
FC, μg/g	1486.00 (603.50, 2904.25)
FL, μg/ml	202.79 (74.12, 443.82)

[a]Continuous and categorical variables are presented as median (IQR) and frequency (%), respectively.

The median [interquartile range (IQR)] and frequency (%) were used to describe continuous and categorical variables, respectively. Kruskal–Wallis and chi-square tests were performed to compare the differences in continuous and categorical variables, respectively, among the different trajectories. The correlation among trjactories of FL, FC, and CRP was calculated using Spearman correlation analysis. Statistical significance was set at P-value < 0.05. Associations between outcomes and trajectories were assessed using a multivariate logistic regression model after adjusting for potential confounders. Model 1 was adjusted for sex and age, while Model 2 was adjusted for variables from Model 1, as well as a history of anti-TNF exposure, history of vedolizumab exposure, baseline concomitant corticosteroid, baseline EMS, and treatment allocation. Subgroup analyses were performed among patients of different sex, age (<40 or ≥40 years old), disease duration (<18 or ≥18 months), and history of TNF exposure to explore whether there was any interaction. Moreover, we performed a receiver operating characteristic (ROC) curve analysis to evaluate the predictive ability of the biomarker trajectories for outcomes. The area under the ROC curve (AUC) and maximum Youden index were calculated.

We conducted a sensitivity analysis to demonstrate the consistency of our results. First, patients who withdrew prematurely from the study were excluded. Second, we further adjusted for disease duration, body mass index, and variables in Model 2.

All the statistical analyses were performed using R version 3.6.3.

Results

Baseline characteristics

A total of 637 patients were eligible for the trajectory model development (supplementary Fig. 1). As shown in Table 1, 384 (60.3%) patients were male, with a median age and disease duration of 30.0 (IQR: 30.0, 45.0) years and 6.01 (IQR: 2.78, 11.02) years, respec-

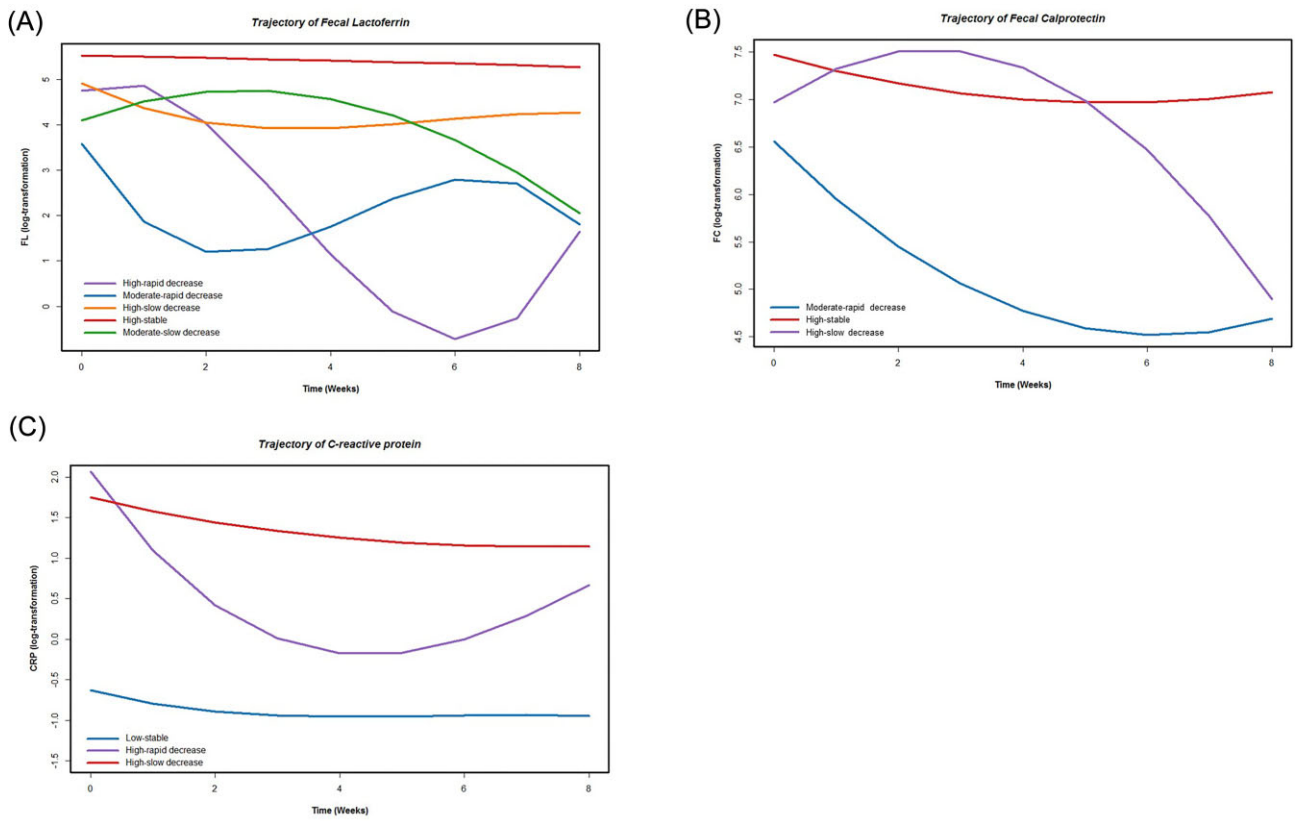

Figure 1. Trajectory of inflammatory biomarkers: (**A**) fecal lactoferrin; (**B**) fecal calprotectin; and (**C**) C-reactive protein.

tively. Regarding biological exposure, 345 (54.2%) patients had a history of exposure to anti-TNF agents, while 121 (19.0%) had exposure to vedolizumab. The median (IQR) baseline partial Mayo score and EMS were 6.00 (5.00, 7.00) and 3.00 (2.00, 3.00), respectively. For 1-year outcome analyses, 405 patients were included (supplementary Table 2, see online supplementary material); 240 (59.3%) were male, 204 (50.4%) had a history of anti-TNF exposure, and 64 (15.8%) had a history of vedolizumab exposure. The other baseline characteristics are described in supplementary Table 2.

Characteristics of inflammatory biomarker trajectories

FL, FC, and CRP levels showed distinct trajectories during the induction therapy (Fig. 1). The biomarker trajectories were named based on their initial concentrations (low, moderate, and high) and variation tendencies (rapid decrease, slow decrease, and stable). The initial concentrations and variation speed of each biomarker trajectory are presented in supplementary Table 3, see online supplementary material.

There were five groups of FL trajectories: moderate-rapid decrease (n = 46), high-rapid decrease (n = 57), moderate-slow decrease (n = 103), high-slow decrease (n = 150), and high-stable (n = 259). In the rapid decrease group (moderate/high-rapid decrease), patients had a lower history of exposure to anti-TNF agents ($P = 0.001$) and vedolizumab ($P < 0.001$) when compared to the slow decrease and high-stable groups (supplementary Table 4, see online supplementary material). For FC, three trajectories were determined: moderate-rapid decrease (n = 124), high-slow decrease (n = 72), and high-stable (n = 435). Patients in the moderate-rapid decrease and high-slow decrease groups were less likely to have anti-TNF ($P < 0.001$) or vedolizumab ($P < 0.001$) exposure than those in the high-stable group (supplementary Table 5, see online supplementary material). For CRP, the three trajectories were labeled as slow-stable (n = 99), high-rapid decrease ($n = 68$), and high-slow decrease ($n = 470$). Patients with a slow-stable trajectory were younger, had a shorter disease duration, and had a lower proportion of anti-TNF and vedolizumab exposure (supplementary Table 6, see online supplementary material). Spearman correlation analyses showed that FL and FC trajectories ($\rho = 0.161$, $P < 0.001$), and FL and CRP trajectories ($\rho < 0.001$, $P = 0.005$) had weak correlations, while no significant correlation was observed between FC and CRP trajectories ($\rho = 0.067$, $P = 0.132$).

Moreover, we assessed the association between biomarker trajectories and endoscopic response at week 8. Moderate-slow decrease, high-slow decrease, and high-stable groups in FL had an adjusted odds ratio (OR) of 0.27 (95% confidence interval (CI): (0.14, 0.50); $P < 0.001$), 0.14 (95%CI: (0.07, 0.25); $P < 0.001$), and 0.12 (95%CI: (0.07, 0.22); $P < 0.001$), respectively, when compared to the rapid decrease group (Table 2). The moderate-rapid decrease group in FC had the largest proportion of patients (79.8%; n = 99) with week-8 endoscopic response, whereas the slow decrease (OR (95%CI): 0.27 (0.14, 0.53); $P < 0.001$) and high-stable groups (OR (95%CI): 0.12 (0.07, 0.20); $P < 0.001$) were less likely to achieve endoscopic response. According to the CRP trajectory, the high-slow decrease group (OR (95%CI): 0.53 (0.33, 0.86); $P = 0.010$), rather than the high-rapid decrease group (OR (95%CI): 1.16 (0.59, 2.29); $P = 0.673$), was significantly associated with a lower likelihood of an endoscopic response than the low-stability group (Table 2). Additionally, we found that combining FC and FL trajectories, as well as CRP concentration, had the highest AUC (0.7594

Table 2. The association between biomarker trajectories and week-8 endoscopic response.

		Univariate analysis		Model 1[a]		Model 2[b]	
	n/N	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Trajectory of FL							
Moderate/high-rapid decrease	82/103	Reference	–	Reference	–	Reference	–
Moderate-slow decrease	57/103	0.32 (0.17, 0.58)	<0.001	0.32 (0.17, 0.58)	<0.001	0.27 (0.14, 0.50)	<0.001
High-slow decrease	60/149	0.17 (0.09, 0.30)	<0.001	0.18 (0.10, 0.31)	<0.001	0.14 (0.07, 0.25)	<0.001
High-stable	106/259	0.18 (0.10, 0.30)	<0.001	0.18 (0.10, 0.30)	<0.001	0.12 (0.07, 0.22)	<0.001
Trajectory of FC							
Moderate-rapid decrease	99/124	Reference	–	Reference	–	Reference	–
High-slow decrease	40/72	0.32 (0.17, 0.60)	<0.001	0.31 (0.16, 0.59)	<0.001	0.27 (0.14, 0.53)	<0.001
High-stable	171/434	0.16 (0.10, 0.26)	<0.001	0.16 (0.10, 0.26)	<0.001	0.12 (0.07, 0.20)	<0.001
Trajectory of CRP							
Low-stable	58/99	Reference	–	Reference	–	Reference	-
High-rapid decrease	45/68	1.38 (0.73, 2.65)	0.322	1.40 (0.74, 2.69)	0.306	1.16 (0.59, 2.29)	0.673
High-slow decrease	212/469	0.58 (0.37, 0.90)	0.016	0.59 (0.38, 0.92)	0.021	0.53 (0.33, 0.86)	0.010

[a]Model 1 was adjusted for sex and age.
[b]Model 2 was adjusted for variables from Model 1 as well as history of anti-TNF exposure, history of vedolizumab exposure, baseline concomitant corticosteroid, baseline EMS and treatment allocation.

(95%CI: (0.7213, 0.7975)) for identifying endoscopic responses (supplementary Table 7, see online supplementary material).

Prognostic value of inflammatory biomarker trajectories

FL trajectory could predict endoscopic, histological, and clinical remission. For endoscopic remission, moderate-slow decrease, high-slow decrease, and high-stable had an OR (95%CI) of 0.38 (0.18, 0.78; $P = 0.010$), 0.47 (0.23, 0.93; $P = 0.032$), and 0.33 (0.17, 0.63; $P = 0.001$), respectively, when compared with the rapid decrease group (Table 3). For histological remission, moderate-slow decrease (OR (95%CI): 0.37 (0.15, 0.87); $P = 0.005$), high-slow decrease (OR (95%CI): 0.22 (0.08, 0.55); $P = 0.001$), and high-stable (OR (95%CI): 0.25 (0.11, 0.55); $P = 0.001$) were all less likely to achieve the 1-year outcome (Table 3). Furthermore, the rapid decrease trajectory group had the highest likelihood for 1-year clinical remission, followed by the moderate-slow decrease (OR (95%CI): 0.40 (0.16, 0.96); $P = 0.046$), high-slow decrease (OR (95%CI): 0.35 (0.14, 0.82); $P = 0.020$), and high-stable (OR (95%CI): 0.23 (0.10, 0.50); $P < 0.001$) trajectory groups (Table 3). The subgroup analyses showed no significant interaction between FL trajectories and sex, age, disease duration, or anti-TNF exposure (supplementary Tables 8–11, see online supplementary material). Furthermore, we performed sensitivity analyses and found consistent results (supplementary Tables 12 and 13, see online supplementary material). However, FC and CRP trajectories were not associated with endoscopic or clinical remission (Table 4). We further assessed the predictive ability of the biomarkers and biomarker trajectories for all 1-year outcomes. The results showed that the FL trajectory had the largest AUCs for predicting endoscopic (0.7054, 95%CI: (0.6516, 0.7592)), histological (0.7442, 95%CI: (0.678, 0.8103)), and clinical (0.7188, 95%CI: (0.6661,0.7715)) remission among all the biomarkers and biomarker trajectories (supplementary Tables 14–16, see online supplementary material).

Patient stratification based on FL and its trajectory

We further explored whether FL trajectories could add value to a single FL measurement (at week eight) for stratifying patients with distinct prognoses. First, we assessed the prognostic value of week-8 FL in each FL trajectory group. We performed ROC analysis to determine the optimal cut-off value for FL and assessed its association with 1-year endoscopic remission. The results showed that FL < 7.05 μg/ml in the moderate-slow decrease trajectory (OR (95%CI): 8.13 (1.76, 50.62); $P = 0.013$) was more likely to achieve endoscopic remission, while FL < 435.40 μg/ml in the high-stable group (OR (95%CI): 3.37 (1.11, 12.89); $P = 0.047$) indicated better outcome (supplementary Table 17, see online supplementary material). Second, we developed a scheme for patient stratification based on FL trajectory and week-8 FL level (Fig. 2). Patients were divided into three groups: good (rapid decrease trajectory or moderate-slow decrease trajectory plus week-8 FL < 7.05 μg/ml), moderate (high-slow decrease trajectory or high-stable trajectory plus week-8 FL < 435.40 μg/ml), and poor (moderate-slow decrease trajectory plus FL ≥ 7.05 μg/ml or high-stable trajectory plus week-8 FL ≥ 435.40 μg/ml) prognosis. Patients with good, moderate, and poor prognoses had distinct probabilities of achieving 1-year endoscopic (52.7%, 30.9%, and 12.8%, respectively) and clinical (73.1%, 62.0%, and 57.9%, respectively) remission (Fig. 2; supplementary Table 18, see online supplementary material).

Discussion

We performed a *post hoc* analysis of the UNIFI trial to demonstrate the trajectory of the three most widely used biomarkers and assessed their predictive ability for 1-year outcomes. We discovered that FC, FL, and CRP levels had distinct trajectories during induction therapy with ustekinumab, and their trajectory characteristics were associated with a history of exposure to anti-TNF agents and vedolizumab. Among these biomarker trajectories, the FL trajectory was the most valuable for predicting clinical, endoscopic, and histological remission. Furthermore, a simple approach based on the FL trajectory and FL concentration could stratify patients with different prognoses. These findings highlight the importance of trajectories in predicting prognosis and provide new insights for clinicians to utilize biomarker trajectories in patient stratification and management.

To the best of our knowledge, this is the first study to investigate inflammatory biomarker trajectories in UC. Distinct trajectories were observed in FL, FC, and CRP; however, they had some

Table 3. The prognostic value of inflammatory biomarker trajectories for 1-year remission.

		Univariate analysis		Model 1[a]		Model 2[b]	
	n/N	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Endoscopic remission							
Trajectory of FL							
Moderate/high-rapid decrease	36/65	Reference	–	Reference	–	Reference	–
Moderate-slow decrease	22/72	0.35 (0.17, 0.71)	0.004	0.36 (0.18, 0.73)	0.005	0.38 (0.18, 0.78)	0.010
High-slow decrease	31/91	0.42 (0.21, 0.80)	0.009	0.46 (0.23, 0.88)	0.020	0.47 (0.23, 0.93)	0.032
High-stable	40/163	0.26 (0.14, 0.48)	<0.001	0.26 (0.14, 0.47)	<0.001	0.33 (0.17, 0.63)	0.001
Trajectory of FC							
Moderate-rapid decrease	32/73	Reference	–	Reference	–	Reference	-
High-slow decrease	18/48	0.77 (0.36, 1.61)	0.489	0.77 (0.36, 1.62)	0.488	0.87 (0.39, 1.92)	0.728
High-stable	82/278	0.54 (0.32, 0.91)	0.021	0.54 (0.32, 0.92)	0.022	0.68 (0.38, 1.22)	0.189
Trajectory of CRP							
Low-stable	25/59	Reference	–	Reference	–	Reference	–
High-rapid decrease	23/53	1.04 (0.49, 2.21)	0.913	1.13 (0.53, 2.42)	0.751	1.28 (0.58, 2.87)	0.541
High-slow decrease	84/291	0.55 (0.31, 0.99)	0.043	0.59 (0.33, 1.07)	0.077	0.71 (0.38, 1.35)	0.289
Histological remission							
Trajectory of FL							
Moderate/high-rapid decrease	24/63	Reference	–	Reference	–	Reference	–
Moderate-slow decrease	12/67	0.35 (0.15, 0.78)	0.012	0.35 (0.15, 0.79)	0.012	0.37 (0.15, 0.87)	0.025
High-slow decrease	9/83	0.20 (0.08, 0.45)	<0.001	0.22 (0.09, 0.50)	0.001	0.22 (0.08, 0.55)	0.001
High-stable	18/153	0.22 (0.11, 0.44)	<0.001	0.21 (0.10, 0.43)	<0.001	0.25 (0.11, 0.55)	0.001
Trajectory of FC							
Moderate-rapid decrease	24/69	Reference	–	Reference	–	Reference	–
High-slow decrease	8/45	0.41 (0.15, 0.98)	0.052	0.38 (0.14, 0.93)	0.041	0.38 (0.13, 0.98)	0.053
High-stable	32/260	0.26 (0.14, 0.49)	<0.001	0.26 (0.14, 0.49)	<0.001	0.29 (0.14, 0.57)	<0.001
Trajectory of CRP							
Low-stable	17/57	Reference	–	Reference	–	Reference	–
High-rapid decrease	6/50	0.32 (0.11, 0.86)	0.030	0.34 (0.11, 0.91)	0.039	0.32 (0.10, 0.91)	0.039
High-slow decrease	41/270	0.42 (0.22, 0.83)	0.010	0.45 (0.23, 0.88)	0.018	0.48 (0.24, 1.02)	0.050
Clinical remission							
Trajectory of FL							
Moderate/high-rapid decrease	57/66	Reference	–	Reference	–	Reference	–
Moderate-slow decrease	50/72	0.36 (0.14, 0.83)	0.020	0.35 (0.14, 0.82)	0.019	0.40 (0.16, 0.96)	0.046
High-slow decrease	62/91	0.34 (0.14, 0.75)	0.010	0.34 (0.14, 0.75)	0.011	0.35 (0.14, 0.82)	0.020
High-stable	90/162	0.20 (0.09, 0.41)	<0.001	0.20 (0.09, 0.41)	<0.001	0.23 (0.10, 0.50)	<0.001
Trajectory of FC							
Moderate-rapid decrease	55/74	Reference	–	Reference	–	Reference	-
High-slow decrease	32/48	0.69 (0.31, 1.54)	0.362	0.68 (0.30, 1.51)	0.334	0.84 (0.36, 1.97)	0.687
High-stable	180/277	0.64 (0.35, 1.12)	0.131	0.64 (0.35, 1.13)	0.133	0.80 (0.42, 1.48)	0.481
Trajectory of CRP							
Low-stable	46/60	Reference	–	Reference	–	Reference	–
High-rapid decrease	37/54	0.66 (0.29, 1.51)	0.330	0.66 (0.28, 1.52)	0.329	0.68 (0.28, 1.65)	0.402
High-slow decrease	185/289	0.54 (0.28, 1.01)	0.062	0.54 (0.27, 1.01)	0.062	0.62 (0.30, 1.22)	0.177

[a]Model 1 was adjusted for sex and age.
[b]Model 2 was adjusted for variables from Model 1 as well as history of anti-TNF exposure, history of vedolizumab exposure, baseline concomitant corticosteroid, baseline EMS and treatment allocation.

common features. For instance, trajectories with rapidly decreasing patterns were more frequently observed in biologic-naïve patients, whereas slow-decreasing or highly stable patterns were correlated with a history of exposure to anti-TNF agents. Ustekinumab has been shown to have unsatisfactory efficacy in patients already treated with anti-TNF agents, especially in those who do not respond to these agents, compared with anti-TNF-naïve patients.[14,15] The efficacy of ustekinumab is first manifested by the degree and speed of inflammation control, which could be reflected in the trajectories of inflammatory biomarkers. Therefore, it is unsurprising that biomarker trajectories are associated with a history of biological exposure. Moreover, our study showed that the FC and FL trajectories were related to the week-8 endoscopic response. The percentage of patients with endoscopic response in the rapid decrease, slow decrease, and highly stable groups was ~80%, 50%, and 40%, respectively. As FC and FL have been demonstrated to be useful for assessing endoscopic activity in UC,[16] we further assessed the predictive value of biomarkers and biomarker trajectories for identifying endoscopic response. We found that the combination of FC, FL trajectories, and CRP concentration was the most accurate for identifying the endoscopic response, with an AUC of 0.7594. Although more high-quality research is needed to validate this finding, it provides an alternative tool for clinicians to evaluate patient response status and may

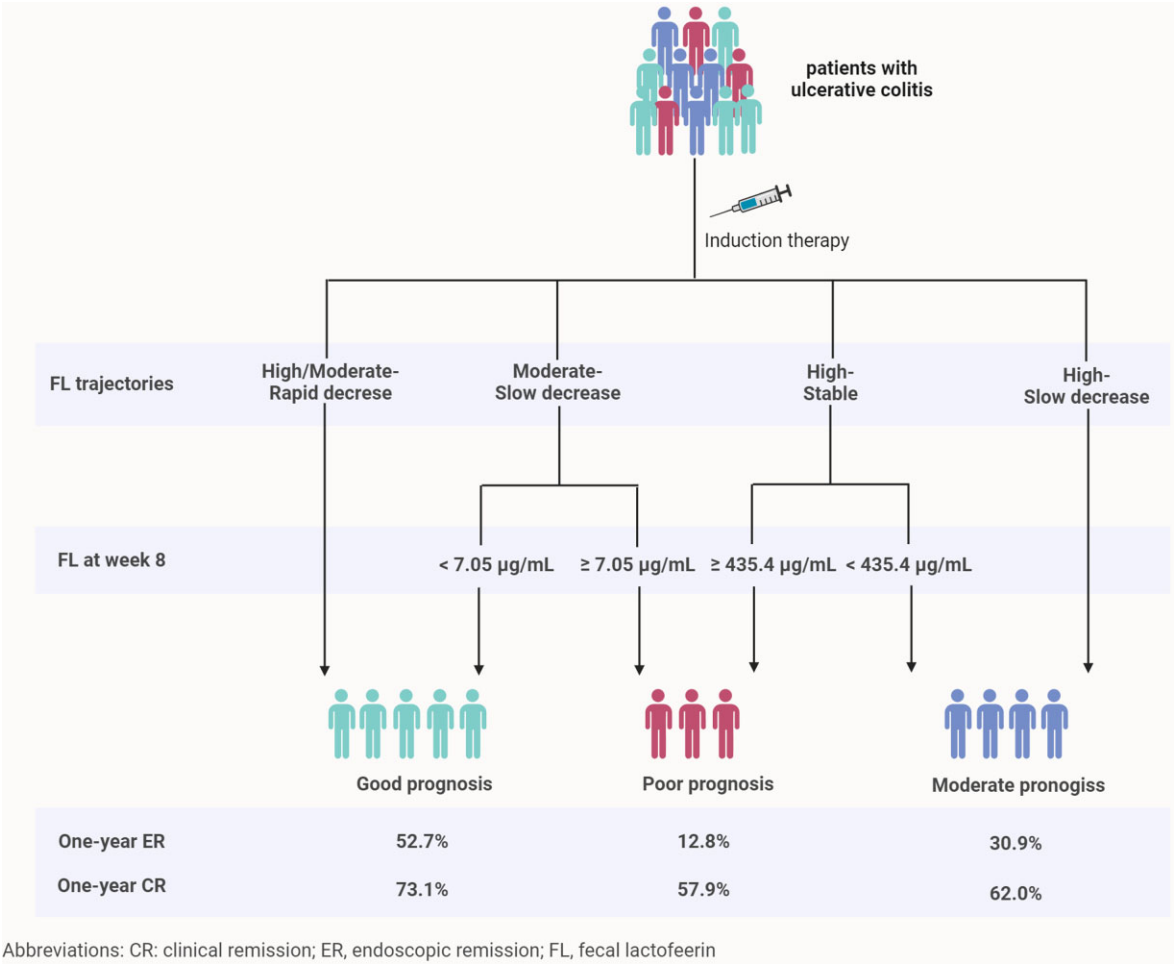

Figure 2. Scheme for patient stratification based on FL trajectory and week-8 FL level.

reduce the need for invasive endoscopic procedures in patients with UC.

Importantly, the FL trajectory could predict 1-year remission. Previous studies have demonstrated that FL is a prognostic factor for UC. Frin *et al.* recruited 31 UC patients who received infliximab and found that the FL level at week 14 could predict sustained response at week 52.[17] Moreover, Gisbert *et al.* conducted a multicenter prospective cohort study and revealed that a positive FL test was a risk factor for relapse in patients with clinical remission.[18] Similar results were also found in other studies.[19,20] Recently, we performed another *post hoc* analysis based on the data from the UNIFI trial and revealed that FL concentration could be an early predictor of long-term disease remission and risk of colectomy in UC.[7] However, it is unclear whether the trajectory of FL can predict therapeutic outcomes, especially endoscopic remission, which has been recommended as the most important long-term therapeutic target in UC.[21] Our study fills the gap in this field. In our study, the rapid decrease trajectory of FL suggested the highest likelihood of 1-year remission, followed by the slow decrease trajectory, whereas the highly stable trajectory indicated the worst therapeutic outcomes. Additionally, we discovered that patients with distinct FL trajectories (e.g. high-rapid decrease and moderate-slow decrease) had different prognoses but similar FL concentrations at week 8. This phenomenon highlights the need to consider the overall trend and process of FL changes rather than relying solely on FL concentration at a single time to predict further therapeutic outcomes accurately.

We further investigated whether combining the FL trajectory and single FL measurements could better predict the prognosis and could be applied to patient stratification. FL at week 8 was shown to differentiate patients with different prognoses in groups with a moderate-slow decrease and high-stable trajectories. Therefore, we developed a scheme for patient stratification based on FL trajectory and concentration. In this scheme, patients identified as having good, moderate, and poor prognosis have likelihoods of 52.7%, 30.9%, and 12.8% of achieving 1-year endoscopic remission. This patient-stratification scheme has several advantages. First, it provides high accuracy and clear stratification. Second, the assessment process is simple and does not require complex mathematical equations or scoring systems. Third, because only one biomarker is measured, it is relatively cost-effective and non-invasive. These features render this scheme promising for use in clinical practice.

Our study has some limitations. First, it included patients with moderate-to-severe UC. Therefore, the findings of our study cannot be applied to patients with mild or severe acute UC. Second, our study could not investigate the association between biomarker trajectories and colectomy because of the lack of long-term follow-up data. However, we assessed the association between biomarker trajectories and endoscopic remission, which

are associated with the risk of colectomy and are recommended as the principal long-term treatment targets in UC.[21] Third, this study only included patients who received ustekinumab, instead of other biologics, such as infliximab, adalimumab, and vedolizumab. Thus, whether the present study findings could be applied to UC patinets using other biologics is still unclear and necessitates more evidence. Finally, we did not perform an external validation of the patient stratification scheme. Therefore, the validity and generalizability of our scheme require further research. Soon, we plan to perform a real-world cohort study to validate the predictive ability of our patient stratification scheme.

In conclusion, this study described the characteristics of biomarker trajectories during induction therapy and found that the FL could predict 1-year remission in patients with UC. Furthermore, a scheme based on FL trajectories and concentrations can better stratify patients. However, further studies are required to validate these findings.

Supplementary data

Supplementary data is available at *PCMEDI*online.

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Author contributions

S.Z. and M.C.: conceptualization; funding acquisition; writing—review and editing; supervision. R.C.: data curation; formal analysis; methodology; writing—original draft preparation; project administration. L.L.: formal analysis; methodology; writing—original draft preparation; project administration. Y.T.: supervision; methodology.

Conflict of interest

All authors declare that there is no conflict of interest.

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