ORIGINAL ARTICLE

Faecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Crohn's disease treatment

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Abstract

Objective. Serial monitoring data for faecal calprotectin and lactoferrin during Crohn's disease (CD) therapy are scarce. The aim of this research was to study the behaviour of faecal biomarkers during CD therapy. ***Material and methods.*** Adult CD patients ($n = 19$) needing therapy enhancement were prospectively recruited. The simple endoscopic score for Crohn's disease (SES-CD) was administered before and 4–6 months after therapy. At baseline and at 2–3 and 4–6 months, patients provided faecal samples for measurements of calprotectin and lactoferrin. ***Results.*** Of 19 patients, seven were endoscopic responders, three were partial responders and nine were non-responders. During therapy, both faecal-biomarker concentrations decreased significantly in responders: median calprotectin from 1282 µg/g (range 156–2277 µg/g) to 73 µg/g (range 7–2222; $P = 0.005$) and lactoferrin from 233 µg/g (range 2.8–802 µg/g) to 0.0 µg/g (range 0.0–420 µg/g; $P = 0.005$), and these changes correlated significantly with changes in the SES-CD. In non-responders, changes in faecal biomarkers were non-significant: calprotectin decreased from 1017 µg/g (range 53–3928 µg/g) to 223 µg/g (range 35–15 330 µg/g; $P = 0.594$) and lactoferrin from 22.5 µg/g (range 2.1–629 µg/g) to 13.0 µg/g (range 3.5–1259 µg/g; $P = 0.515$). ***Conclusions.*** The faecal neutrophil-derived proteins calprotectin and lactoferrin are reliable surrogate markers of mucosal improvement. Endoscopic responders achieved normalization of faecal biomarkers, whereas in the majority of endoscopic non-responders these markers remained abnormal.

Key Words: *Calprotectin, colonoscopy, endoscopy, faecal biomarkers, lactoferrin, simple endoscopic score for Crohn's disease*

Introduction

Clinical remission has traditionally been the target of Crohn's disease (CD) therapy and many clinical treatment trials have used a Crohn's disease activity index (CDAI) of below 150 as a score indicative of remission [1]. A considerable number of patients in clinical remission, however, have ongoing intestinal inflammation, which may progress to disease complications such as strictures [2]. Endoscopy is an important tool for assessing disease activity in CD [3]. Endoscopically assessed mucosal healing achieved with anti-tumour necrosis factor (TNF)-blocking agents reduces disease-related hospitalizations and

surgery [4]. With new medications, mucosal healing has been suggested as a target for therapy. The validated score and gold standard for the scoring of endoscopic findings in CD has been the Crohn's disease index of severity (CDEIS) [5]. This score is, however, complex and difficult to use in everyday clinical practice. The simple endoscopic score for Crohn's disease (SES-CD)–validated in five independent European centres–is a fairly new activity score for CD and correlates closely with the CDEIS [6].

Colonoscopic monitoring of the therapy response in CD is time-consuming and costly. The faecal granulocyte-derived biomarkers calprotectin and lactoferrin have proven to be reliable tools in

distinguishing active inflammatory bowel disease (IBD) from irritable bowel syndrome in several studies [7–9]. The single-stool calprotectin assay correlates closely with a 4-day faecal excretion of ^{111}In-labelled granulocytes, a reliable measure of intestinal inflammation [10]. Both faecal calprotectin and lactoferrin are non-specific markers of gut inflammation; in addition to IBD, elevated stool levels may be detectable, for example in colorectal cancer, infective diarrhoea and enteropathy induced with non-steroidal anti-inflammatory drugs [11–14]. Faecal calprotectin and lactoferrin correlate closely with the CDEIS and SES-CD [15,16] and have many features of an ideal test for the monitoring of therapy: they are cheap, safe, non-invasive and easily measurable from a single stool sample with enzyme-linked immunosorbent assays (ELISAs) [17,18]. In a study of CD patients receiving TNF-α-blocking agents, both faecal calprotectin and lactoferrin decreased in parallel with endoscopically detected mucosal improvement [19]. Data monitoring therapy success using serial measurements of faecal biomarkers are as yet limited. The aim of our study was to explore the value of faecal biomarkers as surrogate markers of endoscopically detected mucosal healing in CD patients needing therapy enhancement with conventional oral medications.

Material and methods

Patients

At the Helsinki University Central Hospital Clinic of Gastroenterology we prospectively recruited 19 CD patients (mean age 31.2 years, range 19–51 years) referred for ileocolonoscopy between January 2005 and March 2007. Of these 19 patients, four had newly diagnosed CD. After baseline endoscopy, based on a global assessment of disease activity, CD therapy was enhanced with conventional oral medications: treatment with budesonide ($n = 2$), mesalamine ($n = 2$) or long-term metronidazole ($n = 1$); treatment with systemic corticosteroids or budesonide and simultaneous initiation of azathioprine or 6-mercaptopurine ($n = 11$) or methotrexate ($n = 1$); treatment with systemic corticosteroids and simultaneous initiation of maintenance therapy with mesalamine ($n = 1$); and initiation of azathioprine only ($n = 1$). Systemic corticosteroids were gradually tapered off within 3 months, except in two patients receiving low-dose prednisone, until the follow-up endoscopy. Budesonide therapy lasted until the second endoscopy in two patients. Endoscopic response to therapy was evaluated 4–6 months (median 5.2 months, range 3.9–6.7 months)

after the start of individualized therapy. During all endoscopies, experienced gastroenterologists (T. S., H. N., M. F.) scored the ileocolonoscopy findings according to the SES-CD [6]. For the SES-CD, four endoscopic variables were scored from 0 to 3 in the five bowel segments (ileum, right, transverse, left and sigmoid colon and rectum): extent of affected surface (none = 0; < 50% = 1; 50–75% = 2; > 75% = 3); presence and size of ulcers (none = 0; ulcer diameter 0.1–0.5 cm = 1; 0.5–2 cm = 2; > 2 cm = 3); extent of ulcerated surface (none = 0; < 10% = 1; 10–30% = 2; > 30% = 3); and presence and type of narrowings (none = 0; single, can be passed = 1; multiple, can be passed = 2; cannot be passed = 3) [5]. In this study, a SES-CD between 0 and 2 suggested inactive disease, 3–6 mildly active, 7–15 moderately active and ≥ 16 severely active [20]. As validated SES-CD scores for endoscopic response are lacking, somewhat arbitrarily we defined endoscopic response as achievement of SES-CD ≤ 2 (mucosal healing) or a two- or three-class decrease in the SES-CD from the baseline score, i.e. change from endoscopically severely active disease to mildly active or inactive or from moderately active to inactive. A partial response was defined as a one-class change in the SES-CD, i.e. from endoscopically moderately active disease to mild disease, or from endoscopically severely active disease to moderate disease. A non-response was defined as no change or worsening of the SES-CD score. Clinical activity was assessed according to the CDAI [1]; a CDAI < 150 indicated clinically inactive disease and scores ≥ 150 active disease [21].

Faecal biomarkers and blood tests

Patients provided stool samples for measurements of calprotectin and lactoferrin three times during the study period: at baseline; at a scheduled appointment 2–3 months from baseline; and at response endoscopy (4–6 months after baseline endoscopy). Faecal samples were stored at –40°C until analysis. Faecal calprotectin was measured by means of a quantitative ELISA (PhiCal Test; Calpro AS, Oslo, Norway; NovaTec Immundiagnostica GmBH, Dietzenbach, Germany) and a calprotectin value < 100 µg/g was quoted as normal [22,23]. Lactoferrin was measured by means of a quantitative ELISA (IBD-SCAN; Inverness Medical, Princeton, NJ; Techlab, Blacksburg, VA) with a normal value of < 7.25 µg/g of stool (the baseline value quoted by the manufacturer). Patients provided blood samples for measurements of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) at baseline and at 2–3 and 4–6 months.

Ethics

For participation in this study, all patients gave their informed written consent, approved by the ethics committee of the hospital. The study was registered in a publicly accessible hospital registry (register number 198283).

Statistics

Values are presented as medians, with ranges in parentheses. For data analysis we used SPSS for Windows software (Version 15.0; SPSS Inc., Chicago, IL). The Wilcoxon signed-rank test served to explore changes between related variables. For correlation analyses we used the two-tailed non-parametric Spearman's rank order correlation (r). Significance was set at 0.05.

Results

Variables at baseline

Table I shows patient characteristics. At baseline, 17 of 19 endoscopies reached the ileum: one endoscopy remained incomplete due to technical problems and another one due to an impassable strictured valvula. The median SES-CD in baseline ileocolonoscopy was 12 (3–27) and the CDAI 156 (–7 to 419). Serum CRP was 9 mg/l (< 5 to 211 mg/l) and ESR 16 mm/h

Table I. Patient characteristics.

	n (%)
Gender, female/male	9/10 (47/53)
Disease duration, years, mean	6.8 (range 0-27)
Disease behaviour	
Inflammatory ± perianal disease	13 (68)
Stricturing ± perianan disease	6 (32)
Disease location	
Ileum	5 (26)
Colon	7 (37)
Ileocolon	7 (37)
Smoking no/yes	12/7 (63/37)
Prior operation no/yes	16/3 (84/16)
Maintenance therapy at baseline	
No therapy	7 (37)
Mesalamine/sulphasalazine only	8 (42)
Azathioprine only	1 (5)
Mesalamine/sulphasalazine and azathioprine	3 (16)

(4–79 mm/h). Faecal calprotectin was 1141 µg/g (53–6453 µg/g) and lactoferrin 68.5 µg/g (2.1–802.1 µg/g). Baseline calprotectin concentrations exceeded 1000 µg/g in 10 cases (53%) and baseline lactoferrin concentrations exceeded 70 µg/g in seven cases.

Although 18 (95%) calprotectin and 16 (84%) lactoferrin values were above the normal range, only 10 patients (53%) had a CDAI value > 150, indicating clinically active disease.

At baseline, one patient with ileal disease had low concentrations of both calprotectin (53 µg/g) and lactoferrin (5.3 µg/g) and two other patients with ileal disease had normal lactoferrin concentrations of 2.1 and 2.8 µg/g, respectively (calprotectin was mildly elevated, at 160 and 156 µg/g, respectively).

Variables after treatment

Changes in endoscopic and clinical scores and faecal biomarkers are presented in Table II. Of 19 post-treatment endoscopies, one remained incomplete due to an impassable strictured valvula. As a consequence of therapy, the median SES-CD decreased significantly ($P = 0.006$) to 7 (0–26), serum CRP to < 5 mg/l (< 5–37 mg/l, $n = 18$, $P = 0.003$) and ESR to 6 mm/h (0–37 mm/h, $P = 0.006$). During therapy, faecal calprotectin decreased significantly to a median concentration of 136 µg/g (7–15 326 µg/g, $P = 0.016$). Lactoferrin decreased to 5.6 µg/g (0.0–1259 µg/g, $P = 0.077$). Faecal biomarkers according to therapy response are shown in Table III. During therapy, 7 of 19 patients (37%) were responders, five of whom (83%) achieved a normal post-treatment calprotectin concentration and six (86%) a normal lactoferrin concentration. Mucosal healing (post-treatment SES-CD 0–2) occurred in five patients (26%). Calprotectin was normal in four of these patients (median 39 µg/g, range 11–166 µg/g). Lactoferrin was normal in four patients, and median lactoferrin concentration was 0.0 µg/g (0.0–28.6 µg/g)

Of 19 patients, three were partial responders. In responders or partial responders, calprotectin decreased significantly from a baseline concentration of 1282 µg/g (156–6450 µg/g) to 73 µg/g (7–2222 µg/g, $P = 0.005$). Lactoferrin decreased significantly from 233 µg/g (2.8–802 µg/g) to 0.0 µg/g (0.0–419 µg/g, $P = 0.005$). Figure 1 shows concentrations of faecal biomarkers according to endoscopic response.

Of 19 patients, nine (47%) failed to achieve endoscopic improvement (non-responders). Faecal biomarkers in these non-responders showed non-significant changes (Table III). Of non-responders, eight (89%) had abnormal post-treatment calprotectin concentrations (132–15 330 µg/g). Post-treatment

Table II. Endoscopic and clinical activity scores and faecal biomarkers during the study.

	SES-CD	CDAI	F-calprotectin µg/g	F-lactoferrin µg/g
Baseline	12 (3–27)	156 (−7–419)	1141 (53–6453)	68.5 (2.1–802)
2 to 3 months	–	62 (−5–144)	218 (36–2169)	7.9 (0.0–389)
4 to 6 months	7 (0–26)*	70 (13–183)*	136 (7–15330)*	5.6 (0.0–1259)

*$P < 0.05$ between baseline and post-treatment values. SES-CD, the simlple endoscopic score for Crohn's disease; CDAI, Crohn's disease activity index.

lactoferrin was abnormal in six patients (67%) (10.6–1259 µg/g). Despite persistently active disease in the terminal ileum, one patient had a normal post-treatment calprotectin concentration.

After treatment, the CDAI dropped to 70 (13–183, $P = 0.008$). Post-treatment CDAI indicated clinically active disease in only one endoscopic non-responder with high faecal-biomarker concentrations (calprotectin 15–330 µg/g, lactoferrin 1259 µg/g).

Discussion

Our study showed that, despite therapy, calprotectin and lactoferrin remained abnormal in the majority of endoscopic non-responders or partial responders, whereas in all responders both biomarkers decreased significantly from their baseline concentrations and reached normal or only slightly elevated concentrations.

Many cross-sectional studies have detected elevated calprotectin and lactoferrin concentrations in clinically active CD [7,9,18,24,25]. Furthermore, in endoscopically active IBD, faecal calprotectin and lactoferrin concentrations have been higher than in inactive disease [15,26,27]. Roseth et al. [28] showed a normal faecal calprotectin concentration to be a good predictor of mucosal healing. In that study, some patients had provided calprotectin samples during an earlier active IBD phase and these concentrations were significantly higher than those in remission

[28]. In 31 ulcerative colitis (UC) patients followed during a 12-month period, both faecal calprotectin and lactoferrin concentrations were significantly higher in the active than the inactive disease phase [29]. Only a few prospective data are available regarding the behaviour of faecal markers during CD therapy. During anti-TNF-therapy, both faecal calprotectin and lactoferrin decreased significantly and correlated with endoscopic improvement [19,30]. In five paediatric patients with clinically active CD, lactoferrin appeared to be a sensitive and specific non-invasive biomarker of intestinal inflammation, and a helpful tool for monitoring infliximab treatment response [31]. Wagner et al. [32] followed 27 UC and 11 CD patients with endoscopically active IBD. After 8 weeks of individualized enhancement of therapy, faecal calprotectin declined significantly in all IBD patients, but in CD–with response being defined as a decrease in clinical Harvey–Bradshaw score to < 5–the difference remained non-significant [32].

Only a third of patients achieved endoscopic remission during therapy. In these patients, both faecal biomarkers appeared to be good surrogate markers of mucosal healing. However, with individualized conventional therapy, the majority of patients failed to achieve mucosal healing and their faecal markers remained abnormal. Almost all patients in our study received induction with topical or systemic corticosteroids (79%) and 68% started with an immunomodulatory therapy. It is possible that in our study the anti-inflammatory effect was mainly achieved with

Table III. Faecal biomarkers according to endoscopic response.

	Calprotectin baseline	Calprotectine post-treatment	Lactoferrin baseline	Lactoferrin post-treatment	SES-CD baseline	SES-CD post-treatment
Responders or partial responders n=0	1282 (156–6450)	73 (7–2222) p=0.005	233 (2.8–802)	0.6 (0.0–420) p=0.005	16 (3–26)	3 (0–13) p=0.005
Non-responders n=9	1017 (53–3928)	223 (35–15330) p=0.594	22.5 (2.1–629)	13.0 (3.5–1259) p=0.515	11 (5–27)	11 (4–26) p=0.888

Values presented as medians. Range in parenthesis. p-values given between baseline and post-treatment values. SES-CD, the simple endoscopic score for Crohn's disease.
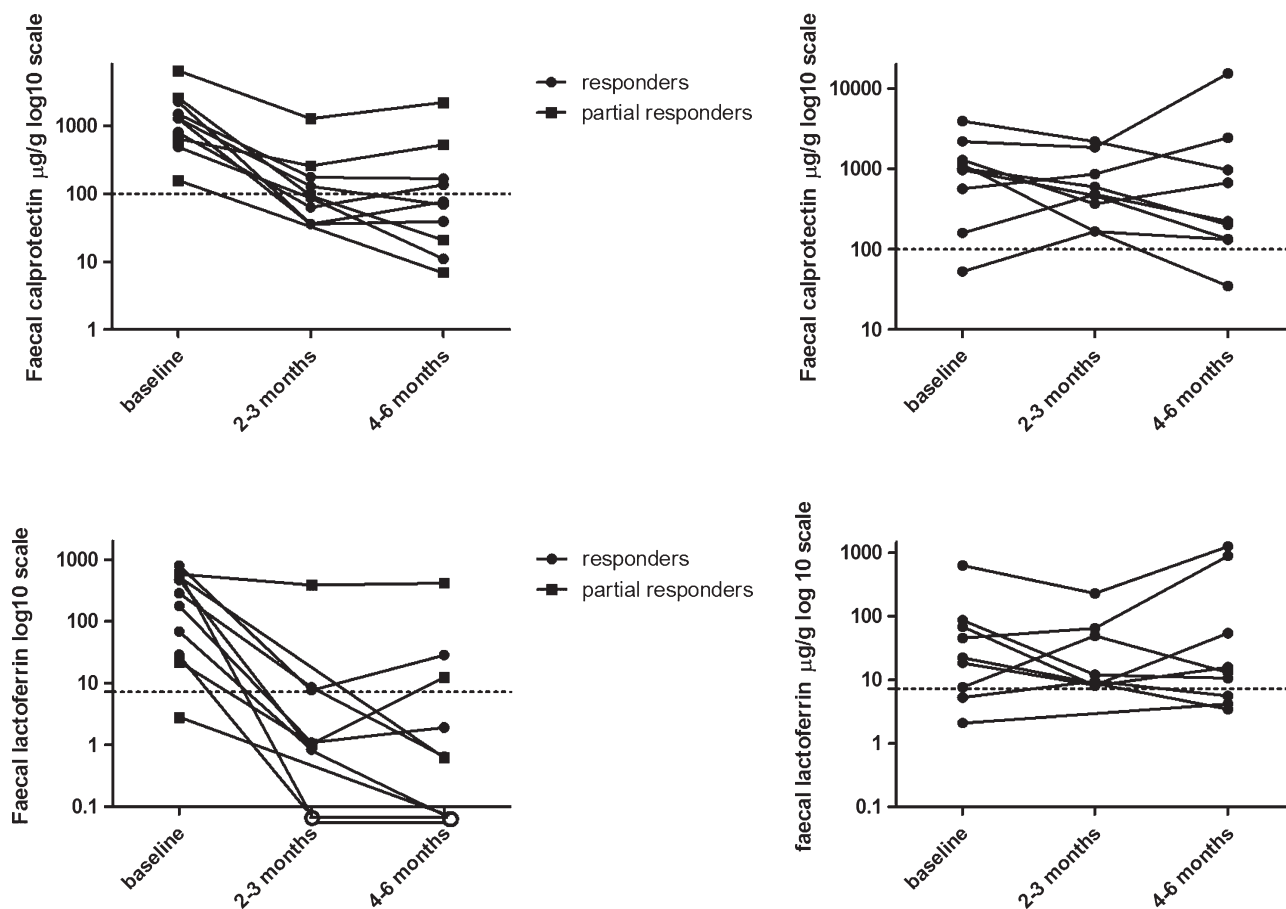
Figure 1. Faecal-biomarker concentrations of each patient according to endoscopic response. Faecal lactoferrin in responders or partial responders: at 2–3 months, two values were zero and at 4–6 months, four values were zero.

corticosteroids, because the effect of thiopurines takes several months to complete. Our finding is in keeping with earlier studies showing incomplete mucosal healing in patients treated with corticosteroids [33]. Our previous paediatric study [22] has shown that, despite clinical remission, faecal calprotectin concentrations often remain abnormal in IBD patients after corticosteroid therapy, possibly due to ongoing intestinal inflammation. Also, lactoferrin concentrations seem to remain higher in patients being weaned from steroids than in patients with inactive disease [34].

Our study has some limitations. The number of recruited patients was fairly small and the follow-up time fairly short. The results of the current study, however, parallel those of previous monitoring studies of patients treated with anti-TNF-α medications [18,29,30]. One may also consider the absence of a standardized therapy to be a weakness. However, faecal markers seem to work as surrogate markers of mucosal improvement regardless of the given therapy. Due to the limited number of patients and a relatively short follow-up time, our result should be

confirmed in a larger and longer study. Thirdly, we chose values for the SES-CD response somewhat arbitrarily. Validated SES-CD values for endoscopic response are lacking, but validated SES-CD values for remission and mildly, moderately or severely active disease have recently been developed [20].

In the study design we included both faecal neutrophil-derived markers, because at that time only a few data were available concerning their behaviour during IBD therapy. Based on our results, however, parallel measurement of these neutrophil-derived markers seems unnecessary. Either of these surrogate markers is suitable for monitoring of therapy success.

According to the current study, normalization of a faecal marker seems to be a good predictor of mucosal healing during CD therapy, making endoscopic monitoring of treatment success less necessary. On the other hand, endoscopic examination of faecal-marker non-responders–even those who are asymptomatic–may be necessary for guidance of second-line therapy.

In conclusion, the faecal biomarkers calprotectin and lactoferrin are valuable objective tools for

monitoring CD therapy and may discriminate between therapy responders and non-responders. Our results need to be confirmed in a study with a larger study population and a longer follow-up time.

Acknowledgements

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