

Timely Monitoring of Inflammation by Fecal Lactoferrin Rapidly Predicts Therapeutic Response in Inflammatory Bowel Disease

Dario Sorrentino, MD, FRACP,*† and James M. Gray*

Background: Fecal lactoferrin (FL) levels may mirror drug-induced changes in inflammation in ulcerative colitis and Crohn disease in a timely way and could be used to assess loss of response (LOR) to biologics.

Methods: This study is a retrospective outcome review in 61 patients on adalimumab, infliximab, or vedolizumab managed in our center and followed for 6 to 24 months. Patients were 1) in clinical remission or 2) were experiencing possible LOR.

Results: For group 1, in 71% of 31 patients, FL slowly increased during the therapeutic interval ($R^2 = 0.769$; $P < 0.001$), thus reflecting increasing inflammation as drug concentrations decreased. In the remaining patients, FL was undetectable throughout the therapeutic interval because of a stronger suppression of inflammation. For group 2, in 30 patients negative for infections, FL levels measured 1 to 3 days after infusion/injection compared to preadministration values either increased (nonresponders)—in these patients the medication was switched to another class; partially decreased (partial responders)—the therapeutic interval was shortened; or were normal throughout (responders)—causes for symptoms unrelated to disease activity were found for all. After FL-based management, 3-month standardized clinical scores were normalized in both partial responders (0.58 ± 0.21 vs 0.13 ± 0.09 ; $P < 0.001$) and nonresponders (0.81 ± 0.17 vs 0.12 ± 0.08 ; $P < 0.001$), and FL levels dropped by up to 99%.

Conclusions: Levels of FL reflect drug-induced changes in mucosal inflammation in a timely way, thus enabling rapid assessment of therapeutic response in patients with ulcerative colitis and with Crohn disease. In patients with suspected LOR, FL levels before and after infusion/injection accurately separated responders, partial responders, and nonresponders. The strategy proposed here is simple, accurate, and easily applicable to clinical practice.

Key Words: inflammatory bowel diseases, therapeutic response, fecal lactoferrin

INTRODUCTION

Response to therapy in inflammatory bowel disease (IBD)—both ulcerative colitis (UC) and Crohn's disease (CD)—has classically been monitored by the severity of clinical symptoms¹ and by endoscopy, a more objective but also more expensive and invasive tool. More recently, in patients on biologics, the measurement of drug trough levels (TL) and of antidrug antibodies—a strategy known as therapeutic drug monitoring (TDM)—has been widely used to assess/manage loss of response (LOR) and primary nonresponse (PNR).² Although TDM-based management may improve outcomes in IBD,^{2,3} this strategy is expensive and has practical shortcomings.⁴⁻⁹ Ideally, it is the individual patient disease burden that therapy should target.^{4, 10, 11}

Fecal markers of inflammation, specifically fecal lactoferrin (FL) and fecal calprotectin (FC), seem to be accurate indicators of intestinal mucosa inflammation,¹ but their definitive role in IBD management still needs to be elucidated.¹² In particular, it is unclear whether stool markers can accurately indicate immediate therapy-induced changes in IBD activity, a necessary feature for the management of LOR. Previous studies have shown that FC levels in patients with IBD on biologics increase during the therapeutic interval—thus reflecting in a timely way the increasing inflammatory activity resulting from the progressive neutralization of the medication.¹³ We have since confirmed such findings with FL and leveraged them to routinely evaluate patients' immediate response to treatment when experiencing an apparent clinical flare. In this retrospective study we report the relevant data.

Received for publications June 13, 2020; Editorial Decision September 24, 2020.

From the *IBD Center, Division of Gastroenterology, Virginia Tech Carilion School of Medicine, Roanoke, Virginia, USA; †Department of Clinical and Experimental Medical Sciences, University of Udine School of Medicine, Udine, Italy

Presented at: This study was presented in abstract form in *Gastroenterology* in the proceedings of Digestive Disease Week 2020, Chicago, IL.

Conflicts of interest: Dario Sorrentino has received consulting fees from Abbott/AbbVie, Schering-Plough, MSD, Janssen Research & Development LLC, Centocor Inc., TechLab, Hoffmann-LaRoche, Giuliani, Schering-Plough, and Ferring; research grants from AbbVie, Janssen Research & Development LLC, Schering-Plough, TechLab, Centocor, Takeda, Medtronic, the Broad Foundation, and the Helmsley Foundation; and serves on the speakers bureau of AbbVie and the national faculty of Janssen.

Address correspondence to: Dario Sorrentino, MD, FRACP, Division of Gastroenterology, 3 Riverside Circle, Roanoke, VA 24016 (drsorrentino@carilionclinic.org).

© 2021 Crohn's & Colitis Foundation. Published by Oxford University Press on behalf of Crohn's & Colitis Foundation.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

doi: 10.1093/ibd/izaa348
Published online 27 January 2020

METHODS

Study Design and Study Population

This retrospective study enrolled 61 patients with UC or CD diagnosed and staged according to established criteria,¹⁴ treated with biologics (infliximab, adalimumab, and vedolizumab), and monitored as standard of care in our IBD center through the measurement of FL levels. Five of these patients were treated with infliximab-dyyb (Inflixtra), but no distinction was made between Inflixtra and infliximab-Remicade. The FL levels and clinical/laboratory data were collected from the patients' electronic medical records. Data collected involved 2 different groups of patients.

The first group (therapeutic interval group) consisted of patients on maintenance monotherapy in clinical remission; data included FL at baseline (measured within 1 month of initiating therapy) and FL measured at different times during the biologic therapeutic interval (8 weeks for vedolizumab/infliximab, 2 weeks for adalimumab). The objective of this study was to confirm our original observation (obtained with FC) showing that in patients responding to biologics, fecal inflammatory markers mirror mucosal inflammation, which is inversely correlated to serum drug concentrations, in a timely way.¹³ It was anticipated that FL fluctuation during the therapeutic interval would not be detected in patients in profound/biologic remission.

The second group (patients with suspected LOR) included 30 patients on maintenance monotherapy with biologics apparently experiencing a disease flare as judged by pain and/or diarrhea and/or bleeding in whom stool

studies (*Clostridioides difficile*, stool culture, ova and parasites) had excluded infection. No steroids or other medications were prescribed before the index FL tests. The FL levels were measured 1 to 3 days before and 2 to 3 days after infusion/injection (Fig. 1). Patients with elevated FL levels before infusion/injection were managed according to changes observed afterward. When FL returned to normal levels, patients were considered full responders and non-IBD causes of symptoms were sought. When FL decreased but did not return to normal, patients were considered partial responders and the therapeutic interval was decreased (by 2-4 weeks for infliximab/vedolizumab and by 7 days for adalimumab). If patients were already on the shortest interval, then an immunomodulator was added. When FL levels increased after infusion/injection, patients were considered nonresponders and the medication was switched to a new one. We anticipated such increases in inflammation and FL levels in nonresponders because of the progressive, time-dependent nature of uncontrolled disease activity. If patients failed or had already failed the available biologic agents, then they underwent surgery. Patients in whom the FL levels were normal before infusion/injection (in all of them levels remained normal afterward) were also considered full responders and no therapeutic changes were made. However, non-IBD causes for the symptoms were sought.

Outcome data in this group included clinical assessment (see "Assessment of Clinical Disease Activity" below) and additional predrug infusion/injection FL measurements and/or imaging/colonoscopy performed at 3 months after the index FL determination. Patients were also followed in the long term.

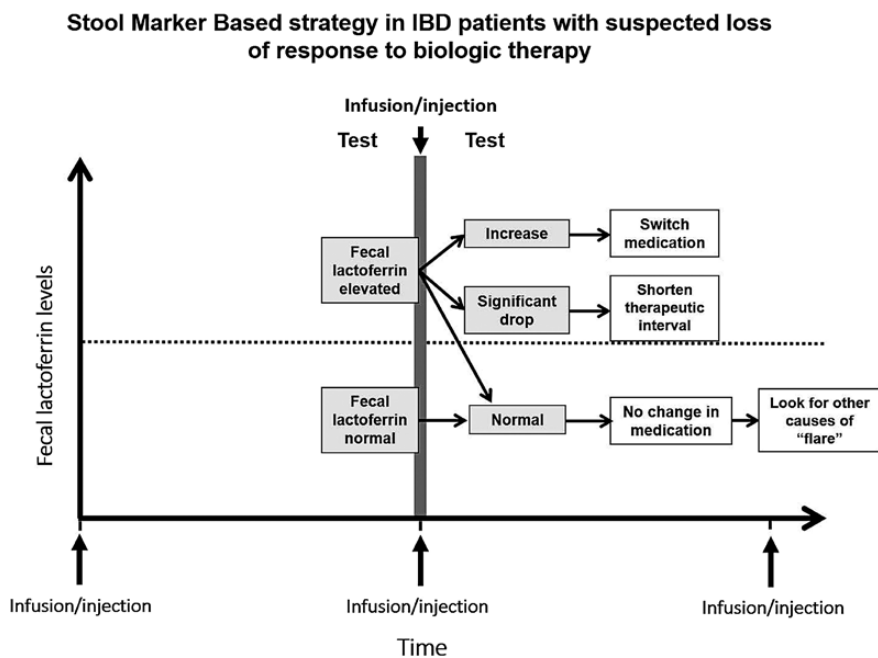


FIGURE 1. Stool marker-based strategy for the evaluation of patients with IBD with suspected LOR to biologics.

The goal of this study was to show that a stool marker–based approach can separate responders from partial responders and nonresponders based on symptom resolution and improvement of objective parameters of disease activity.

There was no direct patient involvement. The study conformed to the guidelines of the 1975 Declaration of Helsinki as reflected by Carilion Clinic Ethical Committee approval. No patient consent was deemed necessary.

Biomarker Testing

The FL was measured in samples collected during the first bowel movement of the day by the LACTOFERRIN SCAN (TECHLAB, Blacksburg, VA), an enzyme-linked immunosorbent assay. Values ≤ 7.24 $\mu\text{g/mL}$ are considered normal¹⁵. At the Carilion Clinic Laboratory, the lower limit to which the linearity of results has been validated is 6.25 $\mu\text{g/mL}$ —which was the absolute value reported and analyzed for all results ≤ 6.25 $\mu\text{g/mL}$. The FL values are presented by standard decimal approximations of the real number by excess or defect. Routine blood and stool studies were performed using established methods.

Assessment of Clinical Disease Activity

Clinical assessment was based on the Harvey-Bradshaw Index (HBI) for CD and the Partial Mayo Scoring Index (PMSI) for UC.¹⁶ For HBI, scores of <5 , 5 to 7, 8 to 16, and >16 define remission, mild, moderate, and severe disease activity. The same activities are defined by the scores of 0 to 1, 2 to 4, 4 to 6, and 7 to 9 for PMSI.

Statistical Analysis

Therapeutic interval group

This analysis focused on the relationship between FL values and the timing of test determination during the therapeutic interval for the 3 medications. Because the therapeutic intervals differed for the 3 medications (2 weeks for adalimumab and 8 weeks for infliximab/vedolizumab) and because the individual absolute FL values also differed among patients, the data were normalized to percentage of baseline FL values (values before starting any therapy) and percentage of therapeutic interval. All treatments were considered together because when treatment was considered as a second variable in the model and when interaction of treatment and timing was considered, neither one was found to be statistically significant. A repeated-measures model using the percentage of FL as the outcome of the model was run initially, which showed the residuals to be right-skewed. Hence, FL values were log-transformed and percentages of baseline ln (FL) were calculated, with the resulting model residuals appearing much closer to being normally distributed. Data were then analyzed again with a repeated-measures model using R version 3.6.1 and specific packages.¹⁷

¹⁸ The results of the analysis of variance *F* test for the effect of therapeutic interval were calculated as “conditional” R^2 , a parameter that includes random subject variability.¹⁹

Patients with suspected LOR

These patients were divided into 2 subgroups: those who experienced a decrease in FL after treatment and those who experienced an increase in FL after treatment (see Fig. 1). These 2 subgroups were modeled separately. Patients with FL levels within normal limits before and after drug infusion/injection were not included in the analysis—because by definition they were not experiencing a true disease flare. This analysis was a descriptive analysis to summarize the changes in FL after drug administration relative to baseline. As in the first (therapeutic interval) group, FL values were log-transformed before analysis. Analysis of HBI and PMSI before and after treatment (at 3-month follow-up) was performed by independent (2-sample) *t* tests. To uniformly analyze the data, these clinical indices were rescaled to be between 0 and 1 by dividing each score by the maximum value for each scale; for HBI, the value 16 was used as the maximum value because all the scores >16 are considered severe (and 16 was also the highest value of the HBI in the dataset).

TABLE 1. Therapeutic Interval Group Characteristics

Demographics	n = 31
Age, y, median (IQR)	46 (24)
Sex (men/women)	14/17
Smoker (% current, former, never)	16, 45, 39
Disease characteristics	
Disease type	
CD, n (%)	21 (68)
UC, n (%)	10 (32)
Disease duration, y, median (IQR)	6 (9)
Biologic, n (%)	
Infliximab	18 (58)
Vedolizumab	6 (19)
Adalimumab	7 (23)
CD location, n (%)	
L1: ileal	4 (20)
L2: colonic	6 (26)
L3: ileocolonic	11 (54)
CD behavior, n (%)	
B1: nonstricturing, nonpenetrating	16 (74)
B2: stricturing	3 (16)
B3: penetrating	2 (10)
UC location (%)	
E1: Ulcerative proctitis	1 (10)
E2: Left-sided (distal)	2 (20)
E3: Extensive (pancolitis)	7 (70)

TABLE 2. FL During Therapeutic Interval

Patient number, disease type, location-extent	Patients Treated With Infliximab—FL Values (µg/mL)		
	Baseline	Interval First Value (week number)	Interval Last Value (week number)
1 UC—E3	9888	6.25 (3)	622 (5)
2 UC—E3	1653	6.25 (5)	19 (6)
3 UC—E2	362	6.25 (4)	142 (6)
4 UC—E2	541	6.25 (1)	43 (6)
5 CD—L3	400	6.25 (5)	9 (6)
6 CD—L3	407	6.25 (1)	6.25 (8)
7 CD—L3	330	72 (3)	194 (8)
8 CD—L2	437	47 (1)	238 (8)
9 UC—E3	444	19 (3)	85 (4)
10 CD—L1	9	6.25 (1)	6.25 (6)
11 CD—L3	362	6.25 (2)	241 (8)
12 CD—L1	67	6.25 (1)	6.25 (7)
13 CD—L2	448	6.25 (3)	6.25 (8)
14 CD—L2	7285	683 (1)	1007 (5)
15 CD—L3	5923	6.25 (1)	18 (8)
16 CD—L2	583	61 (3)	65 (7)
17 CD—L3	218	36 (1)	152 (8)
18 UC—E3	4262	6.25 (1)	10 (8)
Patient number, disease type, location-extent	Patients Treated With Vedolizumab—FL Values (µg/mL)		
	Baseline	Interval First Value (week number)	Interval Last Value (week number)
19 UC—E3	4097	10 (1)	40 (7)
20 UC—E3	4019	73 (1)	380 (6)
21 UC—E1	46	6.25 (3)	6.25 (5)
22 UC—E3	3978	66 (1)	420 (6)
23 CD—L2	1464	66 (4)	172 (8)
24 CD—L3	337	6.25 (3)	6.25 (6)
Patient number, disease type, location-extent	Patients Treated With Adalimumab—FL Values (µg/mL)		
	Baseline	Interval First Value (day number)	Interval Last Value (day number)
25 CD—L2	654	6.25 (3)	6.25 (12)
26 CD—L3	297	6.25 (5)	6.25 (10)
27 CD—L3	166	29 (2)	98 (13)
28 CD—L3	97	6.25 (7)	6.25 (10)
29 CD—L3	434	43 (4)	68 (13)
30 CD—L1	57	20 (3)	30 (12)
31 CD—L1	93	10 (4)	63 (13)

E1 indicates proctitis; E2, left sided colitis; E3, pancolitis; L1, ileal; L2, colonic; L3, ileocolonic.

RESULTS

Therapeutic Interval Group

Table 1 shows the clinical features of the 31 patients in clinical remission tested for FL at various timepoints during the therapeutic interval. Of these, 68% had CD and 32% had UC, 58% were on infliximab, and 19% and 23% were on vedolizumab and adalimumab, respectively. Most patients with

CD had nonstricturing/nonpenetrating colonic or ileocolonic disease, and most patients with UC had pancolitis.

Table 2 shows the FL values at baseline (before starting therapy) and at an early and a late timepoint during the 8-week (infliximab/vedolizumab) or 14-day (adalimumab) therapeutic interval. Baseline FL values varied among patients. However, in all patients FL values dropped significantly (up to 99.9%) after the start of therapy, consistently with observed clinical response. In

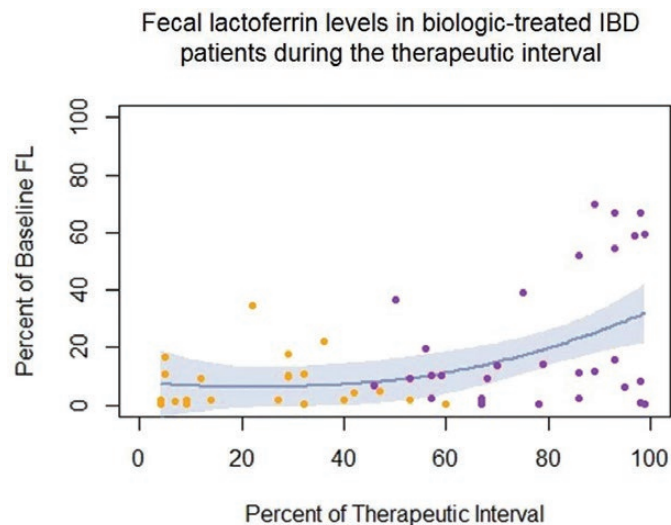


FIGURE 2. Loess smoother of FL changes during the therapeutic interval in patients with IBD treated with biologics. Colored points denote patients' first (yellow) and subsequent (purple) measurements.

22/31 patients (71%), FL levels increased moving toward the infusion/injection day, with the highest values recorded immediately before drug administration and the lowest values immediately afterward. The results of the analysis of variance *F* test for the effect of therapeutic interval showed a significant ($P < 0.001$; conditional $R^2 = 0.769$) progressive increase of FL over time. In 9/31 (29%) of patients, FL levels were below detectability throughout the interval and were excluded from the analysis. On average, patients with undetectable FL values during the therapeutic interval had baseline FL levels 9 times lower than patients with detectable levels. This finding suggests that standard medication dosage/interval induced a deeper remission (with persistently undetectable FL) in patients with a lower disease burden.

Fig. 2 is a scatterplot with a Loess smoother that shows the pattern of percentage of baseline FL levels as a percentage of therapeutic interval. Supplementary Fig. 1 is a line graph that connects the individual patient values and shows the upward trend for all patients. Hence, as the therapeutic interval progresses, FL levels increase—more so toward the end of the interval—thus reflecting the surge in inflammation as the drug concentration decreases over time.¹³

Patients With Suspected LOR

There were 30 consecutive patients on maintenance therapy experiencing a possible disease flare (newly reported pain and/or diarrhea and/or bleeding). Their features are shown in Table 3. Their mean age was 44 years, 57% were men, 63% had CD, 67% were on infliximab, 30% were on adalimumab, and 3% were on vedolizumab. The majority of patients with CD had colonic or ileocolonic nonstricturing/nonpenetrating disease. The majority of patients with UC had pancolitis.

TABLE 3. Characteristics of Patients With Suspected LOR

Demographics	n = 30
Age, y, median (IQR)	44 (29)
Sex (men/women)	17/13
Smoker (% current, former, never)	14, 41, 45
Disease characteristics	
Disease type	
CD, n (%)	19 (63)
UC, n (%)	11 (37)
Disease duration, y, median (IQR)	9 (11)
Biologic, n (%)	
Infliximab	20 (67)
Vedolizumab	1 (3)
Adalimumab	9 (30)
CD location, n (%)	
L1: ileal	3 (16)
L2: colonic	7 (37)
L3: ileocolonic	9 (47)
CD behavior, n (%)	
B1: nonstricturing, nonpenetrating	16 (84)
B2: stricturing	2 (11)
B3: penetrating	1 (5)
UC location (%)	
E1: Ulcerative proctitis	2 (18)
E2: Left-sided (distal)	3 (27)
E3: Extensive (pancolitis)	6 (55)

The FL values before and after drug administration (see Fig. 1) separated 3 subgroups. In the first subgroup, FL was elevated before infusion/injection and continued to increase thereafter ("nonresponders"). In the second subgroup, FL was also elevated before infusion/injection but decreased significantly without returning to normal after infusion/injection ("partial responders"). A third subgroup displayed normal FL values after drug administration and in 9/10 patients even before drug administration ("responders").

Table 4 shows the clinical picture of these 3 subgroups of patients at the time of drug administration, the FL values before and after infusion/injection, the management strategy, and the 3-month outcomes—including the clinical indices before/after the management decision and the follow-up FL measured before infusion/injection (also expressed as a percentage of premanagement values). The total follow-up time and additional changes in disease management are also shown in Table 4. In the first subgroup, the biologic was switched (in most patients to a different class). Two of these patients failed the new medication and required surgery. One of these patients had already failed other biologics and directly opted for surgery. One patient underwent urgent surgery because of severe colitis and

TABLE 4. Patients With Suspected LOR: Clinical Picture, FL Levels, Management, and Follow-Up

Nonresponders									
Patient Number	Disease Type	Clinical Picture at Infusion/Injection (medication type and frequency)	FL (µg/mL) Pre-/Postmedication (± % Δ)	IBD Care Management	FL (µg/mL) Change in Care (± % Δ)	HBI (before/after management decision)	PMSI (before/after management decision)	Total Follow-Up	
1	UC	Moderate diarrhea, abdominal pain (infliximab q8w)	80/94 (+15%)	Medication change to vedolizumab	21 (-78%)	N/A	4/0	6 months; no clinical changes	
2	UC	Moderate diarrhea, abdominal pain, rectal bleeding (infliximab q8w)	90/249 (+64%)	Medication change to vedolizumab	46 (-81%)	N/A	6/2	18 months; later failure of vedolizumab; now on tofacitinb	
3	UC	Moderate diarrhea, abdominal pain, rectal bleeding (adalimumab q2w)	146/216 (+32%)	Medication change to vedolizumab	15 (-93%)	N/A	7/1	12 months; no clinical changes	
4	UC	Severe diarrhea, abdominal pain, rectal bleeding (infliximab q4w—had failed adalimumab and vedolizumab)	983/3143 (+69%)	Total abdominal colectomy with ileostomy	N/A	N/A	8/0	24 months; no clinical changes	
5	CD	Severe diarrhea, abdominal pain, rectal bleeding (adalimumab q2w)	243/381 (+36%)	Medication change to infliximab	6.25 (-97%)	12/1	N/A	24 months; no clinical changes	
6	CD	Moderate diarrhea, abdominal pain (vedolizumab q8w)	313/450 (+30%)	Medication change to adalimumab	62 (-86%)	12/4	N/A	14 months; no clinical changes	
7	UC	Severe diarrhea, abdominal pain (infliximab q8w)	878/928 (+5%)	Medication change to vedolizumab	6.25 (-93%)	N/A	8/2	12 months; no clinical changes	
8	UC	Severe diarrhea, abdominal pain (infliximab q6w; steroid-resistant)	3442/3577 (+3%)	Total abdominal colectomy after failing vedolizumab	125 (-97%)	N/A	9/1	24 months; developed pouchitis—controlled by antibiotics	
9	UC	Diarrhea, abdominal pain, rectal bleeding (infliximab q8w—had failed adalimumab)	313/330 (+5%)	Total abdominal colectomy after failing vedolizumab	N/A	N/A	8/1	24 months; no clinical changes	
10	UC	Severe diarrhea, abdominal pain, rectal bleeding (infliximab q4w)	2081/3593 (+22%)	Total abdominal colectomy	N/A	N/A	9/1	Died of complications at surgery	

TABLE 4. Continued

Partial responders									
Patient Number	Disease Type	Clinical Picture at Infusion/Injection (medication type and frequency)	FL (µg/mL) Pre-/Postmedication (± % Δ)	IBD Care Management	FL (µg/mL) Response to Change in Care (± % Δ)	HBI (before/after management decision)	PMSI (before/after management decision)	Total Follow-Up	
11	UC	Severe diarrhea, abdominal pain (infliximab q8w)	744/573 (-23%)	Shortened interval to 4 weeks	6.25 (-92%)	N/A	5/0	48 months; normal colonoscopy at 24 months	
12	CD	Mild diarrhea, abdominal pain (infliximab q8w)	2059/519 (-75%)	Shortened interval to 4 weeks	66 (-97%)	12/5	N/A	18 months; at 12 months normal colonoscopy and FL = 6.25 µg/mL	
13	CD	Diarrhea (infliximab q8w)	42/22 (-50%)	Shortened interval to 5 weeks	N/A	16/3	N/A	6 months; FL = 6.25 µg/mL	
14	CD	Moderate diarrhea, abdominal pain (infliximab q8w)	239/41 (-83%)	Shortened interval to 6 weeks	7 (-97%)	8/2	N/A	6 months; no changes, then had to stop infliximab for prostate cancer	
15	CD	Moderate diarrhea, abdominal pain (infliximab q8w)	96/20 (-80%)	Shortened interval to 6 weeks	26 (-73%)	13/4	N/A	24 months; no clinical changes	
16	CD	Moderate diarrhea, abdominal pain, rectal bleeding (adalimumab q2w)	108/38 (-65%)	Shortened interval to 1 week	6.25 (-95%)	6/2	N/A	12 months; no clinical changes	
17	CD	Mild diarrhea (adalimumab q2w)	235/91 (-61%)	Shortened interval to 1 week	N/A	6/1	N/A	12 months; developed flare on adalimumab q1w with FL 341/181—switched to infliximab q8w; at 6 months FL = 16 µg/mL	
18	CD	Moderate diarrhea and abdominal pain (infliximab q8w)	477/62 (-87%)	Shortened interval to 6 weeks	6.25 (-99%)	6/2	N/A	24 months; no clinical changes	
19	UC	Diarrhea, rectal bleeding (infliximab q8w)	6622/815 (-88%)	Shortened interval to 4 weeks	6.25 (-99%)	N/A	5/0	18 months; no clinical changes	
20	CD	Moderate diarrhea and abdominal pain (infliximab q4w)	3291/561 (-83%)	Addition of azathioprine	6.25 (-99%)	7/2	N/A	24 months; no clinical changes	

∞ TABLE 4. Continued

Patient Number	Disease Type	Clinical Picture at Infusion/Injection (medication type and frequency)	Responders							Additional Notes and Total Follow-Up
			FL (µg/mL) Pre/Postmedication (± % Δ)	IBD Care Management	FL (µg/mL) Change in Care (± % Δ)	HBI (before/after management decision)	PMSI (before/after management decision)			
21	CD	Blood in stool (infliximab q8w)	18/6.25 (−65%)	No action	N/A	2/2	N/A	N/A	Blood was urinary because of urinary stones; abatement of symptoms after stone surgery; 12 months	
22	CD	Rectal bleeding (infliximab q8w)	6.25/6.25 (0%)	No action	6.25 (0%)	2/2	N/A	N/A	Blood because of hemorrhoids; abatement of symptoms after hemorrhoidectomy; 48 months	
23	UC	Moderate diarrhea (adalimumab q2w)	6.25/6.25 (0%)	No action	6.25 (0%)	N/A	3/0	N/A	Symptoms suspected from food poisoning; symptoms improved within 1 week; 24 months	
24	CD	Moderate diarrhea (adalimumab q2w)	6.25/6.25 (0%)	No action	6.25 (0%)	10/0	N/A	N/A	Short gut syndrome; symptoms relieved after specific management; 18 months	
25	CD	Moderate diarrhea and abdominal pain (infliximab q8w)	6.25/6.25 (0%)	No action	N/A	15/15	N/A	N/A	No inflammation at upper and lower endoscopy, capsule endoscopy, imaging; likely nerve entrapment after previous abdominal surgery; managed symptomatically; 24 months	
26	CD	Abdominal pain (infliximab q8w)	6.25/6.25 (0%)	No action	6.25 (0%)	2/0	N/A	N/A	Umbilical hernia and gastroparesis; abatement of symptoms after surgery and treatment of gastroparesis; 12 months	
27	CD	Mild diarrhea (Adalimumab q2w)	6.25/6.25 (0%)	No action	N/A	3/0	N/A	N/A	Symptoms abated after reduction in dietary fiber intake; 12 months	
28	CD	Rectal bleeding (adalimumab q2w)	6.25/6.25 (0%)	No action	6.25 (0%)	2/2	N/A	N/A	Blood because of hemorrhoids; no further action; 48 months	
29	CD	Diarrhea and bloating (infliximab q8w)	6.25/6.25 (0%)	No action	6.25 (0%)	4/0	N/A	N/A	Symptoms abated after reduction in dietary fiber intake; 24 months	
30	CD	Abdominal discomfort (adalimumab q2w)	6.25/6.25 (0%)	No action	6.25 (0%)	1/1	N/A	N/A	Symptoms spontaneously abated in 4 days, likely because of food poisoning; 12 months	

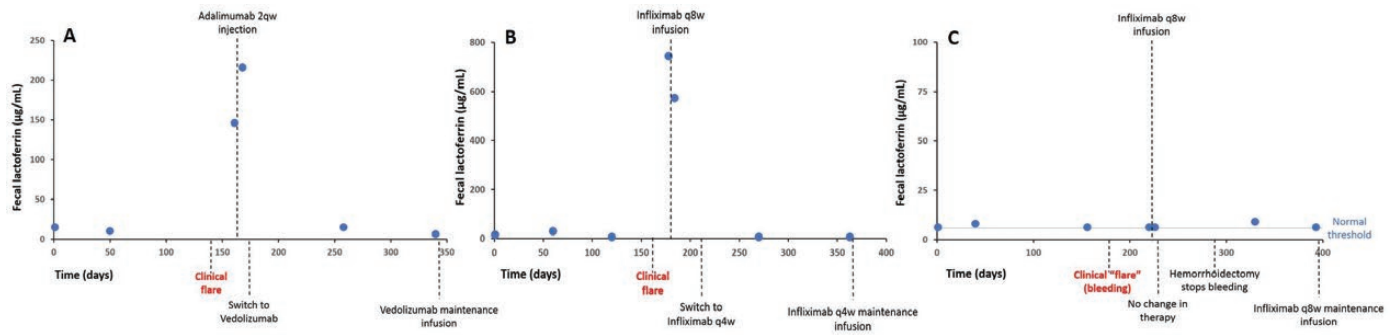


FIGURE 3. FL levels in patients with IBD apparently losing response after a period of remission. A, Nonresponder (patient 3; see Table 4): FL levels before drug administration were elevated and increased further thereafter. The medication was switched and the patient regained response. B, Partial responder (patient 11; see Table 4): FL levels before drug administration were elevated and decreased afterward. The medication therapeutic interval was shortened and the patient regained response. C, Responder (patient 22; see Table 4): FL levels before drug administration were normal and did not change after drug administration. No changes were made in IBD medications and other causes of symptoms were sought (in this patient, bleeding resulted from hemorrhoids and ceased after surgery). Patient continued to respond to IBD medication. Day 0 arbitrarily indicates the time of the initial observation for each patient.

died of complications during surgery. Total follow-up time in this group averaged 18 months. In the second subgroup, FL decreased on average by approximately 70% after infusion/injection. In these patients, the therapeutic interval was decreased by 2 to 4 weeks (infliximab/vedolizumab) or 1 week (adalimumab). In 1 patient, another medication (azathioprine 3 mg/Kg) was added because the patient was already on the shortest biologic therapeutic interval. Total follow-up time in this group averaged 19 months. In the third subgroup of patients (responders), the therapy was left unchanged and other causes for the symptoms were sought and found for all.

In most patients, hemorrhoids were found to be responsible for rectal bleeding while pain and diarrhea were attributed to a number of causes including nerve entrapment, hernia, short gut syndrome, and food poisoning, which mostly resolved after adequate management. Total follow-up time in this group averaged 23 months. Fig. 3 graphically illustrates the results for 3 different patients representing nonresponders, partial responders, and responders.

Disease severity was scored before and after (at 3 months) the management decision for all subgroups. Analysis of standardized clinical scores showed that in nonresponders the initial scores (at time of drug administration) were higher than in partial responders ($P = 0.002$). However, the subgroup-tailored management strategy led to a significant decrease in standardized clinical scores for both partial responders (0.58 ± 0.21 vs 0.13 ± 0.09 ; $P < 0.001$) and nonresponders (0.81 ± 0.17 vs 0.12 ± 0.08 ; $P < 0.001$). The FL level was measured (immediately before drug administration) at 3 months in all patients, except for those who had undergone surgery. It decreased on average by 88% and 97% compared to corresponding values at the time of index drug administration in nonresponders and partial responders, respectively.

DISCUSSION

LOR and PNR to biologic agents occur frequently in IBD.^{20, 21} Traditionally, management has involved empirical

drug dose escalation with subsequent medication switch in the absence of symptomatic improvement.^{20, 21} More recently, TDM has gained popularity over this approach. A number of studies have indeed shown that TDM may impact the management of LOR and PNR and has also helped clarify some of their mechanistic aspects.^{2, 3} However, several clinical issues (most notably the absence of universally applicable TL) limit the routine use of TDM strategies in daily practice.^{4-9, 11, 13, 22} A more practical approach would be to directly evaluate medication impact on disease activity and inflammatory burden—of which FL/FC are excellent indicators.²³⁻²⁹ However, their precise role in IBD management is still partly undefined,¹² and in particular it is unclear whether fecal markers may reflect immediate drug efficacy in a timely way—a feature needed to manage LOR.

In this retrospective study, we collected data generated in our clinical practice confirming initial FC data by our group¹³ and providing evidence that FL levels bear a fine time-sensitivity to drug-generated changes in inflammation. In 71% of patients in clinical remission, FL levels increased during the therapeutic interval with the highest values recorded immediately before infusion/injection—mirroring increasing inflammatory activity because of depletion of circulating drug levels in a timely way.¹³ Hence, the majority of patients with IBD successfully treated with biologics had FL levels above normal, especially approaching drug administration. This finding is significant because complete mucosal healing and normal fecal markers correlate with better outcomes in the long term.^{30, 31} However, dose escalation in all such patients would increase costs and potentially increase the risk of adverse effects.³² In patients with persistently normal FL levels during the entire therapeutic interval, baseline FL values were almost an order of magnitude lower than in the other patients, suggesting that the standard biologic dose/interval was sufficient to afford a tight control of inflammation and could in theory even be de-escalated.^{33, 34} Because fecal marker levels change during the therapeutic interval in

most patients, individual single-point values should be interpreted in the context of their timing of testing in relation to drug administration. For example, a normal value immediately before infusion/injection is more suggestive of deep remission than a normal value immediately after drug administration.

The fecal marker time sensitivity to drug-induced changes in inflammation was confirmed and leveraged in the group of patients with suspected LOR. In these patients, FL measured immediately before and immediately after infusion/injection distinguished 3 groups of patients: one group had normal FL levels that remained normal after drug administration (responders), another group had elevated FL levels that partly decreased after infusion/injection but remained well above the normal threshold (partial responders), and a third group showed an increase in FL levels after drug administration (nonresponders). The increase in FL after infusion/injection in the latter group was expected because in the absence of therapeutic response, inflammation would continue to increase with time. The first group was managed conservatively, and a cause other than LOR for the apparent flare was actively sought (and found) in most of the patients (eg, hemorrhoids causing rectal bleeding). In nonresponders the medication was switched or surgery was performed for multiple biologic failures, with excellent clinical results at the 3-month follow-up time. In partial responders the therapeutic interval was decreased, with reinduction of clinical remission in most patients at 3 months. Furthermore, in the latter 2 groups the 3-month FL (performed at the end of the new drug/new therapeutic interval) dropped on average by 88% and 97%, respectively.

The average total follow-up time in the 3 groups of patients was 20 months (range, 4-48 months) during which most patients in each group continued to be successfully managed with the strategy adopted at the time of disease flare. However, 1 patient among the nonresponders and 1 patient among the partial responders developed resistance to the new medication. More important, 4 patients among the nonresponders needed surgery, as opposed to none in the other groups. Multiple biologic failures over time were more common among nonresponders⁴ than among partial responders and responders (1 and 0, respectively). This result suggests that biologic failures identify a group of difficult-to-treat patients: those more likely to undergo surgery.

The main limitation of our study is its retrospective nature, reflecting the clinical practice and strategies applied in our center. The patient population studied here was diverse in terms of disease type, location and extent, medications and routes used (intravenous vs subcutaneous), length of therapeutic interval, and previous biologic use. However, such heterogeneity is actually one of the strengths of the study, confirming that this strategy is applicable across the entire spectrum of disease expression and biologic therapies, a crucial feature for its practical routine use. However, adopting this strategy may be challenging if the disease only involves a short small bowel

segment, when fecal marker levels may not be always elevated.³⁵ Nevertheless, as shown herein, any baseline FL level significantly above normal may be the only prerequisite to apply this strategy.

Notably, in patients experiencing a possible flare, steroids or any other effective fast-acting medications given after symptom onset and before/during the double FL testing could affect the results. In our patients no concomitant medications were given in this period. If clinically needed (ie, patients experiencing symptoms several weeks before the next drug infusion/injection), steroids could be initiated and kept on a stable dose well before and after the double FL testing—an approach that is classically used in clinical trials.

Finally, TL and ADA could have added insightful information to the study, but they were not determined. Future prospective studies should compare TDM to the strategy proposed here—in terms of both accuracy and costs—and to endoscopy and symptom-based management. They should also clarify whether FL should be tested at different times according to the rate of drug concentration decline during the therapeutic interval, identify potential FL cutoff levels in different scenarios, and clarify whether FC could be used in place of FL.

CONCLUSIONS

The timely sensitivity of FL to drug-generated changes in inflammation provides the rationale for an accurate and inexpensive stool marker-based strategy to manage LOR. Larger, prospective studies may be needed to confirm our findings and evaluate any potential complementary role for TDM.³⁶

SUPPLEMENTARY DATA

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

Supplementary Figure 1. FL changes during the therapeutic interval in patients with IBD treated with biologics: line graph connecting individual patient values (represented by the same color).

ACKNOWLEDGMENTS

We thank Dr. Kim Love for help with data analysis and James Boone for critically reviewing the manuscript.

REFERENCES

1. D'Inca R, Caccaro R. Measuring disease activity in Crohn's disease: what is currently available to the clinician. *Clin Exp Gastroenterol*. 2014;7:151-161.
2. Vande Casteele N, Herfarth H, Katz J, et al. AGA Institute technical review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. *Gastroenterology*. 2017;153:835-857.e6.
3. Kennedy NA, Heap GA, Green HD, et al.; UK Inflammatory Bowel Disease Pharmacogenetics Study Group. Predictors of anti-TNF treatment failure in anti-TNF-naïve patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. *Lancet Gastroenterol Hepatol*. 2019;4:341-353.

4. Sorrentino D, Nguyen V, Henderson C, et al. Therapeutic drug monitoring and clinical outcomes in immune mediated diseases: the missing link. *Inflamm Bowel Dis*. 2016;22:2527–2537.
5. D'Haens G, Vermeire S, Lambrecht G, et al.; GETAID. Increasing infliximab dose based on symptoms, biomarkers, and serum drug concentrations does not increase clinical, endoscopic, and corticosteroid-free remission in patients with active luminal Crohn's disease. *Gastroenterology*. 2018;154:1343–1351.e1.
6. Pariente B, Pineton de Chambrun G, Krzysiek R, et al. Trough levels and antibodies to infliximab may not predict response to intensification of infliximab therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18:1199–1206.
7. Ungar B, Chowers Y, Yavzori M, et al.; ABIRISK Consortium. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut*. 2014;63:1258–1264.
8. Gibson DJ, Ward MG, Rentsch C, et al. Review article: determination of the therapeutic range for therapeutic drug monitoring of adalimumab and infliximab in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2020;51:612–628.
9. Wright EK, Kamm MA, De Cruz P, et al. Anti-TNF therapeutic drug monitoring in postoperative Crohn's disease. *J Crohns Colitis*. 2018;12:653–661.
10. Roblin X, Boschetti G, Duru G, et al. Distinct thresholds of infliximab trough level are associated with different therapeutic outcomes in patients with inflammatory bowel disease: a prospective observational study. *Inflamm Bowel Dis*. 2017;23:2048–2053.
11. Billiet T, Cleynen I, Ballet V, et al. Evolution of cytokines and inflammatory biomarkers during infliximab induction therapy and the impact of inflammatory burden on primary response in patients with Crohn's disease. *Scand J Gastroenterol*. 2017;52:1086–1092.
12. Boon GJ, Day AS, Mulder CJ, et al. Are faecal markers good indicators of mucosal healing in inflammatory bowel disease? *World J Gastroenterol*. 2015;21:11469–11480.
13. Sorrentino D, Marino M, Dassopoulos T, et al. Low dose infliximab for prevention of postoperative recurrence of Crohn's disease: long term follow-up and impact of infliximab trough levels and antibodies to infliximab. *Plos One*. 2015;10:e0144900.
14. Benevento G, Avellini C, Terrosu G, et al. Diagnosis and assessment of Crohn's disease: the present and the future. *Expert Rev Gastroenterol Hepatol*. 2010;4:757–766.
15. Langhorst J, Boone J. Fecal lactoferrin as a noninvasive biomarker in inflammatory bowel diseases. *Drugs Today (Barc)*. 2012;48:149–161.
16. Peyrin-Biroulet L, Panés J, Sandborn WJ, et al. Defining disease severity in inflammatory bowel diseases: current and future directions. *Clin Gastroenterol Hepatol*. 2016;14:348–354.e17.
17. R: A Language and Environment for Statistical Computing. Version 3.6.1. R Foundation for Statistical Computing; 2015. Accessed December 18, 2020. <https://www.r-project.org/>.
18. Bates D, Maechler M, Bolker B, et al. Fitting linear mixed-effects models using lme4. *J Stat Softw*. 2015;67:1–48.
19. Nakagawa S, Schielzeth H. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods Ecol Evol*. 2013;4:133–142.
20. Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol*. 2009;104:760–767.
21. Danese S, Fiorino G, Reinisch W. Review article: causative factors and the clinical management of patients with Crohn's disease who lose response to anti-TNF- α therapy. *Aliment Pharmacol Ther*. 2011;34:1–10.
22. Chaparro M, Guerra I, Iborra M, et al. Infliximab serum levels do not predict remission after the induction phase in Crohn's disease patients. *Gastroenterology*. 2015;148:S849.
23. Sachar DB; Biomarkers Task Force of the IOIBD. Role of biomarkers in the study and management of inflammatory bowel disease: a “nonsystematic” review. *Inflamm Bowel Dis*. 2014;20:2511–2518.
24. Colombel JF, Panaccione R, Bossuyt P, et al. Effect of tight control management on Crohn's disease (CALM): a multicentre, randomised, controlled phase 3 trial. *Lancet*. 2018;390:2779–2789.
25. El-Matary W, Abej E, Deora V, et al. Impact of fecal calprotectin measurement on decision-making in children with inflammatory bowel disease. *Front Pediatr*. 2017;5:7.
26. Dai C, Jiang M, Sun MJ, et al. Fecal lactoferrin for assessment of inflammatory bowel disease activity: a systematic review and meta-analysis. *J Clin Gastroenterol*. 2020;54:545–553.
27. Goutorbe F, Goutte M, Minet-Quinard R, et al. Endoscopic factors influencing fecal calprotectin value in Crohn's disease. *J Crohns Colitis*. 2015;9:1113–1119.
28. Rubio MG, Amo-Mensah K, Gray JM, et al. Fecal lactoferrin accurately reflects mucosal inflammation in inflammatory bowel disease. *World J Gastrointest Pathophysiol*. 2019;10:54–63.
29. Vieira A, Fang CB, Rolim EG, et al. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. *BMC Res Notes*. 2009;2:221.
30. Yzet C, Diouf M, Le Mouel JP, et al. Complete endoscopic healing associated with better outcomes than partial endoscopic healing in patients with Crohn's disease. *Clin Gastroenterol Hepatol*. 2020;18:2256–2261.
31. Kennedy NA, Jones GR, Plevis N, et al. Association between level of fecal calprotectin and progression of Crohn's disease. *Clin Gastroenterol Hepatol*. 2019;17:2269–2276.e4.
32. Srinivasan A, Vasudevan A, McFarlane A, et al. Anti-TNF re-induction is as effective, simpler, and cheaper compared with dose interval shortening for secondary loss of response in Crohn's disease. *J Crohns Colitis*. 2018;12:280–288.
33. Sorrentino D, Paviotti A, Terrosu G, et al. Low-dose maintenance therapy with infliximab prevents postsurgical recurrence of Crohn's disease. *Clin Gastroenterol Hepatol*. 2010;8:591–599.e1.
34. Pouillon L, Lamoureux A, Pineton de Chambrun G, et al. Dose de-escalation to adalimumab 40 mg every three weeks in patients with inflammatory bowel disease—a multicenter, retrospective, observational study. *Dig Liver Dis*. 2019;51:236–241.
35. Sorrentino D, Nguyen VQ. Clinically significant small bowel Crohn's disease might only be detected by capsule endoscopy. *Inflamm Bowel Dis*. 2018;24:1566–1574.
36. Dreesen E, Baert F, Laharie D, et al. Monitoring a combination of calprotectin and infliximab identifies patients with mucosal healing of Crohn's disease. *Clin Gastroenterol Hepatol*. 2020;18:637–646.e11.