

Role of Fecal Calprotectin in Monitoring Response to Therapy in Inflammatory Bowel Diseases

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Abstract

Inflammatory bowel diseases (IBDs) are chronic intestinal disorders characterized by a typically relapsing course. Disease flares occur in a random way and are often unpredictable. In search to provide noninvasive, cheap and rapid methods able to help in diagnosis and monitoring of IBD activity, within the last years, fecal neutrophil-granular proteins, like calprotectin, have been largely studied. Different studies showed a good diagnostic accuracy of fecal calprotectin (FC) in IBDs and a close correlation between levels of this marker and degree of IBD activity.

More recently, emerging interest has rising on the role of FC in assessing response to therapy and predicting relapse in IBD. We performed a MEDLINE search for more recent articles published on this topic.

Encouraging results show that FC represents a reliable monitoring tool to assess response to treatment, significantly more accurate than serum markers and clinical parameters. Normalization of FC concentrations (FCCs) results as an accurate indicator of endoscopic healing. FC also appears to have a good diagnostic precision in predicting IBD relapse, possibly more in ulcerative colitis than in Crohn's disease.

However, mainly for this last topic, available evidences, although promising, are still heterogeneous and not sufficiently strong. Assessment of usefulness and predictive value of FC according to different medications, frequency of determinations, the establishment of validated cut-off, should be better evaluated in larger and prospective studies.

Keywords: Fecal calprotectin; IBD; Intestinal inflammation; Therapy

Role of Fecal Biomarkers in Inflammatory Bowel Diseases

Inflammatory bowel diseases (IBDs), Crohn's disease (CD) and ulcerative colitis (UC), are chronic intestinal disorders characterized by a typically relapsing course. Disease flares occur in a random way and are often unpredictable [1]. Detection of the presence of intestinal inflammation is a primary criterion for the diagnosis of IBD and for the differentiation from other diseases such as irritable bowel syndrome (IBS) [2].

Physicians commonly use a combination of symptoms, clinical examination, laboratory indices, radiology, and endoscopy with histology to make the diagnosis, to assess the severity, and to predict the outcome of IBDs [1]. In particular, grading of intestinal inflammation is crucial for the assessment of disease activity and for tailoring of therapy [1,2]. Clinical classification systems based on subjective criteria, like Crohn's disease activity index (CDAI), are not reliable and result inappropriate for the assessment of inflammatory activity [3]. Endoscopy and histology remain the current gold standard method for detecting and monitoring bowel inflammation. Nevertheless, endoscopy has the disadvantage of being invasive, time consuming and not well tolerated by patients, mainly because frequent monitoring is often required to assess treatment efficacy and severity

of disease. Moreover, risk of potential complications should be considered.

Active UC and CD are characterized by a 10-fold or more increased migration of neutrophils from the circulation to the gut, as shown by ¹¹¹indium-labeled neutrophils abdominal scintigraphy and the 4-day fecal collection measuring fecal ¹¹¹indium-labeled neutrophils excretion [4]. Nevertheless, also this technique is expensive; it involves exposure to ionizing radiation and a prolonged faecal collection.

To overcome these limitations, within the last years, various laboratory markers have been investigated in search to provide noninvasive, cheap and rapid methods able to help in diagnosis and monitoring of IBD activity. The most widely used laboratory parameters of inflammation, like erythrocyte sedimentation rate (ESR) and C reactive protein (CRP), result not sufficiently specific or sensitive and poorly correlate with symptoms and endoscopic scores of disease activity [5,6].

Leucocyte migration leads activated neutrophil cells to release several neutrophil-granular proteins, like calprotectin, lactoferrin and polymorphonuclear neutrophil elastase, which can be easily detected and measured in feces [7]. Fecal markers have the theoretical advantage of higher specificity for the diagnosis of gastrointestinal diseases, because their levels are not raised in extra-digestive processes [8].

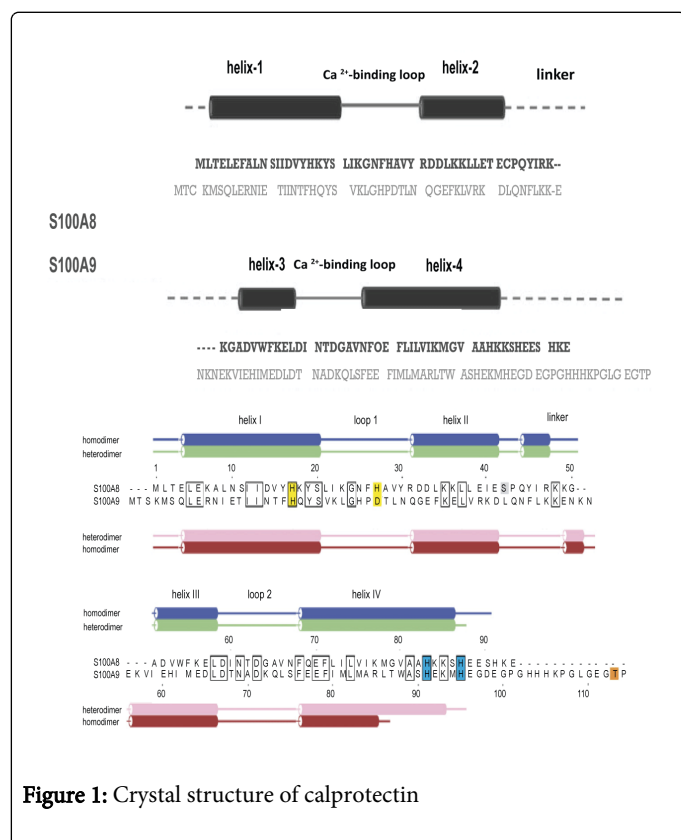
In the last years, a strong correlation between fecal markers concentration and fecal excretion of ¹¹¹indium labeled neutrophils has

been reported [8]. Moreover, several studies showed that these fecal markers more closely correlate with endoscopic findings than CRP, blood leucocytes, and other clinical scores and may have a main role in discriminating active IBD from inactive IBD [7].

Fecal Calprotectin

Calprotectin belongs to S100 proteins, a family of proteins sharing a common Ca²⁺-binding helix-loop-helix motif, the so-called “EF-hand”. S100A8 and S100A9 form the heterooligomer calprotectin. Human S100A8 comprises 93 amino acid residues and has a molecular mass of 10.8 kDa while S100A9 comprises 113 amino acid residues and has a molecular mass of 13.2 kDa [9].

Each S100 protein is composed of two EF-hand motifs. EF-hand I comprises helix I (residues 4–20 in S100A8, residues 7–23 in S100A9), the non-canonical Ca²⁺-binding loop (residues 21–30 in S100A8, residues 24–33 in S100A9), and helix II (residues 31–41 in S100A8 and residues 34–44 in S100A9). EF-hand II comprises helix III (residues 51–58 in S100A8, residues 56–66 in S100A9), the canonical Ca²⁺-binding loop (residues 59–67 in S100A8, residues 67–75 in S100A9), and helix IV (residues 68–86 in S100A8 and residues 76–94 in S100A9). The two EF-hands are separated by linker regions of different lengths in both proteins (residues 42–50 in S100A8 and residues 45–55 in S100A9) [9,10] (Figure 1).



Calprotectin mainly derives from neutrophils, and to a lesser extent, from monocytes and reactive macrophages and it accounts for approximately 60% of the total protein of the cytosol [8,11]. It has a relevant antimicrobial activity, by competing for zinc and by inhibiting zinc dependent enzymes [12]. As a consequence, increased concentrations of calprotectin can be measured at different sites

(plasma, synovial fluid, urine, liquor, saliva and feces), when an inflammation process with recruitment of neutrophils is ongoing [13,14].

Calprotectin can be assayed in simple buffer extracts of small faecal samples [11] and it shows excellent stability in feces at room temperature for as long as a week [15,16]. Its quantification is inexpensive, easy and commonly performed by a commercially available ELISA immunoassay. Up to now, an optimal cut-off has not been identified yet [17]. Nowadays, is commonly thought that concentrations of <50 µg/g are normal; concentrations of 50–100 µg/g represent a weakly positive test; concentrations of >100 µg/g indicate a positive result [18].

Different studies showed a good diagnostic accuracy of fecal calprotectin (FC) in IBD in respect to IBS or healthy subjects [7,8,19-24]. At this regards, in von Roon et al. meta-analysis' including 9 studies, FC showed an overall sensitivity and specificity of 95% and 91% respectively for the identification of patients with IBD, when compared with those without [24]. Also in this work, the precision of FC for the diagnosis of IBD appeared to be superior to serological markers [24]. In a similar more recent analysis including 670 adults with suspected IBD undergoing endoscopy, van Rhee et al., found that in adults, the sensitivity and specificity of FC was 0.93 and 0.96 [25]. Therefore, it has been suggested that testing for FC could represent a useful screening tool for identifying patients who are most likely to need endoscopy for suspected IBD, leading to a reduction in the number of specialist referrals for further invasive endoscopic investigations, as well as their associated costs [25,26]. However, although the high predictive negative value, normal concentrations of FC cannot rule out diagnosis of all organic diseases. FC, in fact, should not be considered as a marker of organic intestinal disease at all, rather it represents an accurate marker of “neutrophilic intestinal inflammation” [23].

A close correlation between fecal calprotectin concentrations (FCCs) and degree of IBD activity has also been reported [3,8,12,22,23,27-29]. Sipponen et al. evaluated the correlation of FCCs with the Crohn's disease index of severity (CDEIS) in 77 CD patients, showing that FC could discriminate inactive from all the other activity groups, with a sensitivity and specificity in predicting endoscopically active disease (CDEIS ≥ 3) of 70% and 92% respectively, at a cut-off level of 200 µg/g [28]. As reported, FC presented the best accuracy for detection of endoscopically active disease, conversely than other serum markers and clinical scores. Schoepfer et al. reported that in 134 UC patients undergoing colonoscopy and scored according the endoscopic and clinical section of the Rachmilewitz Index for activity monitoring, the overall accuracy for the detection of endoscopically active disease (score ≥ 4) was 89% for FC (at a cut-off of 50 µg/g), 73% for Clinical Activity Index, 62% for elevated CRP, and 60% for leukocytosis [3].

As the same, when correlated to the Simple Endoscopic Score for Crohn's disease (SES-CD), the overall accuracy for detection of endoscopically active disease was 87% for calprotectin (at a cut-off 70 µg/g), 66% for elevated CRP, 54% for blood leukocytosis, and only 40 % for the CDAI ≥ 150 [30].

Whereas these evidences are strongly supported, emerging interest on the role of this marker in assessing response to therapy and predicting relapse is rising. These topics will be better focused in the following sections of this work.

Role of Fecal Calprotectin in Assessment of Therapy Response

The clinical course of IBD is often unpredictable. So far, different prognostic models have been developed to identify non-responding patients, but they mostly rely on subjective clinical parameters and are rarely used in clinical practice. It is important to closely monitor these patients and to evaluate their responses to treatments, since failure to identify the non-responders within an appropriate period leads to increased morbidity and eventual mortality.

Recent studies suggest “mucosal healing”, defined by the absence of lesions [31], as the therapeutic goal for treatment of IBDs [8,27-29]. Mucosal healing is regarded as the major endpoint definition for remission [32] and as a surrogate marker for more effective control of disease, predicting a better course of CD [33]. Recently Ardizzone et al. [34] showed that this also applied to those with newly diagnosed UC and that patients who achieved early mucosal healing after the initial therapy had a more favorable disease course than others.

Therefore, to avoid multiple and uncomfortable endoscopic examinations, biological markers able to recognize the achievement of a mucosal healing have been evaluated. In consideration of its close correlation with disease activity, FC has been suggested as a reliable marker able to assess response to treatment.

In 90 patients with acute severe UC, with overall high basal FCCs (median, 020.0 µg/g), significantly higher FCCs have been reported in those who failed medical therapy and required emergency colectomy (, 200 µg/g vs 887,0 µg/g), with a trend toward higher levels in both steroid nonresponders (100.0 µg/g vs. 863.5 µg/g), as well as in infliximab nonresponders (795.0 µg/g vs. 920.5 µg/g) [4].

In a prospective study, Kolho et al. measured FCCs in 15 pediatric IBD patients receiving glucocorticoid therapy, showing that FCCs declined in line with clinical improvement, but seldom fell in normal ranges. This result suggested that although the achievement of a clinical remission, complete FCC normalization was more difficult, perhaps because of the persistence of a subclinical inflammation, mainly in CD [35]. Nevertheless, histological follow-up was not performed in this study and FCCs were only compared to clinical evaluation.

More recent studies investigated correlation of FC with response to biological therapies, in particular infliximab. Sipponen et al. measured FCCs in 15 CD patients before and 3 months after starting anti TNF-α therapy. They showed that FCCs significantly declined in treatment responders and normalized in almost all those who reached endoscopic remission (scored by using the CDEIS) [36]. In particular, median FCCs fell from 1173 µg/g to 130 µg/g, and in the 5 patients who achieved endoscopic remission (CDEIS<3), median FCCs declined from 1891 µg/g to 27 µg/g, while remaining substantially unchanged from basal levels in nonresponder subjects. Nevertheless, this study was designed to explore fecal markers only during the induction (0 and 8 weeks) therapy, leaving behaviour of these markers during maintenance treatment unanswered [35]. In another study, 64 CD patients on infliximab therapy were evaluated for clinical response and remission using the Crohn's disease activity index (CDAI), the Harvey-Bradshaw index score, SES-CD score, CRP, and FC [37]. Endoscopic activity demonstrated a stronger correlation with FC and CRP than with the clinical index. Neither the clinical index nor CRP were reliable at identifying endoscopic remission. FC alone (with a cut-off of 94 µg/g) identified endoscopic remission with a sensitivity of 84% and specificity of 74% [37].

Although the role of TNF-α in the pathogenesis of ulcerative colitis has been debated, infliximab has been proven to be effective also in this condition, resulting in a significant corticosteroid-sparing effect. De Vos et al. correlated FC levels with endoscopic response to infliximab in a cohort of 53 patients with active UC [37]. Endoscopic remission, defined by a Mayo score of 0 to , was achieved in 58% of the patients and was paralleled by a decrease of FCCs to <50 µg/g in all patients achieving remission. Moreover, a sharp decrease of FCCs as early as week 2 from the initial infusion was well correlated with endoscopic remission at week 10. FCCs <50 µg/g or a decrease of 80% predicted remission with a specificity of 67% and sensitivity of 54% [31].

These encouraging results on small sample size showed that fecal calprotectin could represent a reliable marker of response to treatment, playing a significant role in clinical practice, mainly allowing early evaluation of response to treatment and prompt identification of patients in need of dose intensification or additional treatment modifications [2]. Nevertheless, these data should be confirmed in larger trials (Table 1).

Author	No of IBD patients (active disease)	Results	Treatment
Ho [4]	UC 90	High FCC in patients failing medical therapy and requiring emergency colectomy	Steroid and infliximab
Sipponen [36]	CD 15	Direct correlation between FCC and endoscopic remission	Infliximab (induction phase)
af Björkesten [37]	CD 64	A FCC cut off 94 µg/g has sensitivity 84% and specificity 74% in identify endoscopic remission	infliximab
De Vos [31]	UC 53	Normalization of FCC in all patients achieving remission	infliximab
IBD: Inflammatory Bowel Disease; UC Ulcerative Colitis; CD: Crohn's Disease; FCC: Fecal Calprotectin Concentration			

Table 1: Main studies on role of fecal calprotectin in assessment of therapy response.

Role of Fecal Calprotectin in Assessment of Relapse Prediction

Patients with IBD often experience relapse of disease, with variable consequences [1]. It is thought that, even in cases of successful treatment, subclinical inflammation of the intestinal wall, not commonly detected by the most widely used laboratory parameters of inflammation, may persist, significantly contributing to risk of relapse [5]. Nevertheless, if flare occurrence is reliably predicted, early treatment regimens may be promptly introduced, leading to lower rate of morbidity.

From recent studies, FC appears as a promising marker able to predict IBD relapse [5,9,38,39]. Tibble et al. reported that in 80 IBD patients in clinical remission, basal FCCs of 50 mg/g (≅ 250 µg/g) or more, predicted a 13-fold increased risk for relapse within a year [11]. On the contrary, CRP and ESR resulted unable to predict IBD relapse [11]. In a following study, Costa et al. included 38 CD and 41 UC

patients in remission, showing that baseline FCCs of 150 µg/g or more were predictive for a relapse in the next year [5]. They found a high sensitivity for both CD (87%) and UC (89%), while specificity was much lower in CD (43%) compared with UC (82%). Also in this study, ESR and CRP were not predictive of relapse [5]. It is probable that the different specificity of FC in predicting relapse in UC and CD could reflect differences in the inflammatory pattern of these two diseases, since clinical remission in UC is more frequently accompanied by endoscopic and histological normalization than in CD [5].

García-Sánchez et al. evaluated 69 CD and 69 UC patients in clinical remission for at least 3 months [39]. Thirty-nine (30%) suffered from relapse. FCCs were higher among the patients with relapse than in those that remained in remission (444 µg/g vs 112 µg/g). Patients with CD and FCCs >200 µg/g relapsed 4 times more often than those with lower marker concentrations. In UC, FCCs >120 µg/g were associated with a 6-fold increase in the probability of disease activity outbreak. The predictive value was similar in UC and CD with colon involvement, than in patients with ileal disease [39].

On the other hand, Laharie et al. evaluated FCCs and CRP in 65 patients with steroid-refractory luminal CD on infliximab therapy, in clinical remission off steroids at week 14 [40]. They showed that 23 of 50 (46%) experienced clinical relapse during the first year of follow-up. Median FCCs at week 14 were similar in patients with and without clinical relapse (200 and 150 µg/g respectively). Neither FC nor CRP at baseline and at week 14 could predict relapse even when CD location subgroup analysis was considered [40]. However, endoscopic evaluation was not performed in follow-up period, and disease relapse, the primary endpoint in the present study, was exclusively clinically-based.

In a prospective multicenter study enrolling 87 UC adult patients in clinical remission under infliximab maintenance therapy (defined as partial Mayo score 3 at all times with an endoscopic score of 0 to 1 at week 52), patients who experienced relapse had significantly higher

FCCs (median >300 mg/kg) 3 months before the flare [41]. Moreover, two consecutive calprotectin measurements of >300 mg/kg with a 1-month interval were identified as the best predictor of a flare (61.5% sensitivity and 100% specificity). FCCs at the moment of relapse were significantly better correlated with endoscopic index than the CRP concentration (area under the curve, 0.85 vs 0.58 [41]).

Furthermore, in another recent study by Lason et al. [42], 69 patients with new onset of UC were included after the initial treatment, and were followed up after 3 months and then yearly for 3 years. FCCs measured 3 months after the diagnosis were higher in patients experiencing a relapsing disease course compared with those with a mild disease course during 1 year and 3 years of follow-up, conversely than for CRP and Mayo score. Interestingly, patients with FCC >262 µg/g at the 3 months follow-up, had an increased risk of experiencing a relapsing disease course during the first 3 years after onset, compared with those with lower levels [42].

Therefore, FC has been suggested as a valuable marker in the clinical management of these patients. However, this study exclusively included patients with new onset of UC, and investigated all patients 3 months after the diagnosis, even those who were not in clinical remission, limiting possibility of direct comparison with other studies enrolling patients on clinical remission at the time of inclusion.

Finally, in a recent meta-analysis by Mao et al. [43], a total of 672 IBD patients (318 UC and 354 CD) from six different studies were analyzed. The pooled sensitivity and specificity of FC to predict relapse of quiescent IBD was 78% and 73%, respectively, with comparable results between UC and CD. Nevertheless, in CD patients the predictive value of FC in isolated small bowel CD was not assessed due to insufficiency of available data, and compared with all enrolled CD patients, FC appeared to be more accurate in ileocolonic and colonic CD. The Authors concluded that FC is useful to predict relapse in quiescent IBD patients, as a simple and noninvasive marker [43].

Author	No of IBD patients (in clinical remission)	Results
Tibble [11]	43 CD 37 UC	Basal FCC ≥ 250 µg/g predicts a 13-fold increased risk for relapse within a year
Costa [5]	38 CD 41 UC	Basal FCC ≥ 150 µg/g is highly predictive for a relapse in the next year
Garcia-Sanchez, [39]	69 CD 69 UC	CD: basal FCC ≥ 200 µg/g predicts a 4-fold increased risk for relapse in the next year UC: basal FCC ≥ 120 µg/g predicts a 6-fold increased risk for relapse
Laharie, [40]	65 CD	No showed correlations between FCC and risk for relapse
De Vos, [41]	87 UC	Basal FCC ≥ 300 µg/g correlate with relapse in the next year. Two consecutive value >300 µg/g with an 1 month interval is the best predictor of flare
Lason, [42]	69 UC	Basal FCC > 262 µg/g has an increased risk of relapse during the first 3 years after onset
Mao, [43]	318 UC 354 CD	The pooled sensitivity and specificity of FC to predict relapse of quiescent IBD was 78% and 73%, respectively, with comparable results between UC and CD.
IBD: Inflammatory Bowel Disease; UC: Ulcerative Colitis; CD: Crohn's Disease; FCC: Fecal Calprotectin Concentration		

Table 2: Main studies on role of fecal calprotectin in assessment of relapse prediction.

It should be considered that the cut-off value differs accordingly to the specific assay used and the results of these few studies are not directly comparable, also in consideration of the differences in patient selection, in the extent of disease and remission time. Therefore, although FC appears to have a good diagnostic precision in predicting IBD relapse, possibly more in UC than in CD, larger prospective studies are mandatory to assess usefulness and predictive value of biomarkers according to medications and duration of remission, to assess the period of time between increase of FC and the occurrence of clinical relapse, as well as to establish the frequency of FC determinations (Table 2).

Conclusions and Future Perspectives

Role of FC has been largely studied in the last years. A good diagnostic precision in discriminating IBD and functional intestinal diseases has been widely reported, thus leading to propose FC as a filter to avoid unnecessary procedures in patients who are most likely to need endoscopy for suspected IBD. FCCs better correlate with IBD activity (more in UC than in CD patients) than the other classically recommended inflammatory parameters (ESR, CRP).

Prognostic role of FC is still matter of debate. Encouraging results showed a better correlation between FC and response to treatment, than with serum markers and clinical score. In particular, normalization of FC resulted as an accurate indicator of endoscopic healing. Nevertheless, large randomized prospective clinical trials are still lacking and these results need to be confirmed.

FC appears to have a good diagnostic precision in predicting IBD relapse, possibly more in UC than in CD, as well as, more in ileocolonic and colonic than in ileal disease. However, available evidences are still heterogeneous and not sufficiently strong, although promising. Assessment of usefulness and predictive value of FC according to different medications and duration of remission should be clarified. As the same, the frequency of FCC determinations, and the establishment of validated cut-off, should be better evaluated in larger prospective studies.

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