Journal of Inflammation Research Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

Developing and Externally Validating a Simple Index Based on the Nonlinear Relationship of Fecal Calprotectin and Long-Term Outcomes in Ulcerative Colitis

Jieqi Zheng[1,2,*], Danping Zheng[1,*], Zinan Fan[2,*], Li Li[1], Rirong Chen[1], Shenghong Zhang[1,3]

[1]Department of Gastroenterology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, People's Republic of China; [2]Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, People's Republic of China; [3]Guangxi Hospital Division of The First Affiliated Hospital, Sun Yat-Sen University, Nanning, People's Republic of China

*These authors contributed equally to this work

Correspondence: Shenghong Zhang; Rirong Chen, Department of Gastroenterology, The First Affiliated Hospital, Sun Yat-Sen University, 58 Zhongshan Road 2, Guangzhou, People's Republic of China, Tel +86-20-87755766, Fax +86-20-87332916, Email shenghongzhang@163.com; zhshh3@mail.sysu.edu.cn; chenrr29@mail.sysu.edu.cn

Background: The possible nonlinear association with therapeutic outcomes in ulcerative colitis may contribute to the inconclusive cutoff values of fecal calprotectin (FC). We aimed to explore the nonlinear association between FC levels and long-term therapeutic outcomes in patients with ulcerative colitis and establish a clinically applicable FC index.

Methods: We included patients treated with vedolizumab or adalimumab from the VARSITY (n=661) and GEMINI 1 (n=620) studies as discovery and validation cohorts, respectively. The primary outcome was endoscopic remission at week 52 (Mayo Endoscopic Score 0). Restricted cubic splines were used to model nonlinearity between FC and long-term outcomes. Cutoff values were determined using piecewise regression to establish the FC index. Multivariable logistic regression and receiver operating characteristic curve analyses were performed to assess its predictive value.

Results: A nonlinear approximate enantiomorphic "J-shaped" association was observed between post-induction FC levels and long-term outcomes. Cutoff values of 180, 500, and 1300 μg/g were selected to construct the FC index; a higher index was significantly associated with a poorer outcome (P for trend <0.05). Furthermore, the FC index had an area under the receiver operating characteristic curve of 0.7095 [95% CI: 0.6621–0.7569], 0.6856 [95% CI: 0.6427–0.7284], 0.7527 [95% CI: 0.7084–0,7971], and 0.7630 [95% CI: 0.7110–0.8150] in predicting long-term endoscopic remission, clinical remission, histological remission, and disease clearance, respectively, approximately comparable to continuous FC, and superior to dichotomous FC.

Conclusion: The FC index is a promising indicator of therapeutic outcomes and may guide clinicians' therapeutic decisions.

Keywords: fecal calprotectin, ulcerative colitis, nonlinearity, long-term outcome

Introduction

Ulcerative colitis (UC), a major form of inflammatory bowel disease (IBD), is a chronic progressive disease, characterized by alternating periods of remission and activity. It can lead to intestinal dyskinesia, fibrosis, and neoplasia.[1,2]

The development of biologics has provided a superior treatment option for patients with UC.[3] However, their efficacy may decrease over time. A previous study showed that 23–46% of patients with IBD receiving tumor necrosis factor (TNF) antagonists experienced a loss of response to therapy after 12 weeks of treatment,[4] and the cumulative incidences of loss of response to vedolizumab treatment have been reported as 20% and 35% at six and 12 months, respectively.[5] Therefore, exploring predictors of long-term therapeutic outcomes in patients with UC receiving biologics is urgently required, to allow early intervention and therefore improve prognosis.

Received: 24 September 2024
Accepted: 14 December 2024
Published: 19 December 2024

Calprotectin is a calcium- and zinc-binding protein found predominantly in neutrophils. Its presence in feces implies that neutrophils migrate toward and infiltrate the intestinal tract.[6] Fecal calprotectin (FC) has been utilized effectively to monitor disease activity, predict clinical relapse, and track therapeutic responses in patients with UC.[7,8] Although various cutoff values of FC, range from 50μg/g to 500μg/g,[7,9,10] were demonstrated to predict therapeutic outcomes, the optimal cutoff value fails to reach a consensus, which hampers the clinical utilization of FC. The inconclusive cutoff value of FC may be contributed to sampling time, saving procedures, detecting methods and its nonlinear association with therapeutic outcomes. Previous studies have disclosed the nonlinear relationship between long-term outcomes and inflammatory biomarkers, such as neutrophil-to-lymphocyte ratio.[11,12] However, it is still unclear whether the association between long-term therapeutic outcomes and FC are linear or not.

This study investigated the nature of the association between FC levels and long-term therapeutic outcomes in patients with UC treated with biologics. Additionally, we constructed a novel FC index and assessed its ability to predict long-term outcomes.

Methods

Study Design

This is a post-hoc analysis of two Phase III, randomized controlled trials, VARSITY (NCT02497469) and GEMINI 1 (NCT00783718), which recruited adult patients with moderately to severely active UC, defined as a total Mayo score of 6–12 and a Mayo endoscopic score (MES) of ≥2.[13,14] We included patients from the VARSITY trial as a discovery cohort to explore the nonlinear association between FC levels and long-term therapeutic outcomes, and to construct and evaluate a novel simple FC index. Patients from the GEMINI 1 trial were included as the validation cohort to verify the nonlinearity, and to test the predictive power of the FC index.

As the data were previously collected and presented anonymously, local ethics approval and informed consent were unnecessary.

Participants

Details of the design and eligibility criteria for the VARSITY and GEMINI 1 studies have been published previously.[13,14] Patients enrolled in the VARSITY study were randomly assigned to receive either intravenous infusions of vedolizumab or subcutaneous injections of adalimumab. FC levels of patients were examined at weeks 14, 30, and 52, and clinical, endoscopic, and histological activities were assessed at weeks 14 and 52.[13] The GEMINI 1 trial randomly assigned patients to receive intravenous vedolizumab or placebo. FC levels were measured at weeks six, 30, and 52, and evaluations of clinical and endoscopic activities were performed at weeks six and 52.[14] Patients from the two studies who received biologics regularly during both induction and maintenance therapy met the eligibility criteria for this study.

Variables

The FC level at the end of the induction therapy (week 14 in VARSITY and week six in GEMINI 1) was the primary variable of interest. FC levels were analyzed using the new FC index established in this study, dichotomous FC (with cutoff values of 250, 125, and 100 μg/g), and continuous FC (per 100 units).

Data on patient demographic and clinical characteristics, including sex, age, disease duration, smoking history, previous exposure to TNF antagonists, treatment allocation, baseline concomitant medications (corticosteroids, immunomodulators, or 5-aminosalicylic acid), FC level, partial Mayo score (PMS), and MES at baseline were collected for comparison and confounding factor adjustment. Sex, smoking history, previous exposure to TNF antagonists, treatment allocation, and baseline concomitant medications were categorical variables; all other variables were continuous data.

Outcomes

The primary outcome was endoscopic remission (ER) at the end of maintenance therapy (week 52), defined as a MES of 0. In addition, clinical remission (CR, PMS <3 and no subscore >1), endoscopic improvement (EI, MES ≤1), histologic remission (HR, highest Geboes score <2.0), histological improvement (HI, highest Geboes score <3.2), and disease

clearance (DC, MES=0, PMS=0, and highest Geboes score <2.0) at week 52 were secondary outcomes. Specific details about the PMS, MES, and Geboes scores have been published previously.[15,16] Patients who dropped out of the trial prematurely were deemed to have failed to achieve therapeutic outcomes.

Statistical Analyses

Continuous variables are presented as medians and interquartile ranges (IQR), and categorical variables as numbers and percentages. Patients without post-induction FC data and those with missing outcome data were excluded from the corresponding analysis.

In the discovery cohort, restricted cubic splines (RCS) and logistic regression analyses were used to model the nonlinear association between FC levels and long-term outcomes, with adjustment for potential confounders, including age, sex, disease duration, smoking history, previous exposure to TNF antagonists, treatment allocation, and baseline concomitant corticosteroid use, PMS, and MES. The reference value (odds ratio [OR]=1) was set as the median and the four knots were set as the 5th, 35th, 65th, and 95th percentiles of FC concentrations. Piecewise logistic regression was further employed to determine the specific inflection points where the association between FC levels and primary outcomes changes. The analysis was conducted using the R package "segmented", which applies likelihood ratio tests and iterative approximation techniques to identify the best-fitting segmented model and a bootstrap resampling method to determine the confidence intervals (CI) for estimated inflection points. FC levels were then categorized into groups to construct a simple FC index according to the estimated inflection points and median. For ease of use, cutoff values were rounded to the nearest hundred, except for values less than 350 μg/g, which were rounded to the nearest ten as previously reported cutoff values were primarily in this range.[17–20] Univariable and multivariable logistic regression analyses were conducted to investigate the predictive value of the FC index in predicting therapeutic outcomes. Three models were applied for the piecewise and multivariable regression described above to adjust for confounding factors. Model 1 was adjusted for sex and age. Model 2 was further adjusted for disease duration, smoking history, and previous exposure to TNF antagonists. Model 3 was adjusted for the covariates in Model 2, as well as treatment allocation, and baseline concomitant corticosteroid use, PMS, and MES. The unadjusted and adjusted ORs were calculated with 95% CI. Then the FC index, originally an ordinal variable, was treated as a continuous variable to calculate the P values for trend. Receiver operating characteristic (ROC) curves were generated and analyzed to compare the prediction accuracy of the FC index with dichotomous FC (with cutoff values of 250, 125, and 100 μg/g) and continuous FC. The area under the curve (AUC) and 95% CIs were calculated. DeLong's test was used to compare the ROC curves. Exploratory subgroup analyses stratified by age (<40 or ≥40 years), sex, smoking history, previous exposure to TNF antagonists, and biologic agents (vedolizumab or adalimumab) were performed, and the interactions between the FC index and grouping variables were tested.

Sensitivity analyses were performed to verify the consistency of results. We (1) performed additional RCS analyses with three knots (10th, 50th, and 90th percentiles) and five knots (5th, 27.5th, 50th, 75.5th, and 95th percentiles) to test the robustness of the nonlinear association, (2) evaluated the predictive value of the FC index constructed using the raw inflection values without rounding, and (3) redefined DC as the simultaneous achievement of CR, ER, and HR to further examine the nonlinearity between FC levels and DC, and the association between the FC index and DC.

P<0.05 was deemed to indicate statistical significance. All statistical analyses were conducted using R software version 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Study Populations

The flow chart of participant recruitment was shown in Figure S1, and Table 1 presents the baseline patient characteristics. In the VARSITY, 573 patients (294 receiving vedolizumab and 279 receiving adalimumab) had available FC data at week 14 and were included in the analysis. In the GEMINI 1, 523 patients treated with vedolizumab had available FC data at week six and were included for external validation. No significant differences were observed between the two trials in terms of sex, age, disease duration, or clinical disease activity. However, the proportions of patients with

Table 1 Baseline Characteristic

Variables	Discovery Cohort	Validation Cohort	*P* Value
Number of patients	661	620	
Female, n (%)	276 (41.8)	256 (41.3)	0.91
Age, y, median (IQR)	39.0 (29.0, 51.0)	38.5 (29.6, 49.5)	0.64
Disease duration, y, median (IQR)	4.67 (2.00, 8.98)	4.85 (2.24, 9.04)	0.72
Smoking history, n (%)	197 (29.8)	240 (38.7)	0.001
Previous TNF antagonist therapy, n (%)	126 (19.1)	286 (46.1)	<0.001
Concomitant medications			
Corticosteroid, n (%)	243 (36.8)	325 (52.4)	<0.001
Immunomodulator, n (%)	171 (25.9)	213 (34.4)	0.001
5-aminosalicylic acid, n (%)	463 (70.1)	403 (65.0)	0.06
PMS, median (IQR)	6 (5, 7)	6 (5, 7)	0.22
MES, median (IQR)	3 (2, 3)	3 (2, 3)	0.03
FC, μg/g, median (IQR)	1441 (617, 3176)	859 (364, 1727)	<0.001

Abbreviations: FC, fecal calprotectin; IQR, interquartile range; MES: Mayo endoscopic score; PMS, partial Mayo score; TNF, tumor necrosis factor.

a smoking history (38.7% vs 29.8%, *P*=0.001), previous TNF antagonist exposure (46.1% vs 19.1%, *P*<0.001), and baseline concomitant corticosteroid use (52.4% vs 36.8%, *P*<0.001) were greater in the GEMINI 1 than in the VARSITY. In addition, patients in the VARSITY had significantly higher baseline FC levels than those in the GEMINI 1 (1441 [IQR 617–3176] vs 859 [IQR 364–1727] μg/g, *P*<0.001).

Nonlinearity Between FC Levels and Long-Term Therapeutic Outcomes

At week 52, 160 (26.0%), 281 (45.7%), and 343 (54.5%) patients in the discovery cohort achieved ER, EI, and CR, respectively, and 135 (21.5%), 294 (46.9%), and 57 (8.9%) achieved HR, HI, and DC, respectively. RCS analysis revealed an approximate enantiomorphic "J-shaped" association between post-induction FC levels and long-term therapeutic outcomes, with a *P* value for nonlinear of 0.0037 for DC, and *P* values for nonlinear of <0.001 for other outcomes (Figure 1). Specifically, as the FC level increased, the possibility of patients achieving long-term outcomes decreased rapidly at first, then decreased gently, and finally stabilized. Piecewise regression analysis identified two corresponding inflection points at 176 and 1265 μg/g. After adjusting for potential confounders, the OR (per 100 units) for the occurrence of ER was 0.56 (95% CI: 0.28–1.10, *P*=0.09), 0.87 (95% CI: 0.77–0.98, *P*=0.03), and 1.01 (95% CI: 1.00–1.02, *P*=0.05) in patients with FC levels of <176, 176–1265, and ≥1265 μg/g, respectively (Table S1). Sensitivity analyses revealed consistent nonlinear associations between FC levels and long-term outcomes (Figures S2–S4).

FC Index Prediction of Long-Term Therapeutic Outcomes

Based on the inflection points of 176 and 1265 μg/g, and the median FC level of 486 μg/g, we selected 180, 500, and 1300 μg/g as cutoff values (Figure S5), and divided FC levels into four groups (<180, 180–500, 500–1300, and ≥1300 μg/g), which were assigned scores of 0, 1, 2, and 3, respectively, to form a novel FC index. The numbers of patients in the VARSITY trial with FC indices of 0, 1, 2, and 3 were 199 (34.7%), 92 (16.1%), 118 (20.6%), and 164 (28.6%), respectively; 44.5%, 26.7%, 13.5%, and 10.4% of these patients, respectively, achieved ER at week 52. Logistic regression models adjusted for potential confounders revealed that a higher post-induction FC index was significantly associated with a lower probability of the occurrence of long-term outcomes (*P* for trend <0.001; Figure 2). For ER, patients with a FC index of 1, 2, and 3 had ORs of 0.44 (95% CI: 0.25–0.78, *P*=0.006), 0.22 (95% CI: 0.11–0.40, *P*<0.001), and 0.17 (95% CI: 0.09–0.30, *P*<0.001), respectively, compared with those with a FC index of 0 (Figure 2). Similar trends were observed for the secondary outcomes (Figure 2). Additionally, the ability of the FC index to predict long-term ER was comparable to that of continuous FC (AUC [95% CI]: 0.7095 [0.6621–0.7569] vs 0.7182 [0.6664–0.7700], *P*=0.28), and superior to that of dichotomous FC categorized by 100 μg/g (AUC [95% CI]: 0.6565 [0.6104–0.7027], *P*=0.005), 125 μg/g (AUC [95% CI]: 0.6661 [0.6197–0.7126], *P*=0.01), or 250 μg/g (0.6677 [0.6214–0.7139],

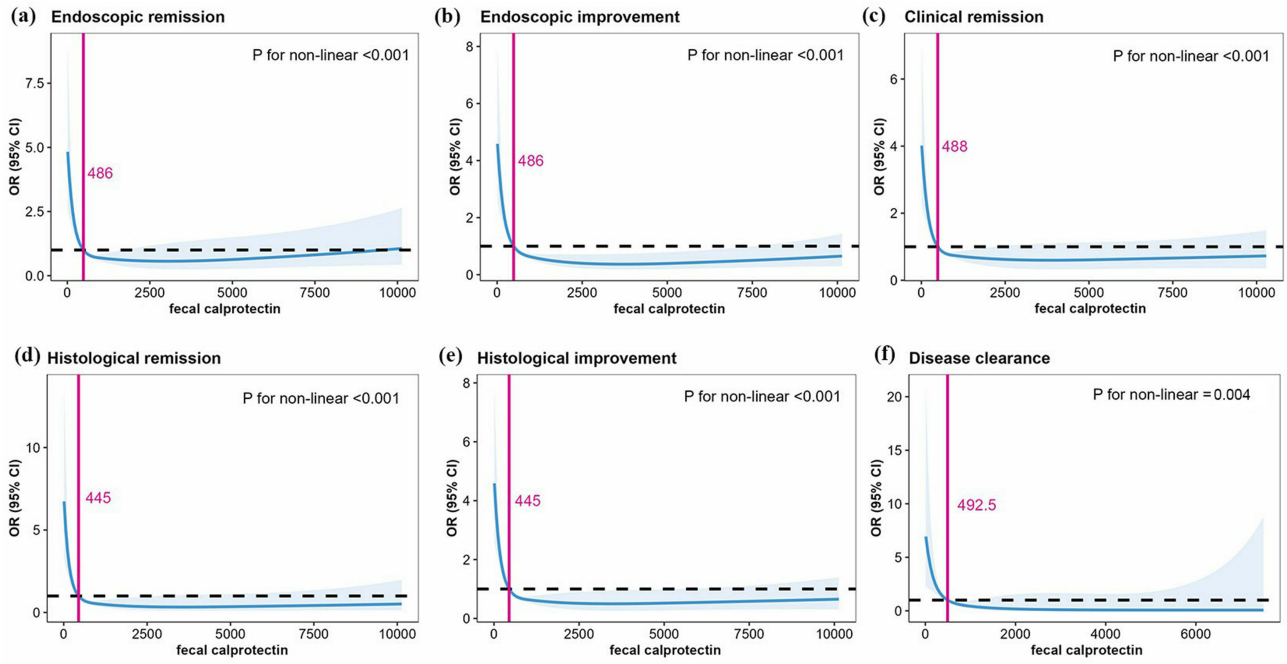

Figure 1 Restricted cubic spline for modelling the nonlinear associations between fecal calprotectin and long-term outcomes in the discovery cohort. (**a–f**) for endoscopic remission, endoscopic improvement, clinical remission, histological remission, histological improvement, and disease clearance, respectively. The part of the curve with fecal calprotectin >7500 $\mu g/g$ (poor convergence) was omitted in (**f**). Spline curves represent odds ratios adjusted for all potential confounders. Blue solid lines are fitted based on logistic regression, with 95% confidence intervals indicated by the shaded areas. The reference point (pink vertical line) is the median of fecal calprotectin levels, with four knots at the 5th, 35th, 65th, and 95th percentiles.

FC index	Events (%)	Unadjusted model	OR [95% CI]	P value	Model 1	OR [95% CI]	P value	Model 2	OR [95% CI]	P value	Model 3	OR [95% CI]	P value
Endoscopic remission													
0	81 (44.51)		Reference			Reference			Reference			Reference	
1	24 (26.67)		0.45 [0.26, 0.78]	0.005		0.46 [0.26, 0.79]	0.006		0.45 [0.25, 0.78]	0.005		0.44 [0.25, 0.78]	0.006
2	15 (13.51)		0.19 [0.10, 0.35]	<0.001		0.20 [0.10, 0.35]	<0.001		0.21 [0.11, 0.38]	<0.001		0.22 [0.11, 0.40]	<0.001
3	16 (10.39)		0.14 [0.08, 0.26]	<0.001		0.14 [0.08, 0.25]	<0.001		0.15 [0.08, 0.26]	<0.001		0.17 [0.09, 0.30]	<0.001
P for trend				<0.001			<0.001			<0.001			<0.001
Endoscopic improvement													
0	129 (70.88)		Reference			Reference			Reference			Reference	
1	43 (47.78)		0.38 [0.22, 0.63]	<0.001		0.37 [0.22, 0.63]	<0.001		0.36 [0.21, 0.61]	<0.001		0.35 [0.20, 0.61]	<0.001
2	39 (35.14)		0.22 [0.13, 0.37]	<0.001		0.22 [0.13, 0.37]	<0.001		0.24 [0.14, 0.39]	<0.001		0.24 [0.14, 0.40]	<0.001
3	33 (21.43)		0.11 [0.07, 0.18]	<0.001		0.11 [0.07, 0.18]	<0.001		0.11 [0.07, 0.18]	<0.001		0.13 [0.08, 0.21]	<0.001
P for trend				<0.001			<0.001			<0.001			<0.001
Clinical remission													
0	142 (76.34)		Reference			Reference			Reference			Reference	
1	50 (54.95)		0.38 [0.22, 0.64]	<0.001		0.38 [0.22, 0.64]	<0.001		0.36 [0.21, 0.61]	<0.001		0.34 [0.20, 0.60]	<0.001
2	55 (47.83)		0.28 [0.17, 0.47]	<0.001		0.28 [0.17, 0.47]	<0.001		0.31 [0.18, 0.51]	<0.001		0.31 [0.18, 0.52]	<0.001
3	55 (35.03)		0.17 [0.10, 0.27]	<0.001		0.17 [0.10, 0.26]	<0.001		0.17 [0.10, 0.27]	<0.001		0.19 [0.12, 0.31]	<0.001
P for trend				<0.001			<0.001			<0.001			<0.001
Histological remission													
0	81 (41.75)		Reference			Reference			Reference			Reference	
1	16 (18.39)		0.31 [0.17, 0.57]	<0.001		0.32 [0.17, 0.58]	<0.001		0.32 [0.17, 0.58]	<0.001		0.28 [0.14, 0.55]	<0.001
2	11 (9.91)		0.15 [0.07, 0.29]	<0.001		0.15 [0.07, 0.29]	<0.001		0.16 [0.07, 0.30]	<0.001		0.13 [0.06, 0.26]	<0.001
3	8 (5.13)		0.08 [0.03, 0.15]	<0.001		0.08 [0.03, 0.15]	<0.001		0.07 [0.03, 0.15]	<0.001		0.09 [0.04, 0.19]	<0.001
P for trend				<0.001			<0.001			<0.001			<0.001
Histological improvement													
0	137 (70.62)					Reference			Reference			Reference	
1	39 (44.83)		0.34 [0.20, 0.57]	<0.001		0.34 [0.20, 0.58]	<0.001		0.33 [0.19, 0.56]	<0.001		0.31 [0.18, 0.54]	<0.001
2	41 (36.94)		0.24 [0.15, 0.40]	<0.001		0.24 [0.15, 0.40]	<0.001		0.26 [0.15, 0.42]	<0.001		0.25 [0.15, 0.42]	<0.001
3	37 (23.72)		0.13 [0.08, 0.21]	<0.001		0.13 [0.08, 0.21]	<0.001		0.13 [0.08, 0.21]	<0.001		0.14 [0.09, 0.23]	<0.001
P for trend				<0.001			<0.001			<0.001			<0.001
Disease clearance													
0	35 (18.42)		Reference			Reference			Reference			Reference	
1	7 (7.78)		0.37 [0.15, 0.83]	0.02		0.39 [0.15, 0.87]	0.03		0.38 [0.15, 0.87]	0.03		0.41 [0.15, 0.99]	0.06
2	4 (3.48)		0.16 [0.05, 0.41]	0.001		0.14 [0.04, 0.38]	<0.001		0.15 [0.04, 0.40]	0.001		0.13 [0.03, 0.36]	<0.001
3	1 (0.61)		0.03 [0.00, 0.13]	<0.001		0.02 [0.00, 0.12]	<0.001		0.02 [0.00, 0.12]	<0.001		0.03 [0.00, 0.16]	0.001
P for trend				<0.001			<0.001			<0.001			<0.001

Figure 2 Associations between FC index with long-term therapeutic outcomes in the discovery cohort. FC index of 0 to 3 represents FC levels of <180, 180–500, 500–1300, and ≥1300 μg/g, respectively. Model 1 was adjusted for sex and age. Model 2 was adjusted for covariates in Model 1 plus disease duration, smoking history, and previous tumor necrosis factor antagonist exposure. Model 3 was adjusted for covariates in Model 2 plus treatment allocations, baseline corticosteroid uses, baseline partial Mayo score, and baseline Mayo endoscopy score.

Abbreviations: CI, confidence interval; FC, fecal calprotectin; OR, odds ratio.

P=0.001) (Table 2). Similarly, there was no statistically significant difference between FC index and continuous FC in predicting long-term CR, although the performance of the FC index was slightly inferior to that of continuous FC for EI, HR, HI, and DC. The superior predictive capability of the FC index over dichotomous FC was maintained for secondary outcomes (Table 2).

Table 2 Receiver Operating Characteristic Curve Analysis for Predicting Long-Term Outcomes in the Discovery Cohort

Variables	AUC [95% CI]	*P* value[†]
Endoscopic remission		
FC index	0.7095 [0.6621, 0.7569]	/
Continuous FC	0.7182 [0.6664, 0.77]	0.28
Dichotomous FC		
Cutoff value =250 μg/g	0.6677 [0.6214, 0.7139]	0.001
Cutoff value =125 μg/g	0.6661 [0.6197, 0.7126]	0.01
Cutoff value =100 μg/g	0.6565 [0.6104, 0.7027]	0.005
Endoscopic improvement		
FC index	0.7247 [0.6831, 0.7662]	/
Continuous FC	0.7405 [0.6977, 0.7832]	0.01
Dichotomous FC		
Cutoff value =250 μg/g	0.6886 [0.6496, 0.7276]	0.002
Cutoff value =125 μg/g	0.6599 [0.6226, 0.6971]	<0.001
Cutoff value =100 μg/g	0.6459 [0.6095, 0.6823]	<0.001
Clinical remission		
FC index	0.6856 [0.6427, 0.7284]	/
Continuous FC	0.697 [0.6533, 0.7407]	0.08
Dichotomous FC		
Cutoff value =250 μg/g	0.6495 [0.6109, 0.6881]	0.003
Cutoff value =125 μg/g	0.6402 [0.6051, 0.6752]	0.004
Cutoff value =100 μg/g	0.6321 [0.5983, 0.6659]	0.002
Histological remission		
FC index	0.7527 [0.7084, 0.7971]	/
Continuous FC	0.7735 [0.7267, 0.8203]	0.01
Dichotomous FC		
Cutoff value =250 μg/g	0.7219 [0.6771, 0.7667]	0.008
Cutoff value =125 μg/g	0.7246 [0.6771, 0.772]	0.07
Cutoff value =100 μg/g	0.7095 [0.6611, 0.7578]	0.02
Histological improvement		
FC index	0.7124 [0.6707, 0.754]	/
Continuous FC	0.7256 [0.6826, 0.7687]	0.04
Dichotomous FC		
Cutoff value =250 μg/g	0.6768 [0.6378, 0.7157]	0.002
Cutoff value =125 μg/g	0.667 [0.6302, 0.7039]	0.002
Cutoff value =100 μg/g	0.6533 [0.6175, 0.6891]	<0.001
Disease clearance		
FC index	0.763 [0.711, 0.815]	/
Continuous FC	0.801 [0.7458, 0.8562]	<0.001
Dichotomous FC		
Cutoff value =250 μg/g	0.7146 [0.6519, 0.7772]	0.003
Cutoff value =125 μg/g	0.7365 [0.6691, 0.8038]	0.16
Cutoff value =100 μg/g	0.7425 [0.674, 0.8109]	0.34

Notes: [†]P value <0.05 was considered as statistically significant difference between the receiver operating characteristic curve of FC index and that of another variable.
Abbreviations: AUC, area under the receiver operator characteristic curve; FC, fecal calprotectin; CI, confidence interval.

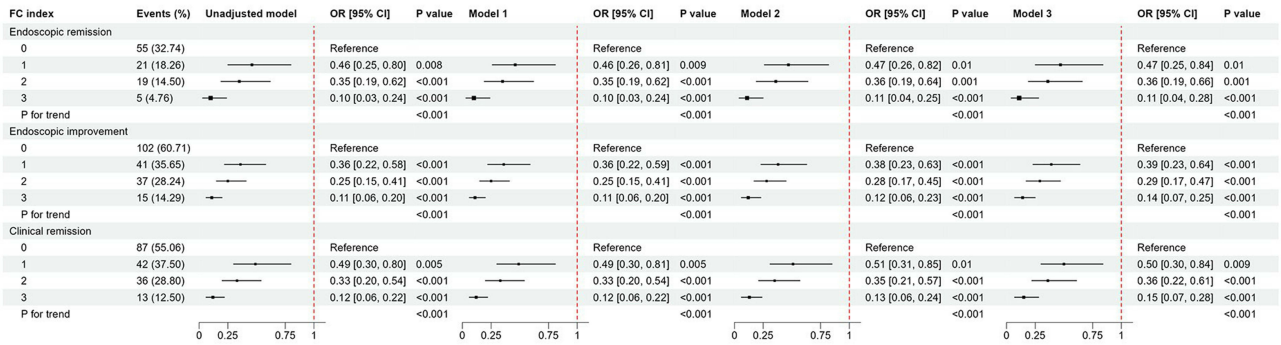

Figure 3 Associations between FC index with long-term therapeutic outcomes in the validation cohort. FC index of 0 to 3 represents FC levels of <180, 180–500, 500–1300, and ≥1300 μg/g, respectively. Model 1 was adjusted for sex and age. Model 2 was adjusted for covariates in Model 1 plus disease duration, smoking history, and previous exposure of tumor necrosis factor antagonist. Model 3 was adjusted for covariates in Model 2 plus treatment allocations, baseline corticosteroid uses, baseline partial Mayo score, and baseline Mayo endoscopy score.
Abbreviations: CI, confidence interval; FC, fecal calprotectin; OR, odds ratio.

No significant interactions between the FC index and grouping variables were observed in subgroup analyses except for smoking history (Tables S2–4) It revealed that the association of the FC index (mainly an FC index of 3) with ER at week 52 was more pronounced in patients with a history of smoking than in those who had never smoked (Table S3). Sensitivity analyses demonstrated that the relationship with long-term outcomes was similarly observed when the FC index was constructed using the unrounded cutoff values, with comparable predictive accuracy for long-term outcomes in this case as well (Tables S5 and S6). Additionally, sensitivity analyses with the redefined DC showed a consistent association between the FC index and long-term DC (Table S7).

External Validation

At the end of maintenance therapy, 109 (19.0%), 212 (36.9%), and 189 (34.3%) patients in the validation cohort achieved ER, EI, and CR, respectively. The enantiomorphic "J-shaped" associations between FC levels at the end of induction therapy and long-term ER, EI, and CR were confirmed in the validation cohort by RCS analysis with four knots, with *P* for nonlinear of 0.001, <0.001, and <0.001, respectively (Figure S6). These findings were repeated following sensitivity analyses with three and five knots (Figure S7). Among patients in the GEMINI 1 study, 169 (32.3%), 115 (22.0%), 132 (25.2%), and 107 (20.5%) had FC indices of 0, 1, 2, and 3, respectively. Patients with an FC index of 1, 2, and 3 were progressively less likely to achieve long-term outcomes than those with an FC index of 0 (*P* for trend <0.001; Figure 3). The FC index had equivalent predictive ability for ER (AUC [95% CI]: 0.6824 [0.6300–0.7347]), EI (AUC [95% CI]: 0.7041 [0.6601–0.7482]) and CR (AUC [95% CI]: 0.6885 [0.6429–0.7340]) to continuous FC, and performed better than dichotomous FC (Table S8), similar to the results from the discovery cohort. Moreover, the FC index performed similarly in predicting long-term therapeutic outcomes across subgroups stratified by age (<40 or ≥40 years), sex, smoking history, previous TNF antagonist exposure, and biologics (Tables S9 and S10).

Discussion

FC, a marker widely used in clinical practice, has attracted considerable attention in UC management and prognosis. Our study revealed a nonlinear association between post-induction FC levels and biological maintenance therapy outcomes in patients with UC. In addition, we demonstrated the advantages of a simple FC index in predicting long-term outcomes, which could facilitate more precise risk stratification of post-induction patients, thus enabling targeted adjustments of treatment regiments. Specifically, when a patient's FC index is 0, indicating a higher chance of long-term ER, continuing current biologic treatment with regular monitoring is preferred. For moderate-to-low chances of ER, treatment escalation options including combining immunomodulators or increasing the dose or frequency of administration, replacing other biologic or small-molecular drugs, or conducting rescue therapy can be selected based on FC index and clinical practice.

To our knowledge, this is the first study to reveal an enantiomorphic "J-shaped" relationship between FC levels and UC treatment outcomes. This relationship suggests that the influence of FC on therapeutic outcomes gradually diminishes

as FC levels increase. Once a certain threshold is surpassed, additional increases in FC levels may have no effect on therapeutic outcomes. These findings not only offer a novel perspective for clinicians regarding the utilization of FC in predicting outcomes in UC, but also remind researchers that investigating nonlinear associations between inflammatory biomarkers and UC outcomes is a crucial preliminary step for future studies. Previous studies have identified nonlinear associations between inflammatory biomarkers and disease development or prognosis in other fields.[11,12,21,22] In UC, inflammatory biomarkers such as C-reactive protein, albumin, and fecal lactoferrin predict long-term outcomes;[23–26] possible nonlinear associations for these biomarkers require further investigation.

Normalization of FC levels has been proposed as an intermediate therapeutic target in UC due to their association with disease activity and prognostic ability.[27] In IBD, FC levels after TNF antagonist induction were showed to predict clinical responses and mucosal healing at one year, with cutoff values of 168 and 121 μg/g, respectively.[19] A recent post-hoc analysis suggested that post-induction FC ≤250 μg/g were associated with CR, ER, and HR at 52 weeks, as well as a reduced probability of future colectomy and hospitalization.[8] In our study, we innovatively applied a more rigorous and rational categorization of FC levels, with a selection of cutoff values based on the nonlinear association between FC levels and long-term outcomes. The predictive power of the FC index was similar to that of continuous FC and significantly exceeded that of dichotomous FC, suggesting that it could reduce information loss associated with dichotomous variables and be clinically applicable. External validation increased our confidence in its predictive ability. Furthermore, published models for long-term outcomes often involved multiple parameters, some of which should be obtained by invasive procedures; yet their predictive power was not that prominent.[28–30] In contrast, the FC index showed significant predictive performance with only one variable. Its non-invasive nature and accessibility emphasize its considerable clinical utility.

Histological healing has been acknowledged as indicating the extent of remission, which is linked to a reduced risk of long-term complications, such as hospitalization, colectomy, and cancer.[27] DC, although a more stringent outcome criterion, remains achievable in UC following the advent of biologics and small molecule drugs, which improve prognosis, and may be attractive in future clinical trials or even clinical practice.[31] Therefore, our study included HR, HI, and DC as secondary outcomes, further illustrating the great predictive potential of the FC index.

Nevertheless, our study has some limitations. First, owing to the post hoc nature of our study, the evidence strength was not as high as in large prospective intervention studies. Second, the small proportion of patients who achieved DC may have contributed to our findings' lack of robustness. Besides, because the validation cohort lacked histological outcomes, external validation was limited to clinical and endoscopic outcomes. However, sensitivity analyses demonstrated consistent associations between the FC index and long-term histological outcomes; thus, we believe that our results are reliable, although further validation is required. Third, while our study focused on patients treated with vedolizumab and adalimumab—two biologic agents with distinct mechanisms of action, namely anti-integrin and anti-TNF therapies, respectively—the broader applicability of our findings to other treatment regimens in UC necessitates additional confirmation. Furthermore, differences in the definition of induction period existed between the discovery and validation cohorts. Although our results suggested that such variations did not affect our affirmation of the predictive value of FC index, further prospective studies are warranted to better define the acceptable post-induction FC measurement time window in real-world clinical settings.

In conclusion, our study demonstrates a nonlinear association between post-induction FC levels and long-term outcomes. Moreover, the FC index established with cutoff values of 180, 500, and 1300 μg/g was indicative of long-term clinical, endoscopic, and histologic outcomes in patients with UC, and may be a promising tool to aid risk stratification, and therefore guide therapeutic decision-making by clinicians.

Abbreviations

AUC, area under the curves; CI, confidence intervals; CR, clinical remission; DC, disease clearance; EI, endoscopic improvement; ER, endoscopic remission; FC, fecal calprotectin; HI, histological improvement; HR, histologic remission; IBD, inflammatory bowel disease; IQR, interquartile ranges; MES, Mayo endoscopic score; OR, odds ratio; PMS, partial Mayo score; RCS, restricted cubic splines; ROC, receiver operating characteristic; TNF, tumor necrosis factor; UC, ulcerative colitis.

Data Sharing Statement

This study used data from the Vivli, Inc., which had an agreement with Takeda. All the data in this study can be obtained from the Vivli, Inc. after agreement.

Ethics Considerations

This post hoc analysis was conducted in accordance with the Declaration of Helsinki. Since the data we used for the analysis were previously collected and anonymized, the Research Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University confirmed that local ethics approval and informed consent were unnecessary.

Acknowledgment

This study is based on the project (Vivli project id #00008266) using data from Takeda which has been made available through Vivli, Inc. Vivli has not contributed to or approved, and is not in any way responsible for, the contents of this publication.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. Jieqi Zheng, Danping Zheng and Zinan Fan are co-first authors and contributed equally to this study.

Funding

This work was supported by the National Natural Science Foundation of China (#82270555, #82070538, #82000520, #82100549) and Guangdong Science and Technology Department (#2021A1515220107, #2020A1515010249). This study was funded by the China Crohn's & Colitis Foundation (CCCF) under Grant No. CCCF-QF-2022B36-7.

Disclosure

All authors declare that there is no conflicts of interest.

References

1. Langholz E, Munkholm P, Davidsen M, Binder V. Course of ulcerative colitis: analysis of changes in disease activity over years. *Gastroenterology*. 1994;107(1):3–11. doi:10.1016/0016-5085(94)90054-x
2. Torres J, Billioud V, Sachar DB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis as a progressive disease: the forgotten evidence. *Inflam Bowel Dis*. 2012;18(7):1356–1363. doi:10.1002/ibd.22839
3. Marcus Harbord RE, Bettenworth D, Karmiris K, et al. Franck carbonnel; for the European crohn's and colitis organisation [ECCO]. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. part 2. *Current Management J Crohns Colitis*. 2023;17(1):149. doi:10.1093/ecco-jcc/jjac104
4. Roda G, Jharap B, Neeraj N, Colombel JF. Loss of response to anti-TNFs: definition, epidemiology, and management. *Clin Transl Gastroenterol*. 2016;7(1):e135. doi:10.1038/ctg.2015.63
5. Shmidt E, Kochhar G, Hartke J, et al. Predictors and management of loss of response to vedolizumab in inflammatory bowel disease. *Inflamm Bowel Dis*. 2018;24(11):2461–2467. doi:10.1093/ibd/izy171
6. Røseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. *Scand J Gastroenterol*. 1999;34(1):50–54. doi:10.1080/00365529950172835
7. Ikhtaire S, Shajib MS, Reinisch W, Khan WI. Fecal calprotectin: its scope and utility in the management of inflammatory bowel disease. *J Gastroenterol*. 2016;51(5):434–446. doi:10.1007/s00535-016-1182-4
8. Dulai PS, Feagan BG, Sands BE, Chen J, Lasch K, Lirio RA. Prognostic value of fecal calprotectin to inform treat-to-target monitoring in ulcerative colitis. *Clin Gastroenterol Hepatol*. 2023;21(2):456–466e7. doi:10.1016/j.cgh.2022.07.027
9. Mosli MH, Zou G, Garg SK, et al. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol*. 2015;110(6):802–819. doi:10.1038/ajg.2015.120
10. Kennedy NA, Warner B, Johnston EL, et al. Relapse after withdrawal from anti-TNF therapy for inflammatory bowel disease: an observational study, plus systematic review and meta-analysis. *Aliment Pharmacol Ther*. 2016;43(8):910–923. doi:10.1111/apt.13547
11. Mu S, Ai L, Fan F, Qin Y, Sun C, Hu Y. Prognostic role of neutrophil-to-lymphocyte ratio in diffuse large B cell lymphoma patients: an updated dose-response meta-analysis. *Cancer Cell Int*. 2018;18:119. doi:10.1186/s12935-018-0609-9

12. Zheng M. Systemic inflammation shapes clinical outcomes in response to immune checkpoint blockade treatment: moving toward optimizing antitumor immunity. *J Immunother Cancer*. 2023;11(3):e006462. doi:10.1136/jitc-2022-006462
13. Sands BE, Peyrin-Biroulet L, Loftus EV Jr, et al. Vedolizumab versus Adalimumab for Moderate-to-Severe Ulcerative Colitis. *N Engl J Med*. 2019;381(13):1215–1226. doi:10.1056/NEJMoa1905725
14. Feagan BG, Rutgeerts P, Sands BE, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2013;369(8):699–710. doi:10.1056/NEJMoa1215734
15. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med*. 1987;317(26):1625–1629. doi:10.1056/nejm198712243172603
16. Jauregui-Amezaga A, Geerits A, Das Y, et al. A simplified geboes score for ulcerative colitis. *J Crohns Colitis*. 2017;11(3):305–313. doi:10.1093/ecco-jcc/jjw154
17. Dulai PS, Battat R, Barsky M, et al. Incorporating fecal calprotectin into clinical practice for patients with moderate-to-severely active ulcerative colitis treated with biologics or small-molecule inhibitors. *Am J Gastroenterol*. 2020;115(6):885–894. doi:10.14309/ajg.0000000000000596
18. D'Amico F, Bonovas S, Danese S, Peyrin-Biroulet L. Review article: faecal calprotectin and histologic remission in ulcerative colitis. *Aliment Pharmacol Ther*. 2020;51(7):689–698. doi:10.1111/apt.15662
19. Guidi L, Marzo M, Andrisani G, et al. Faecal calprotectin assay after induction with anti-tumour necrosis factor α agents in inflammatory bowel disease: prediction of clinical response and mucosal healing at one year. *Dig Liver Dis*. 2014;46(11):974–979. doi:10.1016/j.dld.2014.07.013
20. Theede K, Holck S, Ibsen P, Kallemose T, Nordgaard-Lassen I, Nielsen AM. Fecal calprotectin predicts relapse and histological mucosal healing in ulcerative colitis. *Inflamm Bowel Dis*. 2016;22(5):1042–1048. doi:10.1097/mib.0000000000000736
21. Zhu M, Ma Z, Zhang X, et al. C-reactive protein and cancer risk: a pan-cancer study of prospective cohort and Mendelian randomization analysis. *BMC Med*. 2022;20(1):301. doi:10.1186/s12916-022-02506-x
22. Yang X, Zhao S, Wang S, et al. Systemic inflammation indicators and risk of incident arrhythmias in 478,524 individuals: evidence from the UK biobank cohort. *BMC Med*. 2023;21(1):76. doi:10.1186/s12916-023-02770-5
23. Armuzzi A, Biancone L, Daperno M, et al. Adalimumab in active ulcerative colitis: a "real-life" observational study. *Dig Liver Dis*. 2013;45(9):738–743. doi:10.1016/j.dld.2013.03.018
24. Arias MT, Vande Casteele N, Vermeire S, et al. A panel to predict long-term outcome of infliximab therapy for patients with ulcerative colitis. *Clin Gastroenterol Hepatol*. 2015;13(3):531–538. doi:10.1016/j.cgh.2014.07.055
25. Papamichael K, Rivals-Lerebours O, Billiet T, et al. Long-term outcome of patients with ulcerative colitis and primary non-response to infliximab. *J Crohns Colitis*. 2016;10(9):1015–1023. doi:10.1093/ecco-jcc/jjw067
26. Chen R, Tie Y, Zhang X, Li L, Chen M, Zhang S. Fecal lactoferrin early predicts long-term outcomes in ulcerative colitis: a post-hoc analysis of the UNIFI and PURSUIT trials. *United Eur Gastroenterol J*. 2023;11(6):542–550. doi:10.1002/ueg2.12431
27. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: an update on the selecting therapeutic targets in inflammatory bowel disease (STRIDE) initiative of the international organization for the study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology*. 2021;160(5):1570–1583. doi:10.1053/j.gastro.2020.12.031
28. Vande Casteele N, Jairath V, Jeyarajah J, et al. Development and validation of a clinical decision support tool that incorporates pharmacokinetic data to predict endoscopic healing in patients treated with infliximab. *Clin Gastroenterol Hepatol*. 2021;19(6):1209–1217.e2. doi:10.1016/j.cgh.2020.04.078
29. Dulai PS, Singh S, Vande Casteele N, et al. Development and validation of clinical scoring tool to predict outcomes of treatment with vedolizumab in patients with ulcerative colitis. *Clin Gastroenterol Hepatol*. 2020;18(13):2952–2961.e8. doi:10.1016/j.cgh.2020.02.010
30. Bertani L, Baglietto L, Antonioli L, et al. Assessment of serum cytokines predicts clinical and endoscopic outcomes to vedolizumab in ulcerative colitis patients. *Br J Clin Pharmacol*. 2020;86(7):1296–1305. doi:10.1111/bcp.14235
31. Danese S, Roda G, Peyrin-Biroulet L. Evolving therapeutic goals in ulcerative colitis: towards disease clearance. *Nat Rev Gastroenterol Hepatol*. 2020;17(1):1–2. doi:10.1038/s41575-019-0211-1