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Prognostic significance of faecal calprotectin in patients with inactive inflammatory bowel disease

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Abstract

Background: Faecal calprotectin, an established biomarker used to assess mucosal inflammation, has been shown to correlate with endoscopic activity in inflammatory bowel disease (IBD). Longitudinal monitoring of faecal calprotectin, however, has rarely been employed beyond assessment of therapy response and post hoc analyses of clinical trials.

Aim: We studied whether consecutive measurements of faecal calprotectin every third month are useful for monitoring patients with IBD in clinical remission.

Methods: Patients aged 18 years or older, with a known diagnosis of IBD in clinical remission, were prospectively studied. Patients provided faecal samples every third month and were prospectively followed until the first clinical relapse or the end of the two-year follow-up period. Measurements (EK-CAL, Bühlmann Lab. AG, Switzerland) were done at the end of the study. A Cox model with time-dependent covariates was used for analysis.

Results: Among 104 patients, Crohn's disease (n=49) and ulcerative colitis (n=55), 37 had a relapse. A doubling of faecal calprotectin level between two consecutively collected samples was associated with a 101% increased risk of relapse (HR: 2.01; 95% CI: 1.53-2.65; $p<0.001$). The relative risk of relapse attenuated with time (HR: 0.80; 95% CI: 0.75-0.86; $p<0.001$), by a 20% decrease in risk of relapse per three-month period since the sample was obtained.

Conclusions: By consecutively measuring faecal calprotectin every third month, we quantified the risk of relapse related to faecal calprotectin change and observed attenuation of the risk across time. Our data suggest that longitudinal monitoring of faecal calprotectin is informative in predicting relapse in IBD.

Keywords: Inflammatory bowel disease, longitudinal monitoring, faecal calprotectin, time-dependent covariates

Introduction and Aim

Inflammatory bowel disease (IBD) including the two major forms, Crohn's disease and ulcerative colitis, is an inflammatory condition of the gastrointestinal tract characterized by periods of remission and intermittent relapses. The disease entity is associated with decreased quality of life, especially during relapses when treatment must be intensified. Currently, there are limited possibilities to predict the future disease course for an individual patient. Mucosal healing is presently the best prognostic marker,¹ being associated with reduced risk of clinical flares within the following two-year period.² IBD, however, is a life-long disease. Repeated colonoscopies are generally not accepted by patients and are associated with procedure-related risk and substantial cost.

Inflammatory markers in blood, such as C-reactive protein (CRP) and erythrocyte sedimentation rate, are useful to confirm ongoing mucosal inflammation but of less value to predict a future relapse, since elevated levels of these markers have not been found to precede a clinical flare.^{3, 4} Faecal-based biomarkers, such as faecal calprotectin, might represent a better prognostic tool.^{5, 6} Calprotectin accounts for approximately 40% of the cytosolic content of neutrophils and is released upon neutrophil activation. A correlation between the concentration of faecal calprotectin and endoscopic activity has consistently been reported in both ulcerative colitis and Crohn's disease.⁷⁻¹² Increased faecal calprotectin, measured at a single occasion, has also been associated with an increased risk of a future flare, especially within the following three months. The reliance on single measurements may not be of great help given that IBD activity changes, sometimes rapidly, over time. Recently, post hoc analyses of clinical trials indicate that repeated measurements of faecal calprotectin every second or third month can predict future flares in selected patient populations, such as Crohn's disease patients treated with anti-TNF therapy and ulcerative proctitis patients treated with 5-ASA.^{13, 14} However, any attempt to identify an

absolute cut-off will be limited by pronounced inter-assay differences¹⁵⁻¹⁷ and will probably be disease and phenotype specific. Therefore, we aimed to assess whether consecutive measurements of faecal calprotectin every third month can be used to monitor unselected patients with IBD in clinical remission. More specifically, we wanted to evaluate whether change in faecal calprotectin between consecutively collected samples was associated with increased risk of future flare.

Materials and methods

Patients and study design

This is a 24-month prospective study where patients aged 18 years or older, with a confirmed diagnosis of Crohn's disease or ulcerative colitis, were consecutively screened for participation while attending the outpatient Gastroenterology Clinic at Örebro University Hospital. Patients in clinical remission based on the physician's global assessment¹⁸ were eligible for inclusion irrespective of medication. Exclusion criteria were previous substantial surgical resections or any other systemic disease. Information on disease phenotype according to the Montreal classification¹⁹ was extracted from the medical notes. Patients were asked to fill in a questionnaire and to provide a faecal sample at baseline. A faecal sample and a filled-in questionnaire was subsequently obtained every third month until the first clinical relapse or the end of the 24-month follow-up period. The questionnaire included questions on patient-reported outcome measures (e.g. number of stools/day, abdominal pain, blood and mucus in stool), daily rating of quality of life,²⁰ present IBD therapy, dietary habits, and use of antibiotics or NSAIDs. Patients were followed with scheduled appointments according to clinical routine and instructed to contact the outpatient clinic if gastrointestinal symptoms consistent with a relapse of IBD occurred between appointments during follow up. Relapse was defined as increasing symptoms

necessitating intensified medical therapy or surgery. If a patient did not provide a faecal sample at any of the three-month periods, a written reminder was sent.

Faecal calprotectin measurement

Faecal samples were collected in screw-capped plastic containers, sent the same day by mail to the laboratory, and stored at -70°C . Faecal calprotectin was extracted and analysed with ELISA according to the manufacturer's protocol (EK-CAL, Bühlmann Lab. AG, Switzerland) after all patients had ended the follow up. Consequently, the results of the faecal calprotectin measurements did not influence any clinical decisions. Faecal calprotectin levels were expressed as micrograms of faecal calprotectin per gram of faeces, with the limit of detection (LOD) $10\text{ }\mu\text{g/g}$.

Statistics

Continuous variables with skewed distribution are presented as median and interquartile range (IQR). Differences between groups were compared using the Mann-Whitney U test, and frequencies were compared using the Chi-squared test. Variables that potentially affected sampling completeness were assessed using the logistic regression model.

The predictive value of faecal calprotectin was assessed in the extended Cox regression model that allows for non-proportional hazards by including an interaction term between faecal calprotectin and time. The hazard ratios (HR) with corresponding 95% confidence interval (CI) were calculated. Missing faecal calprotectin values were imputed with the “last observation carried forward” technique.²¹ For faecal calprotectin levels below the LOD $10\text{ }\mu\text{g/g}$, $10/\sqrt{2}$ served as a substitute.²² To comply with the requirement of normal distribution for continuous dependent variables, analyses were carried on base 2 log-transformed faecal calprotectin values.

We performed a sensitivity analysis by including different covariates that might confound the effect of the faecal calprotectin in the Cox regression model. Model 1 included baseline faecal calprotectin, age, and the interaction term between faecal calprotectin and time. Model 2 included time-varied faecal calprotectin, age, sex, and the interaction term between FC and time. Model 3 included time-varied FC, age, sex, and the interaction term between faecal calprotectin and time adjusted for medical treatments (oral corticosteroids, local corticosteroids, oral sulfasalazine, oral 5-aminosalicylic acid, local 5-aminosalicylic acid, immunomodulators, and anti-TNF therapy).

The analysis of linear mixed models, with allowance for dependence of faecal calprotectin values within the diagnosis and the study individual, was used to assess the influence of lifestyle factors, disease activity variables, and current treatments on faecal calprotectin levels. A two-sided p value of <0.05 was considered statistically significant for all analyses.

The Ethics Committee of Uppsala University approved the study (Dnr 2007/291). All patients signed written informed consent.

Results

Patients

In total, 169 patients were screened; 17 patients declined to participate, and 22 patients had active disease (Figure 1). Of the remaining 130 patients, 26 did not provide a faecal sample and were excluded from the analyses. Thus, 104 patients, Crohn's disease ($n=49$) and ulcerative colitis ($n=55$), entered the analysis and provided 525 faecal samples during the follow up, 6 samples of which were discarded due to inadequate packaging. A complete set of faecal samples was obtained from 61 patients, whereas 43 patients did not provide a faecal sample on one or

more occasions. Factors affecting sampling completeness were age at diagnosis ($p=0.005$) and age at enrolment ($p=0.013$), where patients younger than 40 years of age less often provided a complete sample set. A significant correlation between age at diagnosis and age at enrolment was observed ($R=0.585$, $p<0.0001$), necessitating the adjustment of main analysis for only one of these variables.

Figure 1. Flow chart over the patient recruitment process, including information on number of patients providing complete set of samples and number of patients with incomplete set of samples.

During follow up, 37 patients experienced a clinical relapse, and 67 remained in clinical remission. No differences were observed between the patients who relapsed and those who remained in clinical remission in terms of baseline demographics or clinical characteristics, but the proportion of patients treated with oral corticosteroids at baseline seemed to be higher in Crohn's disease and ulcerative colitis patients that relapsed during the study period, $p=0.049$ and $p=0.053$, respectively (Table 1).

Table 1. Data on demographics, clinical characteristics and treatment at baseline in study patients that relapsed or remained in clinical remission during the study.

	Relapse	Remission
Crohn's disease		
n	16	33
Age (years; median (IQR))	50 (42-61)	58 (41-64)
Males	7 (43.8%)	17 (51.5%)
Disease duration (years; median (IQR))	18.5 (9.75-22.5)	25 (11.5-38)
Previous bowel resection	7 (43.8%)	22 (66.7%)
Baseline FC ($\mu\text{g/g}$; median (IQR))	403 (85-590)	171 (62-458)
Location		
L1 (Ileal)	4 (25.0%)	9 (27.3%)
L2 (Colonic)	7 (43.8%)	8 (24.2%)
L3 (Ileocolonic)	5 (31.3%)	16 (48.5%)
Behaviour		
B1 (Non-stricturing/non-penetrating)	8 (50.0%)	10 (30.3%)
B2 (Stricturing)	4 (25.0%)	14 (42.4%)
B3 (Penetrating)	4 (25.0%)	9 (27.3%)
P (Perianal disease)	1 (6.3%)	7 (21.2%)
No treatment	3 (18.8%)	11 (33.3%)
Topical corticosteroids and/or 5-ASA	0 (0%)	1 (3.0%)
Oral 5-ASA or sulfasalazine	5 (31.3%)	9 (27.3%)
Oral corticosteroids	5 (31.3%)	3 (9.1%)
Immunomodulators	8 (50.0%)	16 (48.5%)
Anti-TNF therapy	1 (6.3%)	1 (3.0%)
Ulcerative colitis		
n	21	34
Age (years; median (IQR))	56 (38-65)	59 (49-65)
Males	8 (38.1%)	18 (52.9%)
Disease duration (years; median (IQR))	11 (4.5-16)	20.5 (10-29)
Baseline FC ($\mu\text{g/g}$; median (IQR))	92 (29-551)	100 (33-277)
Extent		
E1 (Proctitis)	2 (9.5%)	4 (11.8%)
E2 (Left-sided)	9 (42.9%)	16 (47.1%)
E3 (Extensive)	10 (47.6%)	14 (41.2%)
No treatment	1 (4.8%)	5 (14.7%)
Topical corticosteroids and/or 5-ASA	2 (9.5%)	2 (5.9%)
Oral 5-ASA or sulfasalazine	10 (47.6%)	22 (64.7%)
Oral corticosteroids	6 (23.8%)	2 (5.9%)
Immunomodulators	7 (33.3%)	12 (35.3%)
Anti-TNF therapy	0 (0%)	2 (5.9%)

Predictive value of baseline faecal calprotectin measurement

The frequency of relapse was 37/104 (35.6%) during the two-year study period in the entire study population regardless of faecal calprotectin. To determine if baseline faecal calprotectin influenced the risk of relapse, we analysed baseline faecal calprotectin measurements in an extended Cox regression model that allowed non-proportional hazards, (Model 1). Holding all other covariates at their means, the risk of relapse was increased by 47% (HR: 1.47; 95% CI: 1.09-1.99, $p=0.012$) per unit increase in log2-transformed baseline faecal calprotectin (Table 3, Model 1). In other words, a doubling of the baseline faecal calprotectin concentration resulted in a 47% increased risk of clinical relapse. Inclusion of age as predictor of relapse in the model did not change the results. A significant interaction between time until relapse and baseline faecal calprotectin was observed ($p=0.017$), which indicates that the proportional hazard assumption does not hold. In other words, the hazard ratio of the baseline faecal calprotectin changed across time.

To further explore the influence of baseline faecal calprotectin on time until relapse, patients were categorized into three groups based on the concentration of faecal calprotectin at baseline (<250 µg/g, 250-500 µg/g, >500 µg/g). A low baseline faecal calprotectin was associated with a lower cumulative relapse frequency, both at 3 months ($p=0.011$) and 12 months ($p=0.014$, Table 2). However, the association did not remain significant when the entire study period of 24 months was taken into account ($p=0.140$).

Table 2. Cumulative number of patients with clinical relapse during the 24-month follow up in relation to baseline concentration of faecal calprotectin.

FC (µg/g) at baseline	Cumulative number of patients with clinical relapse, n (%)
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	0-3 months	0-12 months	0-24 months
<250	2/61 (3.3%)	9/61 (14.8%)	19/61 (31.1%)
250-500	1/21 (4.8%)	5/21 (23.8%)	7/21 (33.3%)
>500	5/22 (22.7%)	10/22 (45.4%)	11/22 (50.0%)
Total	8/104 (7.7%)	24/104 (23.1%)	37/104 (35.6%)

FC; faecal calprotectin

Predictive value of consecutive faecal calprotectin measurements over time

Serial estimations of faecal calprotectin every third month indicated stable concentrations in the patients that remained in remission throughout the follow up (Figure 2). In patients with a clinical relapse, the concentration of faecal calprotectin at the time of relapse ($t=0$) was significantly elevated, 513 $\mu\text{g/g}$ (231-1173), compared to those in sustained remission, 162 $\mu\text{g/g}$ (53-264), $p<0.0001$. At time point -3 months, the concentration of faecal calprotectin was 305 $\mu\text{g/g}$ (72-827) in patients that subsequently relapsed compared to 197 $\mu\text{g/g}$ (51-468) in patients that maintained remission ($p=0.12$). Individual data points of faecal calprotectin are shown in figure 3A-B for time points -6, -3, and 0 months.

Figure 2. Serial estimations of faecal calprotectin in patients with Crohn's disease or ulcerative colitis: time 0 refers to the end of the study period or time of relapse. Data are shown as boxes (median and IQR), separately for patients with relapse and for patients with sustained clinical remission during the study period. FC: faecal calprotectin

Figure 3. Individual data points for serial estimations of faecal calprotectin in patients with Crohn disease or ulcerative colitis. A) Patients that maintained remission: time 0 refers to the end of the study period. B) Patients that had a clinical relapse: time 0 refers to the time of relapse.

3A

3B

To investigate the effect of time-variant faecal calprotectin, we treated faecal calprotectin as a segmented time-variant variable in the extended Cox regression model (Model 2). A doubled risk of relapse was observed per a doubling of the faecal calprotectin concentration (HR: 2.01; 95% CI: 1.53-2.65; $p < 0.001$). There was an interaction between faecal calprotectin and time of follow up (HR: 0.80; 95% CI: 0.75-0.86; $p < 0.001$), which means that the predicted risk of relapse would be decreased by 20% when the time since the faecal calprotectin sample was obtained was increased by a unit, i.e. three months (Table 3, Model 2). In other words, the most recent faecal calprotectin has the strongest association with the relapse. The risk of relapse and the time interaction remained significant when the analysis was performed for Crohn's disease and

ulcerative colitis patients separately, (HR: 2.37; 95% CI: 1.46-3.87; $p=0.001$) and (HR: 1.57; 95% CI: 1.11-2.23; $p=0.012$), respectively.

Potential confounding lifestyle or treatment factors

To explore whether faecal calprotectin levels were systematically confounded by any additional variables other than those included in Model 2 (IBD therapy, patient characteristics, patient reported outcomes, diet, and use of antibiotics or NSAIDs), we used analysis of mixed models design to allow for dependence of faecal calprotectin within a diagnosis and an individual. Male sex and poor general well-being were associated with higher faecal calprotectin levels, ($p=0.03$) and ($p<0.001$), respectively (Supplement Table 1).

Supplement Table 1. Associations between log2-transformed faecal calprotectin and lifestyle-, patient reported outcomes-, and treatment factors by linear mixt modelling.

Variables	Estimate	95% Confidence Interval		Significance, p
		Lower Bound	Upper Bound	
Intercept	6,90	5.21	8.58	<0.001
Male sex	0.76	0.08	1.44	0.03
Age	-0.02	-0.05	0.01	0.14
Antibiotic treatment within the last 12 months	0.10	-0.23	0.42	0.56
Use of NSAID within the last 12 months	0.27	-0.03	0.56	0.08
Gastroenteritis within the last 3 months	-0.03	-0.41	0.35	0.89
Use of specific diet	0.02	-0.59	0.62	0.95
Consumption of bacterial culture within last week	0.01	-0.32	0.34	0.96
Blood in stools	0.34	-0.19	0.86	0.21
Mucous in stools	0.35	-0.04	0.74	0.08
Daily rating of quality of life (IBD today)	0.53	0.26	0.80	<0.001
Number of bowel movements	0.03	-0.13	0.19	0.72
Number of loose stools	0.001	-0.15	0.16	0.98
Oral steroids	-0.48	-1.06	0.10	0.11

Topical steroids	0.87	-0.16	1.89	0.10
Oral 5-aminosalicylic acid	0.52	-0.09	1.13	0.09
Oral sulfasalazine	0.21	-0.52	0.94	0.57
Topical 5-aminosalicylic acid	0.39	-0.54	1.32	0.41
Immunomodulator	-0.15	-0.78	0.47	0.63
Anti-TNF therapy	0.68	-1.23	2.59	0.48

Despite the finding that IBD therapy did not confound the faecal calprotectin levels, we decided to validate this further by adding the treatment options (oral or topical 5-ASA, oral or topical corticosteroid treatment, immunomodulators or anti-TNF) as covariates to the extended Cox regression model (Model 3). The associations remained significant and very similar HRs were observed when the model was adjusted for treatments (Table 3, Model 3). The concentrations of faecal calprotectin according to the treatments are presented in Supplement Table 2.

Table 3. Data from extended Cox regression on predictors of clinical relapse analysed by Model 1, including baseline faecal calprotectin, age, and product of baseline faecal calprotectin x time; Model 2, including time variant faecal calprotectin, age, sex, and product of time-variant faecal calprotectin and time; and Model 3, including time-variant faecal calprotectin, age, sex, product of time-variant faecal calprotectin, and different treatment factors.

Model 1				
Predictors	HR	95% Confidence Interval		P value
		Lower Bound	Upper Bound	
Baseline FC	1.47	1.09	1.99	0.01
Age	0.99	0.97	1.02	0.66
Baseline FC*Time (in 3 months intervals)	0.001	0.0	0.19	0.02

Model 2				
Predictors	HR	95% Confidence Interval		P value
		Lower Bound	Upper Bound	
Time-variant FC	2.01	1.53	2.65	<0.001
Age	1.01	0.98	1.04	0.67
Male sex	1.40	0.56	3.47	0.47

Calprotectin*Time (in 3 months intervals)	0.80	0.75	0.86	<0.001
Model 3				
Predictors	HR	95% Confidence Interval		P value
		Lower Bound	Upper Bound	
Time-variant FC	2.17	1.57	2.99	<0.001
Age	1.01	0.97	1.01	0.74
Male sex	1.41	0.52	3.78	0.50
Oral steroids	1.16	0.40	3.33	0.78
Topical steroids	2.61	0.26	26.01	0.41
Oral 5-aminosalicylic acid	1.48	0.60	3.69	0.40
Oral sulfasalazine	1.54	0.49	4.84	0.46
Topical 5-aminosalicylic acid	0.82	0.08	8.11	0.87
Immunomodulator	1.70	0.70	4.14	0.24
Anti-TNF therapy	0.98	0.08	12.50	0.99
Calprotectin* Time (in 3 months intervals)	0.79	0.73	0.85	<0.001

HR, Hazard ratio; FC, faecal calprotectin.

Supplement Table 2. Faecal calprotectin, median and interquartile range, in relation to ongoing medical treatment during the study period.

	FC µg/g median (IQR)	
	Treated	Not treated
Oral steroids	402 (204-588)	119 (39-402)
Local steroids	465 (312-754)	126 (40-404)
Oral 5-aminosalicylic acid	137 (30-407)	130 (44-414)
Oral sulfasalazine	171 (56-376)	119 (37-425)
Local 5-aminosalicylic acid	178 (49-283)	130 (40-414)
Immunomodulator	81 (28-410)	171 (52-413)
Anti-TNF therapy	263 (114-528)	128 (40-411)

FC, faecal calprotectin

Discussion

In this prospective study, we explored whether the dynamics of faecal calprotectin were associated with the risk of a future flare in an unselected cohort of patients with IBD in clinical remission. We observed a 101% increased risk of clinical relapse within the following three-

month period per unit increase in log₂-transformed consecutive faecal calprotectin measurements. An interaction between faecal calprotectin and time was also detected, corresponding to a 20% decrease in predicted risk of clinical relapse per three-month period since the sample was obtained. In clinical practice this would mean that a twofold increase of faecal calprotectin between two consecutive measurements with a three-month interval translates into a doubled risk of relapse within the following three months. The association between change in faecal calprotectin and risk of disease relapse is not constant over time. Should the patient not develop a clinical relapse within this period, the increased risk during the subsequent three-month period is 20 % smaller. In other words, the most recent faecal calprotectin has the strongest association with relapse.

Numerous studies have revealed that faecal calprotectin correlates with the inflammatory activity in IBD, especially in patients with colorectal inflammation.²³ Tibble et al. studied IBD patients in clinical remission and observed a thirteenfold increase in risk of clinical relapse within the 12-month follow-up period in patients with faecal calprotectin >50 mg/L, although 59 of the 80 patients who experienced a relapse did so within the first three months.³ Since then, many studies have confirmed this pivotal observation, but the proposed cut-off for identifying patients who are at increased risk of relapse differs between the studies.^{9, 24, 25} One explanation for these cut-off differences are the pronounced inter-assay differences.¹⁵⁻¹⁷ Another explanation is that cut-offs are disease and phenotype specific, since differences in the concentrations of FC have been reported between subgroups of patients with IBD, i.e. patients with ileal and colonic Crohn's disease. We did not try to find a specific cut-off value of faecal calprotectin, as this has been known to lead to results that are highly specific to the study sample and, therefore, unlikely to be generalized. Furthermore, it would have led to estimates that are less precise because of the decrease in the number of patients with higher levels.

Faecal calprotectin is a highly dynamic biomarker of intestinal inflammation and is known to vary during the follow-up time. The rationale of our study was based on the assumption that faecal calprotectin may increase while the disease remains without clinical manifestations for some time. We collected repeated measures of faecal calprotectin every three months and analysed them with appropriate technique, such as time-dependent covariate in the extended Cox regression model. This model allows values of a biological variable to be updated every fixed-time interval. Because the value of faecal calprotectin changed every three months, the value introduced at any time in the model was the last known value before that time.

Patients seen in everyday clinical practice tend to be quite heterogeneous. Remarkably, the use of serial measurements of faecal calprotectin for monitoring disease activity has only been explored in highly selected groups so far, such as anti-TNF treated patients and 5-ASA treated ulcerative proctitis patients within a clinical-trial setting.^{13, 14} De Vos et al. reported that sustained deep remission could be predicted by sustained low faecal calprotectin, when measured every four weeks, in patients with ulcerative colitis in clinical remission under infliximab maintenance therapy. On the contrary, in Crohn's disease patients responding to an IFX-induction regimen, faecal calprotectin measurement at week 14 could not predict clinical relapse at one year.²⁶

By analysing consecutively recruited patients with IBD at an outpatient clinic, we extended previous reports on highly selected groups of patients to the general IBD population. Our data show that a twofold increase of faecal calprotectin between two consecutive samples with a three-month interval is associated with a doubled risk of relapse. The corresponding absolute risk depends on the baseline risk associated with faecal calprotectin in the first sample. For example, a patient who experiences an increase in faecal calprotectin from 20 $\mu\text{g/g}$ to 40 $\mu\text{g/g}$ still has a low absolute risk of developing relapse.

As with many other biomarkers, the distribution of faecal calprotectin is right-skewed. To allow analyses of normalised continuous data and to take the dependence of faecal calprotectin within each individual into account, the concentrations of faecal calprotectin were log2-transformed and then analysed in an extended Cox regression model as a continuous trait instead of as a categorical variable.

The variability in the concentrations of faecal calprotectin in patients with IBD depends on disease activity,²⁷⁻²⁹ stool consistency, and time between bowel movements. Lasson et al. found that the longer the time between bowel movements and the looser the stool consistency, the higher the concentrations of faecal calprotectin.³⁰ Associations between faecal calprotectin and age, obesity, physical inactivity, fibre intake, as well as vegetable consumption have also been reported in healthy individuals.³¹ Therefore, we explored the influence of patient characteristics, patient reported outcome measures, use of antibiotics or NSAIDs, as well as dietary effects on faecal calprotectin in our dataset. NSAIDs have previously been associated with increased FC levels,³²⁻³⁴ although the association did not really reach significance in our cohort. Increased concentrations of faecal calprotectin were observed in patients with poor daily rating of quality of life. It can be assumed that this may be caused by symptoms due to active bowel inflammation. However, independent associations with other dimensions of patient reported outcomes, like the number of bowel movements or blood in stool, could not be statistically confirmed in the entire IBD population, probably due to small sample size. In addition, an association between increased faecal calprotectin and male sex was also observed. This has been reported previously,³⁵ but the pathophysiologic explanation for the observed association between sex and concentration of faecal calprotectin can be only speculated on.

The major strength of this study was the prospective measurement of faecal calprotectin over a two-year period in consecutively recruited patients at an outpatient clinic. The fact that the

measurements of faecal calprotectin were performed after completion of the study period and that the clinical decisions were not influenced by the observed results reduced the risk of differential bias. The observation that the results remained significant when adjusting for possible confounders, such as IBD therapy, patient characteristics, diet, and use of antibiotics or NSAIDs, further strengthen this study.

The fact that neither clinical remission at baseline nor clinical relapse during follow up were confirmed by endoscopy is a limitation of the study. However, repeated endoscopies are not accepted by patients and are associated with costs and risks. Similarly, co-monitoring of CRP was not regarded as feasible, since this would require admittance to health care every third month. Some patients were on corticosteroids at baseline, which might have influenced the relapse rate during the study period. The results might also be limited by the fact that 41% of the patients did not provide a faecal sample on one or several occasions. This problem was most pronounced among younger patients. However, none of the patients got feedback on their faecal calprotectin levels since faecal calprotectin was measured after all of the patients had completed their follow up. Another possible weakness of the study is that information on duration of clinical remission at baseline was not included in the analyses, since it is negatively associated with time to clinical relapse.³⁶ Our patient group is heterogeneous, comprising both Crohn's disease and ulcerative colitis patients, regardless of clinical characteristics, such as location, behaviour, and disease extent. Despite that, the predictive value of faecal calprotectin remained statistically significant even when patients with Crohn's disease and ulcerative colitis were analysed separately.

In conclusion, our data suggest that longitudinal monitoring of faecal calprotectin is informative in evaluating risk of relapse in IBD. By consecutive measurement of faecal calprotectin every third month, we quantified the relative risk of relapse associated with faecal calprotectin change

and observed attenuation of the risk across time. Future studies should evaluate whether early medical intervention is useful for the prevention of subsequent relapse in asymptomatic IBD patients with elevated faecal calprotectin levels and examine the cost-effectiveness of this monitoring regimen.

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Authorship statement

YZ, YC, KA, MC, CT and JH contributed equally to the design of the study. YZ, KA and JH collected the data. KA analyzed the stool samples. YZ and YC analyzed the data. YZ wrote the article and all the other authors contributed with critical revision of the manuscript for important intellectual content and interpretation of the data. All authors read and approved the final version of the manuscript.

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