Journal of Crohn's and Colitis, 2015, 33–40
doi:10.1016/j.crohns.2014.06.012
Original Article

Original Article

Does Fecal Calprotectin Predict Short-Term Relapse After Stopping Tnfα-Blocking Agents In Inflammatory Bowel Disease Patients In Deep Remission?

Pauliina Molander[a,*], Martti Färkkilä[b,m], Ari Ristimäki[c], Kimmo Salminen[d], Helena Kemppainen[d], Timo Blomster[e], Ritva Koskela[e], Airi Jussila[f], Henna Rautiainen[g], Markku Nissinen[h], Johanna Haapamäki[b], Perttu Arkkila[b], Urpo Nieminen[b], Juha Kuisma[i], Jari Punkkinen[j], Kaija-Leena Kolho[k,m], Harri Mustonen[l], Taina Sipponen[b]

[a]Maria Helsinki City Hospital and University of Helsinki, Helsinki, Finland [b]Department of Medicine, Division of Gastroenterology, Helsinki University Central Hospital, Helsinki, Finland [c]Department of Pathology, HUSLAB and Haartman Institute, Helsinki University Central Hospital and Genome-Scale Biology, Research Programs Unit, University of Helsinki, Helsinki, Finland [d]Department of Medicine, Division of Gastroenterology, Turku University Central Hospital, Turku, Finland [e]Department of Medicine, Division of Gastroenterology, Oulu University Central Hospital, Oulu, Finland [f]Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, Tampere, Finland [g]Department of Medicine, Division of Gastroenterology, Helsinki University Central Hospital, Jorvi Hospital, Espoo, Finland [h]Department of Medicine, Division of Gastroenterology, Helsinki University Central Hospital, Peijas Hospital, Vantaa, Finland [i]Department of Medicine, Hyvinkää Hospital, Hyvinkää, Finland [j]Department of Medicine, Porvoo Hospital, Porvoo, Finland [k]Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland [l]Helsinki University Central Hospital, Department of Surgery, Biomedicum Helsinki, Finland [m]University of Helsinki, Institute of Clinical Medicine, Department of Medicine, Division of Gastroenterology, Helsinki, Finland

Received March 25 2014; revised June 16 2014; accepted June 29 2014.

[*]Corresponding author at: Department of Medicine, Division of Gastroenterology, Maria Helsinki City Hospital, P.O. Box 6501, Helsinki FIN-00099, Finland. Tel.: +358 50 5252872; fax: +358 9 31034347; E-mail address: pauliina.molander@welho.com

Abstract

Background and aims: This prospective multicenter study examined whether elevated fecal calprotectin (FC) concentrations after stopping TNFα-blocking therapy can predict clinical or endoscopic relapse. In addition, we evaluated the impact of histological remission on the relapse risk.

Methods: We enrolled inflammatory bowel disease (IBD) patients who were in clinical, endoscopic, and FC-based (< 100 μg/g) remission after a minimum 11 months of TNFα-blocking therapy. The patients were followed-up for 12 months after the discontinuation of TNFα-blocking therapy. FC was collected monthly for the first 6 months and thereafter every second month. Ileocolonoscopy was performed at inclusion, at 4 months, at the study end, and at the time of clinical relapse.

Results: Of 52 enrolled patients, 49 (16 Crohn's disease, 33 ulcerative colitis/IBD unclassified) provided the stool samples requested and comprised the study group. During the follow-up, 15/49 (31%) relapsed, whereas 34 (69%) remained in remission. Patients relapsing showed constantly elevated FC levels for a median of 94 (13–317) days before the relapse. Significant increase in median FC levels was seen 2 ($p = 0.0014$), 4 ($p = 0.0056$), and 6 ($p = 0.0029$) months before endoscopic

relapse. Constantly normal FC concentrations during the follow-up were highly predictive for clinical and endoscopic remission. Normal FC concentrations in patients with remission were associated with histological remission.

Conclusion: FC seems to increase and remain elevated before clinical or endoscopic relapse, suggesting that it can be used as a surrogate marker for predicting and identifying patients requiring close follow-up in clinical practice.

Keywords: Crohn's disease; Ulcerative colitis; TNFα-blocking therapy; Histology; Fecal biomarkers

1. Introduction

Crohn's disease (CD), ulcerative colitis (UC), and inflammatory bowel disease unclassified (IBDU) are chronic conditions characterized by periods of remission and episodes of relapse. Commonly, clinical relapses in IBD, especially in CD, are unpredictable. Despite a clinically successful treatment, subclinical smoldering, endoscopically detected inflammation may lead to significant risk of relapse. Several studies have shown that the assessment of subclinical inflammation, using surrogate markers such as fecal calprotectin (FC), may indicate clinical relapse risk in asymptomatic IBD, especially in UC and colonic CD.[1,2] FC, a biomarker of intestinal inflammation, is secreted into the intestinal mucosa during cell activation and death from activated leukocytes, mainly polymorphonuclear neutrophils, and can be detected in stool along the leukocyte shedding in the intestinal lumen.[3,4] FC correlates well with the excretion of indium 111-labeled fecal leukocytes.[5] FC measurement is easy to perform, safe, and simple for the patient and therefore very useful in clinical practice. Importantly, FC correlates well not only with clinical disease activity but also with endoscopic and histological inflammation in IBD patients.[6–9] Furthermore, low FC levels are closely associated with mucosal healing and therefore endoscopic and histological healing could be determined by assessment of FC in a simple stool test.[10] FC has also been studied as a predictor of clinical relapse in CD patients achieving remission with infliximab induction only, but the median FC levels at week 14 were similar in patients with and without clinical relapse.[11] However, we previously showed that a normal FC concentration after induction therapy with tumor necrosis factor α (TNFα)-blocking therapy predicts sustained clinical remission in the majority of IBD patients on scheduled therapy for active luminal disease.[12] Approximately 50% of IBD patients on scheduled maintenance therapy achieve both clinical and endoscopic remission[13] and could be candidates for cessation of anti-TNFα-therapy. After stopping TNFα-blocking therapy, a considerable risk of IBD relapse exists. In our recent study, however, only a third of IBD patients in deep remission at the time of cessation of TNFα-blocking therapy experienced either an endoscopic or both a clinical and endoscopic relapse within 1 year.[14] A biomarker that could reliably identify relapsers during the subclinical phase could enable restart of therapy before symptomatic relapse occurs. In this study, we evaluated the role of FC in serving as such a biomarker.

Thus, the aim of this study was to determinate whether FC concentrations after stopping TNFα-blocking therapy in IBD patients with deep remission can predict clinical or endoscopic relapse. In addition, we evaluated whether the presence of histological inflammation at the time of cessation of TNFα-blocking therapy, despite clinical, endoscopic and FC-based remission, was an indicator of a relapse risk.

2. Materials and methods

2.1 Patients

Fifty-two patients with IBD (17 CD, 30 UC and 5 IBDU) in clinical, endoscopic, and FC-based remission (FC < 100 μg/g), were recruited in this prospective Finnish multicenter study between February 2010 and June 2012. Eligible patients were over 18 years of age, had established IBD diagnoses, had received TNFα-blocking maintenance therapy for at least 11 (median 16, range 11–78) months, and had been in corticosteroid-free remission over the previous 6 months before the inclusion. The maintenance therapy was unaltered during the prospective follow-up after discontinuation of TNFα-blocking therapy. The exclusion criteria included escalation of TNFα-blocking agents during the last six months, history of relapse after stopping TNFα-blocking agents, perianal disease with no other effective medication available, severe arthritis as a concomitant indication for TNFα-blocking therapy, and pregnancy.

2.2 Study design

At baseline, the clinical and demographic data were collected and all patients underwent an ileocolonoscopy. After cessation of TNFα-blocking therapy, the patients were followed up until relapse or up to 12 months. The relapse rates were published in our previous study.[14] Control visits with clinical activity assessment were performed every 4 weeks up to six months and thereafter every 2 months up to the end of the study. At every visit, the patients were asked to provide a stool sample for FC. Endoscopic assessment of disease activity was performed routinely at 4 and 12 months after the drug withdrawal. Additional visits were made if the patients experienced a clinical relapse between the routine follow-up contacts. In case of a clinical relapse, the patients provided a stool sample for measurement of FC and underwent an ileocolonoscopy. The endoscopy was not repeated in patients who had undergone the routine follow-up ileocolonoscopy within 2 months prior to clinical relapse and the endoscopic disease activity had been at least moderate (see below).

2.3 Clinical and endoscopic criteria for remission and relapse

In CD, clinical activity was assessed by the Harvey Bradshaw Index (HBI)[15] and the endoscopic activity by the Simple Endoscopic Score for Crohn's Disease (SES-CD).[16] Clinical remission was defined as HBI ≤ 4 and clinical relapse as HBI ≥ 8 or an increase of > 3 points in HBI to at least an HBI of 5 points.[17] An SES-CD of 0–2 was defined as remission, 3–6 as mildly active disease, 7–15 as moderately active disease, and ≥ 16 as severely active disease.[18,19]

In UC or IBDU, disease activity was assessed, using the Mayo score.[20] Clinical remission was defined as a partial Mayo score (without endoscopic subscore) = 0 and clinical relapse as a partial Mayo score ≥ 3.[20] For the Mayo endoscopic subscore, endoscopic findings were graded as normal (0), mild (1), moderate (2), or severe (3); a subscore of 0–1 was defined as remission and a subscore of ≥ 2 as active disease.[21]

2.4 Histological assessment of disease activity

During all endoscopies, biopsy specimens were taken from the ileum, right, transverse and left colon, and rectum. The biopsies were

collected from the most severely diseased areas or, if no lesions were present, from random sites in each segment. Routine histology was performed on these specimens stained with hematoxylin and eosin. An experienced pathologist (A.R.) scored all histological findings of each segment, according to a score developed for CD[22] and UC findings[21].

CD scoring consisted of epithelial damage (normal = score 0, focal pathology = 1, extensive pathology = 2); architectural changes (normal = 0, moderately, as < 50% disturbed = 1, or severely, as > 50% disturbed = 2); infiltration of mononuclear cells in the lamina propria (normal = 0, moderate increase = 1, severe increase = 2); polymorphonuclear cells in the lamina propria (normal = 0, moderate increase = 1, severe increase = 2); polymorphonuclear cells in the epithelium (if in the surface epithelium = 1, if cryptitis = 2, if a crypt abscess is present = 3); presence of erosion or ulcers (no = 0, yes = 1); and presence of granuloma (no = 0, yes = 1).[22] One modification to the original scoring was made: the number of biopsy specimens affected was not graded. The ileal and colonic scores were counted separately; the sum of the total scores for each four colonic segments represented the total colonic histological activity. The maximum score for the ileum was 13 and for the colon 52. Higher scores indicate more severe histological inflammation. In this study, histological remission was defined as no polymorphonuclear cells in the lamina propria or in the epithelium and histological relapse was defined as the sum of these two scores ≥ 1.

UC scoring consisted of structural changes (normal = score 0, mild abnormality = 1, mild or moderate diffuse or multifocal abnormality = 2, severe diffuse or multifocal abnormality = 3); chronic inflammatory infiltrate (normal = 0, mild increase = 1, moderate increase = 2, marked increase = 3); infiltration of neutrophils in the lamina propria (normal = 0, mild increase = 1, moderate increase = 2, marked increase = 3); neutrophils in the epithelium (none = 0, < 5% crypts involved = 1, < 50% crypts involved = 2, > 50% crypts involved = 3); presence of crypt destruction (none = 0, probable — local excess of neutrophils in part of crypt = 1, probable — marked attenuation = 2, unequivocal crypt destruction = 3); and presence of erosion or ulceration (no = 0, recovering epithelium = 1, probable erosion = 2, unequivocal erosion = 3, ulcer or granulation tissue = 4).[21] Two modifications to the original scoring were made: the infiltration of eosinophils in the lamina propria was not graded and the scores range from 0 to 4, with higher scores indicating more severe histological inflammation. The maximum score was 76. In this study, histological remission was defined as no neutrophils in the lamina propria or in the epithelium and histological relapse was defined as the sum of these two scores ≥ 1.

2.5 Stool samples for fecal calprotectin assays

Prior to inclusion, all patients had an FC concentration < 100 µg/g, the value quoted as normal in our laboratory.[23] The stool samples were stored at – 20 °C until measurement of FC. FC was assayed by a quantitative enzyme immunoassay (CALPRO Calprotectin ELISA Test (ALP); CALPRO AS, Lysaker, Norway).

2.6 Statistics

For data analyses, we used the Statistical Package for the Social Sciences (SPSS version 17.0 and PASW 18) for Windows software (SPSS, Chicago, IL, USA). The Wilcoxon signed rank test was used to test the differences between related variables and the Mann–Whitney U-test and the T-test were used to test the differences between independent variables. Spearman's rank correlation coefficient (rho) was used in correlation analysis between continuous variables. Significance was set at $p < 0.05$. Kaplan–Meier survival analysis served for estimation of relapse-free survival rates, and the log-rank test was used to determine differences between the groups. The cutoff value for FC in predicting the outcomes was calculated, using receiver–operator characteristic (ROC) curve analysis. The optimized cutoff level, by maximizing Youden's index, offered the best combination of sensitivity and specificity. The generalized linear mixed effects model was used to calculate the estimated FC level at relapse in different groups. The FC values are presented as medians and range and calculated at individual time points.

2.7 Ethical statement

The Ethics Committee of Helsinki University Central Hospital (number 348/13/03/01/2009) and the ethics committees at each participating university central hospital approved the study protocol and all documents. All patients attending this study signed an informed consent form. Research study permission number 195/2009 was received from the Department of Medicine, Helsinki University Central Hospital in December 2009.

3. Results

The patient characteristics are shown in Table 1. For further analyses, the subgroups of UC and IBDU were pooled. Of 52 enrolled patients, we excluded three (one CD, two UC) from the final analysis, due to lack of follow-up FC samples. Of 49 patients, 15 (31%, 4 CD, 11 UC) relapsed during the 1-year follow-up and 34 (69%, 12 CD, 22 UC) remained in remission. The mean time after the last TNFα-blocker dose (infliximab n = 14, adalimumab n = 1) to relapse in all patients was 5 (range 2–15) months, in CD 5 (range 3–12) months and in UC 9 (range 2–15) months. Of relapses, nine (60%, two CD, seven UC) were both clinical and endoscopic relapses, whereas two (13%) were only endoscopic (severe endoscopic findings in asymptomatic CD patients). Furthermore, four UC patients experienced clinical relapse, with mild endoscopic activity (Mayo score 1) not fulfilling the predefined criteria for endoscopic relapse, but were considered as relapsers (severe symptoms + mild activity). There was no clinical relapse found without signs of endoscopic activity. Furthermore, no infections were reported during the follow-up.

3.1 FC concentrations and relapse

The median FC values during the follow-up period are shown in Fig. 1. Compared with the baseline FC, patients with relapse (n = 15) had elevated and significantly higher median FC concentration 6 months (120 µg/g, 0–431, n = 6, p = 0.0029), 4 months (108 µg/g, 7–650, n = 8, p = 0.0056), and 2 months (120 µg/g, 0–1867, n = 15, p = 0.0014) before the clinical relapse. Importantly, once FC was elevated in the group of patients with relapse, it remained elevated until clinical or endoscopic relapse occurred. Of 15 relapsers, two patients (13%) with clinical relapse had normal FC levels in all samples provided prior to relapse and also at relapse (one ileal CD and one UC patient with FC of 90 µg/g and no response to restart of therapy and a future panproctocolectomy within 2 months). On the other hand, three UC patients having normal FC levels in all samples provided prior to relapse had elevated FC levels at relapse (266 µg/g, 188–789). Another two UC patients had transiently abnormal FC levels 1–2 months before clinical relapse, but provided normal FC (< 100 µg/g) at the moment of relapse. The CD patient with ileal relapse had no elevation in FC levels during the follow-up. ROC analysis indicated that a cutoff of > 140 µg/g in mean FC values during the six months before the relapse could predict relapse with a 79% specificity and a 53% sensitivity. This analysis indicated also that FC > 200 µg/g measured 2–4 months before clinical relapse could predict relapse with 83% specificity and

Table 1. Patient characteristics at baseline

Characteristic	Crohn's disease n=16	Ulcerative colitis/inflammatory bowel disease unclassified n=33
Gender (female/male)	9/7	14/19
Age at disease onset (median, range)	23 (13–42)	26 (8–45)
Age at TNFα-blocker induction (median, range)	32 (15–52)	32 (13–58)
Active smoker, n (%)	4 (25)	4 (12)
Disease duration (years, median, range)	9 (3–25)	6 (1–35)
Disease behavior (Mb Crohn), n (%)		
Inflammatory (B1)	10 (63)	
Stricturing (B2)	4 (25)	
Penetrating (B3)	1 (6)	
B1±perianal disease	0	
B2±perianal disease	1 (6)	
B3±perianal disease	0	
Disease location	Ileum (L1) 1	Proctitis 0
	Colon (L2) 4	Left colon 14
	Ileocolon (L3) 11	Extensive colitis 19
Previous surgical resection, n (%)	8 (50)	
TNFα-blocking therapy, n (%)		
Infliximab	11 (69)	33 (100)
Adalimumab	5 (31)	
Concomitant medications, n (%)		
Mesalazine	3 (19)	3 (9)
Azathioprine/6-MP	7 (44)	12 (36)
Azathioprine/6-MP+mesalazine	4 (25)	17 (52)
Methotrexate+mesalazine	1 (6)	
Biologic variables (median, range)		
Hemoglobin level (g/L)	135 (109–171)	142 (121–167)
Leukocyte count (10 * 9/L)	5.1 (3.2–10.7)	4.7 (2.8–8.4)
Platelet count (10 * 9/L)	256 (173–437)	248 (118–408)
CRP (mg/L)	<3 (<3–16)	<3 (<3–14)
Fecal calprotectin (µg/g)	52 (7–81)	34 (3–97)
Activity indices (median, range)		
HBI	0 (0–4)	
SES-CD	0 (0–2)	
Partial Mayo score		0 (0–1)
Endoscopic Mayo score		0 (0–1)

6-MP: 6-Mercaptopurine; HBI: Harvey–Bradshaw Index; SES-CD: Simple Endoscopic Score for Crohn's Disease.

50% sensitivity. The generalized estimating equation model showed that at relapse, patients with relapse had higher FC levels (estimated 199 µg/g, 95% CI 103–386 µg/g) than nonrelapsers at the end of the follow-up (49 µg/g, 95% CI 35–69 µg/g). Patients with relapse also showed a significant time-dependent increase in FC values before the relapse; the estimated value at 8 months before the relapse would be 35 µg/g (95% CI 17–73 µg/g) increasing to the above-mentioned value of 199 µg/g at relapse (Table 2). When the relapse rates in patients with FC concentration under 50 µg/g and 50–100 µg/g at baseline were compared, no difference was found (10/27 [37%] versus 5/22 [23%], p = 0.325). When comparing median hemoglobin (Hb), CRP and platelet (PLT) levels at baseline and at the time of relapse, a significant difference was found in CRP and PLT levels (0 [0–1] vs 4 [0–38] mg/L, p = 0.007 and 246 [188–408] vs 268 [201–364] E9/L, p = 0.008 respectively), but not in Hb levels (139 [127–171] vs 136.5 [128–160] g/L, p = 0.242). The elevation of CRP correlated also with the elevation of FC during the follow up (Spearman's rho 0.708, p = 0.01).

3.2 FC concentrations in the remission group

In the remission group, 12/34 patients (35%) had normal FC measurements throughout the study and 22/34 patients (65%) had at least one elevated FC level (>100 µg/g) during the follow-up. Interestingly, the elevation in FC concentration appeared to be transient in the majority of patients (18/22, 82%). Eight patients (three UC and five CD) had two or more subsequently elevated FC concentrations and four of these patients (two UC and two CD) reported mild, but prolonged clinical symptoms. However, the clinical symptoms did not occur simultaneously with the elevated FC measurements. Furthermore, mild symptoms not fulfilling the relapse criteria also appeared in 10/12 (eight UC and four CD) of the patients having normal FC values throughout the study. The median FC level was significantly higher only during the last 2 months of the follow-up (49 µg/g, 0–730, p = 0.0445).

3.3 Histological score at baseline and FC

The histological scores are presented in Fig. 2. Of 49 specimens, 40 (82%) showed no signs of histological inflammation at baseline, whereas mild infiltration of neutrophils in the lamina propria or the epithelium appeared in four patients (8%) and moderate inflammation in five specimens (10%). The histological examination at baseline showed mild to moderate architectural abnormalities in 17 of 49 patients (35%) and basal plasmacytosis in 15 (31%) patients. The median histology score in patients who relapsed was equal with those who remained in remission (p = 0.324). There was no significant difference found in the relapse rate in patients with acute or chronic inflammatory infiltrate at baseline compared to those who

had no signs of inflammation in biopsy specimens (p = 0.221) and the median histology score in patients who relapsed was equal to those who remained in remission (p = 0.324). At 4 months ileocolonoscopy was performed in 41 patients. There was no significant difference found in the relapse rate in patients with acute or chronic inflammatory infiltrate (p = 0.223). At relapse, 3/15 patients (20%) showed mild infiltration of neutrophils in the lamina propria or epithelium in biopsy specimens, 9/15 patients (60%) moderate inflammation, and 2/15 (13%) severe inflammation. Only one CD patient with endoscopic ileitis had normal biopsy specimens. Of patients in remission at 12 months after cessation of TNFα-blocking therapy, 31/34 underwent ileocolonoscopy and showed either histological remission (24/31, 77%) or only mild histological inflammation in specimens (7/31, 23%). Of the 24 patients in full histological remission, 18 provided a stool sample for FC measurement. The majority (16/18, 89%) of these patients had normal (< 100 µg/g) FC concentration (34 µg/g, range 0–178), whereas less than half (40%) of the patients having mild inflammatory activity had normal FC levels (156 µg/g, range 57–340, n = 6). A significant difference in the median FC levels was found between these two groups, p = 0.016. No difference was found in the relapse rate between patients having FC under 50 µg/g and no inflammatory activity in specimens at baseline, compared with the others (p = 0.723).

4. Discussion

In this prospective study, a continuously elevated FC concentration measured after cessation of TNFα-blocking therapy predicted clinical and endoscopic relapse. Patients who remained in clinical remission more often had normal FC concentration (< 100 µg/g) or only transiently elevated FC during the follow-up than patients who experienced clinical or endoscopic relapse. Normal FC concentrations in patients with clinical and endoscopic remission were strongly associated with histological remission. Nevertheless, histological inflammation at the time of cessation of TNFα-blocking therapy did not predict relapse. On the other hand, a rising histological score seems to predict relapse after stopping of TNFα-blocking therapy.

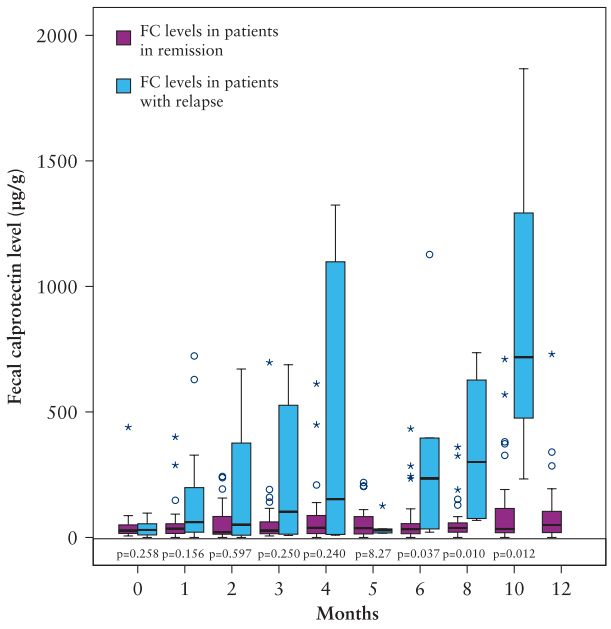

Figure 1. FC levels in patients in sustained remission and in patients with relapse during the follow-up. Boxes indicate interquartile ranges (IQR), with horizontal lines indicating medians, whiskers indicating the upper and lower limits, points indicating values > 1.5 IQR and stars indicating values > 3 IQR.

To the best of our knowledge, this is one of the first studies to demonstrate the predictive value of FC after stopping TNFα-blocking therapy in IBD patients in deep remission with clinical outcome also documented endoscopically. The observational, prospective STORI trial is the largest study exploring the duration of remission in CD after discontinuation of TNFα-blocking therapy.[24] In contrast to our study, in which a normal baseline FC concentration was an inclusion criterion, the FC values at baseline in the STORI trial were elevated up to 318 µg/g in some patients, and an FC value above 300 µg/g was an independent factor associated with relapse. The preliminary data from the STORI trial on CD indicate that FC may be equal to endoscopic assessment in predicting the risk of relapse after cessation of TNFα-blocking therapy, and that FC begins to increase 4–6 months before the clinical relapse, and that the therapeutic strategy could by adapted by measuring these markers every 3–4 months.[25] In other published studies on outcome after discontinuation of TNFα-blocking therapy, FC measurement was not included.[26–28]

Since FC concentration is a reliable parameter of mucosal inflammation activity in IBD,[3] it has also appeared to be a good marker for predicting clinical relapse.[1,29–32] In a pioneer study, Tibble et al.[1] found that in IBD clinical remission, an elevated FC concentration predicts clinical relapse in both CD and UC. This ability of a single FC concentration to predict clinical relapse in IBD patients with clinically quiescent disease was examined in several further studies.[29–33] A recent meta-analysis of these prospective studies showed a pooled sensitivity of 78% and a specificity of 73% for FC in predicting IBD relapse, especially in ileocolonic and colonic CD and UC, demonstrating the usefulness of a simple and noninvasive FC test in predicting relapse in quiescent IBD patients.[2] Furthermore, a large retrospective cohort of CD patients provided compelling evidence that FC measurement could be used to predict disease course, because FC concentrations were significantly higher in patients with progressive CD than in those without disease progression.[34] De Vos et al.[35] recently published a study indicating that two consecutive FC measurements over 300 mg/kg is more specific than a single measurement for predicting relapse in UC patients under maintenance treatment with infliximab. In their study, nearly 80% of the patients in sustained deep remission had at least one FC measurement over 50 mg/kg, similar to our study in which 65% of patients in stable remission had at least one FC measurement over 100 µg/g during the follow-up. De Vos et al. suggested that rather than baseline FC levels, repeated measures of FC may help in adjusting therapy in UC patients before clinical symptoms. This incapability of predicting relapse based on the baseline FC level in previous study, as well as in our own, is most likely due to the fact that all patients were in deep remission and had low FC levels (< 100 µg/g) at the time of inclusion. The results of our study suggest that repeatedly elevated FC levels predict clinical and/or endoscopic relapse, whereas mild, transient clinical symptoms seem to have no correlation with disease outcome. However, patients with ongoing clinical symptoms with constantly elevated FC are potentially the ones to benefit the most from a close follow-up. More importantly, repeated measurements of FC seem to be a useful noninvasive tool for monitoring patients without endoscopy after cessation of TNFα-blocking therapy, as the STORI trial indicated. A repeatedly measured normal FC concentration after stopping anti-TNFα-blocking agents seems to predict clinical and endoscopic remission well. FC levels measured frequently, as

Table 2. General estimating equation model for fecal calprotectin.

	Parameter	95% CI		
	Estimate	Lower	Upper	p
Intercept	1.695	1.550	1.839	<0.0001
Relapse	0.606	0.359	0.853	<0.0001
Time before relapse or end of followup	0.017	0.032	0.001	0.037
Relapse*Time interaction	0.078	0.128	0.028	0.002

Time at relapse is 0 and time before relapse is counted as negative.
Model: log (calprotectin)=intercept+β1*relapse+β2*time+β3*time*relapse, were βs are parameters for each variable.

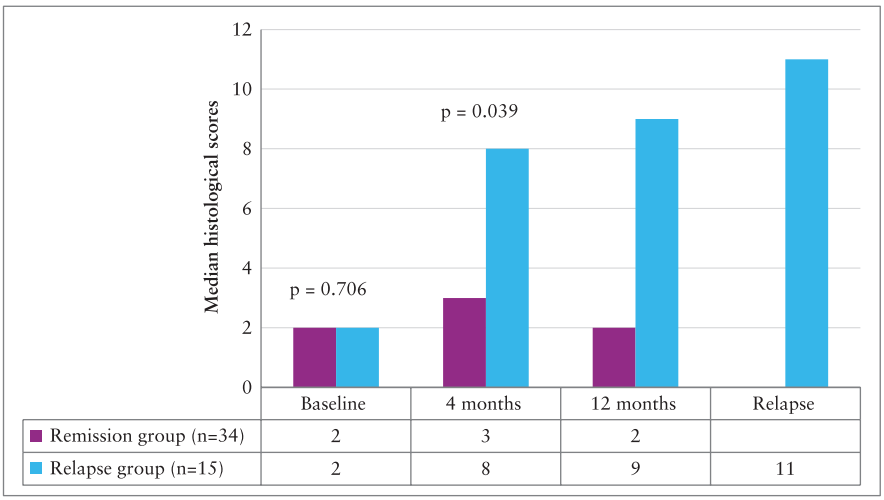

Figure 2. The comparison of median histological scores during the follow-up. Numbers below the histogram showing the median histological score at baseline, at 4 months, 12 months and at the time of relapse.p < 0.05*** (Mann–Whitney U test) comparisons of median FC levels between remission-group and relapse-group.

often as every 4 weeks, could be the most recommendable approach for predicting relapse considering the fairly low sensitivity of a single FC measurement to predict the relapse. Larger studies are needed to determine the optimal interval for measuring FC levels in prediction of relapse, as well as to determine whether treatment could be restarted only by documenting repeatedly elevated FC levels during the follow-up.

The impact of histological healing among IBD patients treated with TNFα-blocking therapy is still to some extent unknown. Over a decade ago, Sandborn et al.[15] stated that the assessment of histological disease activity as a treatment endpoint is not recommended, due to uncertainty in the significance of histological disease activity, the locality of CD inflammation, and the potential for sampling error. Histological changes usually stay behind clinical and endoscopic improvement and, hence, have very little clinical relevance in decision making. More recently, D'Haens et al.[21] stated that histological remission should be considered as a secondary endpoint, at least in clinical trials and the scoring system for histological abnormalities should be used. Importantly, the endpoints for histological remission are still lacking and should be well-defined and validated. Roseth et al.[10] demonstrated that patients with low FC values more often have normal histological findings, which was also seen in our study here and previously.[36] Low-grade infiltration of inflammatory cells in the lamina propria at the time of cessation of TNFα-blocking therapy does not impact the relapse rate.

In our study, over half of the patients in stable remission had some variation in FC level during the follow-up (range 0–730 µg/g). A daily variation in FC excretion in serial samples was demonstrated in an adult population with or without IBD.[5,37] However, a recent study in a large cohort of clinically quiescent CD patients showed a fairly low daily variation in FC concentrations obtained over 3 consecutive days.[38] The use of nonsteroidal anti-inflammatory drugs (NSAIDs) affects FC levels.[39] The regular use of NSAIDs was registered at baseline, but not during the study, which may to some extent have impacted the FC levels in the present study. In our study, up to 27% of patients had normal FC levels at the time of clinical and endoscopic relapse; 75% (3/4) of these patients showed marked elevation of FC levels during the follow-up and could have been detected by closer monitoring, as previously recommended.

We are aware of the weaknesses in our study. The patient group was limited and heterogeneous. We included both CD and UC patients regardless of localization, behavior, or duration of disease. Moreover, the stool samples for FC measurements were provided every month for only over the first 6 months and thereafter every 2 months. More concise intervals for follow-up samples would have been more accurate, but probably more inconvenient for the patients. In addition, several patients failed to provide stool samples according to the study protocol. Clinical scoring and endoscopic scoring were performed by several experienced gastroenterologists, making some interobserver variability in the scoring system possible. Unfortunately, rescoring of the endoscopies was not possible. However, the strength of this study is that ileocolonoscopies with histological biopsy specimens were included in the follow-up protocol. At the end of this study, some patients in remission had rising FC

levels possibly reflecting a later clinical relapse after the 12-month study follow-up period.

In conclusion, FC seems to increase and remain elevated as early as 6 months before clinical or endoscopic relapse, which makes it a useful surrogate marker for predicting relapse in patients with IBD and identifying patients requiring a close follow-up in clinical practice. Transient variation in FC concentrations in patients with stable remission occurs frequently. Constantly normal FC has a high positive predictive value for clinical and endoscopic remission.

Conflict of interest statement

Statement of authorship: study design (PM, MF, TS), data collection (PM, MF, KS, HK, RK, TB, AJ, HR, MN, JH, PA, UN, JK, JP, K-LK, AR, HM, TS), statistical analysis (PM, HM), drafting the manuscript (PM, MF, TS), final reading and approval of the manuscript (all authors).

PM received lecture fees from Abbvie and MSD and consulting fees from Abbvie, MSD, and Tillotts Pharma. MF received consulting fees from MSD, Abbvie, Janssen, Orion Pharma, Medivir, and Roche, and lecture fees from MSD, Abbvie, Bayer, Janssen, and Tillotts Pharma. RK received consulting fees from MSD and lecture fees from Orion Pharma and Tillotts Pharma. TB received lecture fees from Abbvie and Tillotts Pharma and consulting fees from Abbvie. AJ received lecture fees from Abbvie, MSD, and Tillotts Pharma and consulting fees from Abbvie and MSD. PA received lecture fees from Abbvie, MSD, GSK, and Roche. JH received lecture fees from MSD and consulting fees from Tillotts Pharma. HR received lecture fees from MSD, Roche, and Tillotts Pharma. JP received consulting fees from Almirall and Shire. K-LK received consulting fees from MSD, Abbvie, and Tillotts Pharma. TS received consulting fees from MSD, Takeda, and Tillotts Pharma and lecture fees from Abbvie, Medans, MSD, Roche, and Tillotts Pharma. KS, HK, UN, MN, JK, and HM declare no conflicts of interest.

Acknowledgments

We thank study nurses Pirkko Tuukkala, Paula Karlsson, Virpi Pelkonen, and Anne Nikkonen for their excellent assistance. We also thank M.D. Heimo Nurmi, M.D. Perttu Sahlman, and M.D. Ulla Turunen for their help in recruiting the patients. This study was supported by grants from the Helsinki University Central Hospital Research Fund (EVO-grant), Finnish Cultural Foundation, Mary and George C. Ehrnrooth Foundation, Finnish Foundation for Gastroenterological Research, Sigrid Jusélius Foundation, and Foundation for Pediatric Research, and an unrestricted grant from Merck & Co., Inc. Finland.

References

1. Tibble J.A., Sigthorsson G., Bridger S., Fagerhol M.K., Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. *Gastroenterology* 2000;**119**:15–22.
2. Mao R., Xiao Y.-I., Gao X., Chen B.-I., He Y., Yang L. *et al.* Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. *Inflamm Bowel Dis* 2012;**18**:1894–1899.
3. Poullis A., Foster R., Mendall M.A., Fagerhol M.K. Emerging role of calprotectin in gastroenterology. *J Gastroenterol Hepatol* 2003;**18**:756–762.
4. Tibble J., Teahon K., Thjodleifsson B., Roseth A., Sigthorsson G., Bridger S. *et al.* A simple method for assessing intestinal inflammation in Crohn's disease. *Gut* 2000;**47**:506–513.
5. Røseth A.G., Schmidt P.N., Fagerhol M.K. 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**:50–54.
6. Schoepfer A.M., Trummler M., Seeholzer P., Seibold-Schmid B., Seibold F. Discriminating IBD from IBS: comparison of the test performance of fecal markers, blood leukocytes, CRP and IBD antibodies. *Inflamm Bowel Dis* 2008;**14**:32–39.
7. Sipponen T., Savilahti E., Kolho K.L., Nuutinen H., Turunen U., Färkkilä M. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. *Inflamm Bowel Dis* 2008;**14**:40–46.
8. Roseth A.G., Aadland E., Jahnsen J., Raknerud N. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. *Digestion* 1997;**58**:176–180.
9. Limburg P.J., Ahlquist D.A., Sandborn W.J., Mahoney D.W., Devens M.E., Harrington J.J. *et al.* Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. *Am J Gastroenterol* 2000;**95**:2831–2837.
10. Roseth A.G., Aadland E., Grzyb K. Normalization of faecal calprotectin: a predictor of mucosal healing in patients with inflammatory bowel disease. *Scand J Gastroenterol* 2004;**39**:1017–1020.
11. Laharie D., Mesli S., El Hajbi F., Chabrun E., Chanteloup E., Capdepont M. *et al.* Prediction of Crohn's disease relapse with faecal calprotectin in infliximab responders: a prospective study. *Aliment Pharmacol Ther* 2011;**34**:462–469.
12. Molander P., af Björkesten C.G., Mustonen H., Haapamäki J., Vauhkonen M., Kolho K.L. *et al.* Fecal calprotectin concentration predicts outcome in inflammatory bowel disease after induction therapy with TNFa blocking agents. *Inflamm Bowel Dis* 2012;**18**:2011–2017.
13. Molander P., Sipponen T., Kemppainen H., Jussila A., Blomster T., Koskela R. *et al.* Achievement of deep remission during scheduled maintenance therapy with TNFα-blocking. *J Crohn Colitis* 2012;**7**:730–735.
14. Molander P., Färkkilä M., Salminen K., Kemppainen H., Blomster T., Koskela R. *et al.* Outcome after discontinuation of TNFα-blocking therapy in inflammatory bowel disease patients in deep remission. *Inflamm Bowel Dis* 2014;**20**:1021–1028.
15. Sandborn W.J., Feagan B.G., Hanauer S.B., Lochs H., Löfberg R., Modigliani R. *et al.* A review of activity indices and efficacy endpoints for clinical trials of medical therapy in adults with Crohn's disease. *Gastroenterology* 2002;**122**:512–530.
16. Daperno M., D'Haens G., Van Assche G., Baert F., Bulois P., Maunour V. *et al.* Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc* 2004;**60**:505–512.
17. Best W.R. Predicting the Crohn's disease activity index from the Harvey–Bradshaw Index. *Inflamm Bowel Dis* 2006;**12**:304–310.
18. Moskovitz D.N., Daperno M., Van Assche G., Baert F., Gavers A., Sostegni R. *et al.* Defining and validating cut-offs for the Simple Endoscopic Score for Crohn's Disease. *Gastroenterology* 2007;**132**:S1097
19. af Björkesten C.-G., Nieminen U., Sipponen T., Turunen U., Arkkila P., Färkkilä M. Mucosal healing at 3 months predicts long-term endoscopic remission in anti-TNF-treated luminal Crohn's disease. *Scand J Gastroenterol* 2013;**48**:543–551.
20. Schroeder K.W., Tremaine W.J., Ilstrup D.M. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. *N Engl J Med* 1987;**317**:1625–1629.
21. D'Haens G., Sandborn W.J., Feagan B.G., Geboes K., Hanauer S.B., Irvine E.J. *et al.* A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007;**132**:763–786.
22. D'Haens G.R., Geboes K., Peeters M., Baert F., Penninckx F., Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology* 1998;**114**:262–267.
23. von Roon A.C., Karamountzos L., Purkayastha S., Reese G.E., Darzi A.W., Teare J.P. *et al.* Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am J Gastroenterol* 2007;**102**:803–813.
24. Louis E., Mary J.Y., Vernier-Massouille G., Grimaud J.C., Bouhnik Y., Laharie D. *et al.* Maintenance of remission among patients with Crohn's

disease on anti-metabolite therapy after infliximab therapy is stopped. *Gastroenterology* 2012;**142**:63–70.
25. De Suray N., Salleron J., Vernier-Massouille G., Grimaud J.-C., Bouhnik Y., Laharie D. *et al.* Close monitoring of CRP and fecal calprotectin levels to predict relapse in Crohn's disease patients. A sub-analysis of the STORI study. *J Crohn Colitis* 2012;**6**1:P274
26. Clarke K., Regueiro M. Stopping immunomodulators and biologics in inflammatory bowel disease patients in remission. *Inflamm Bowel Dis* 2012;**18**:174–179.
27. Waugh A.W., Garg S., Matic K., Gramlich L., Wong C., Sadowski D.C. *et al.* Maintenance of clinical benefit in Crohn's disease patients after discontinuation of infliximab: long-term follow-up of a single centre cohort. *Aliment Pharmacol Ther* 2010;**32**:1129–1134.
28. Steenholdt C., Molazahi A., Ainsworth M.A., Brynskov J., Thomsen O.Ø., Seidelin J.B. Outcome after discontinuation of infliximab in patients with inflammatory bowel disease in clinical remission: an observational Danish single center study. *Scand J Gastroenterol* 2012;**47**:518–527.
29. Gisbert J.P., Bermejo F., Perez-Calle J.L., Taxonera C., Vera I., McNicholl A.G. *et al.* Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis* 2009;**15**8:1190–1198.
30. Costa F., Mumolo M.G., Ceccarelli L., Bellini M., Romano M.R., Sterpi C. *et al.* Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005;**54**:364–368.
31. García-Sánchez V., Iglesias-Flores E., González R., Gisbert J.P., Gallardo-Valverde J.M., González-Galilea A. *et al.* Does fecal calprotectin predict relapse in patients with Crohn's disease and ulcerative colitis?. *J Crohn Colitis* 2010;**4**:144–152.
32. D' D'Incà R., Dal Pont E., Di Leo V., Benazzato L., Martinato M., Lamboglia F. *et al.* Can calprotectin predict relapse risk in inflammatory bowel disease?. *Am J Gastroenterol* 2008;**103**:2007–2014.
33. Kallel L., Ayadi I., Matri S., Fekih M., Mahmoud N.B., Feki M. *et al.* Fecal calprotectin is a predictive marker of relapse in Crohn's disease involving the colon: a prospective study. *Eur J Gastroenterol Hepatol* 2010;**22**:340–345.
34. Kennedy N.A., Chang J., Guy M.H., Smith T., Loh J.T., Haunschmidt D. *et al.* Elevated faecal calprotectin predicts disease progression in Crohn's disease. *Gut* 2013;**62**:A7
35. De Vos M., Louis E.J., Jahnsen J., Vandervoort J.G., Noman M., Dewit O. *et al.* Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. *Inflamm Bowel Dis* 2013;**19**:2111–2117.
36. Sipponen T., Kärkkäinen P., Savilahti E., Kolho K.-L., Nuutinen H., Turunen U. *et al.* Correlation of faecal calprotectin and lactoferrin with an endoscopic score for Crohn's disease and histological findings. *Aliment Pharmacol Ther* 2008;**28**:1221–1229.
37. Husebye E., Ton H., Johne B. Biological variability of fecal calprotectin in patients referred for colonoscopy without colonic inflammation or neoplasm. *Am J Gastroenterol* 2001;**96**9:2683–2687.
38. Naismith G.D., Smith L.A., Barry S.J.E., Munro J.I., Laird S., Rankin K. *et al.* A prospective single-centre evaluation of the intra-individual variability of faecal calprotectin in quiescent Crohn's disease. *Aliment Pharmacol Ther* 2013;**37**:613–621.
39. Tibble J.A., Sigthorsson G., Foster R., Scott D., Fagerhol M.K., Roseth A. *et al.* High prevalence of NSAID enteropathy as shown by a simple faecal test. *Gut* 1999;**45**:362–366.