The role and utility of faecal markers in inflammatory bowel disease

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Abstract: Crohn's disease and ulcerative colitis are characterized by periods of symptomatic relapse and remission. Diagnosis and assessment of inflammatory bowel disease has so far been based on clinical evaluation, serum parameters, radiology and endoscopy. Faecal markers such as calprotectin or lactoferrin have emerged as new diagnostic tools to detect and monitor intestinal inflammation. This review focuses on their potential clinical applications and limitations in the management of inflammatory bowel disease.

Keywords: calprotectin, Crohn's disease, inflammation, inflammatory bowel disease, lactoferrin, ulcerative colitis

Introduction

Inflammatory bowel diseases (IBDs), mainly consisting of Crohn's disease (CD) and ulcerative colitis (UC), are lifelong intestinal disorders, which are characterized by periods of symptomatic relapse and remission. Disease flares are often unpredictable and significantly affect the patient's quality of life. Diagnosis of IBD has so far been based on a combination of clinical evaluation, serum parameters, radiology and endoscopy including histological examinations.

Stool markers such as calprotectin or lactoferrin have emerged as new diagnostic tools to detect intestinal inflammation. They are noninvasive, rapid, simple and low in cost. Faecal markers include a biologically heterogeneous group of substances that either leak from or are actively released by the inflamed mucosa (see Table 1). Most studies have been performed with faecal calprotectin. Calprotectin is a small calcium-binding protein consisting of two heavy and one light polypeptide chains. It is found in abundance in neutrophilic granulocytes, in which it accounts for 60% of the cytosolic fraction, as well as in monocytes and macrophages [Bjerke *et al.* 1993; Steinbakk *et al.* 1990]. It was first described in 1980 [Fagerhol *et al.* 1980]. It is stable in faeces for up to 7 days at room temperature and has a homogeneous distribution in faeces [Roseth *et al.* 1992].

In active IBD, an increased migration of polymorphonuclear (PMN) neutrophils from the circulation to the inflamed intestinal mucosa has been observed [Roseth *et al.* 1997]. Due to leukocyte shedding in the intestinal lumen, proinflammatory proteins such as calprotectin can be detected and measured in the stool. The concentration of calprotectin is directly proportional to the intensity of the neutrophilic infiltrate in the gut mucosa [Roseth *et al.* 1999].

Separating IBD from irritable bowel syndrome

Patients with IBD and irritable bowel syndrome (IBS) share many clinical symptoms, including abdominal pain, diarrhoea and generalized malaise. A considerable proportion of patients, especially those presenting in primary care, will have functional disorders with no need for invasive examinations. In many cases, IBD and IBS cannot be separated from each other exclusively on the basis of clinical symptoms. Until recently, colonoscopy was required to rule out IBD. However, more than half of patients with symptoms suggesting IBD will have negative endoscopy and be diagnosed with IBS. The same applies to children. Up to 70% of children and teenagers referred to a paediatric gastroenterology centre with suspected IBD do not have the disease [van der Vijver *et al.* 2012]. Remaining a clinical

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Table 1. Faecal markers in clinical use.

Faecal marker	Main source
S100A8/S100A9 (Calprotectin)	Neutrophils, monocytes from cytoplasm and epithelial cells
S100A12	Neutrophils from cytoplasm
Lactoferrin	Mucosal epithelial cells and neutrophils
M2-PK	Expressed by rapidly dividing cells
Neopterin	Activated macrophages
Metalloproteinases	Different cell types including activated neutrophils
Myeloperoxidases	Activated neutrophils
Polymorphonuclear elastase	Activated neutrophils

challenge, an easy, noninvasive and reliable marker would be preferable to distinguish IBD and IBS.

In 1992 it could be shown for the first time that faecal calprotectin is increased in patients with UC and CD [Roseth *et al.* 1992]. Initial studies on faecal calprotectin focused on its accuracy to separate patients with IBD from those with IBS. It has been confirmed in a variety of clinical studies that calprotectin is a useful diagnostic tool to discriminate between these two patient groups. The positive predictive value (PPV) of calprotectin has been described between 70% and 100%, the negative predictive value (NPV) between 51% and 91% [Carroccio *et al.* 2003; Costa *et al.* 2003; Dolwani *et al.* 2004; Konikoff and Denson, 2006; Schoepfer *et al.* 2007, 2008]. A meta-analysis of 13 studies by van Rhee and colleagues found that calprotectin had a pooled sensitivity of 93% and a pooled specificity of 96% to diagnose IBD in adults [van Rhee *et al.* 2010]. A larger review [von Roon *et al.* 2007] including 30 studies calculated a sensitivity of 95% and specificity of 91% for differentiating IBD from non-IBD diagnoses. In a recent meta-analysis, Gisbert and McNicholl combined data from 2475 patients and found a mean sensitivity of 83% and a mean specificity of 84% of calprotectin to distinguish inflammatory from noninflammatory disease [Gisbert and McNicholl 2009]. A slightly higher diagnostic accuracy was calculated for CD than for UC.

In children, faecal calprotectin seems to have a comparable sensitivity, but lower specificity. Diamanti and colleagues examined a cohort of children with abdominal pain and altered bowel habits [Diamanti *et al.* 2010]. They found a sensitivity of 100%, but a specificity of only 68% for diagnosing IBD. In the meta-analysis of van Rhee, the specificity of faecal calprotectin in

children and teenagers was significantly lower (76% *versus* 96% in adults) whereas the sensitivity remained equally high (92% *versus* 93%) [van Rhee *et al.* 2010]. These results are in line with the data published by Henderson and colleagues who performed the largest meta-analysis of calprotectin in paediatric IBD (PIBD) so far [Henderson *et al.* 2014]. They found that calprotectin had a very high sensitivity (98%) but only modest specificity (68%) to diagnose PIBD.

Correlation of calprotectin and disease activity

Subsequently, the correlation of calprotectin concentrations and clinical indices as well as endoscopic and histological activity in IBD has been examined. Most studies described a weak correlation of calprotectin and clinical activity indices [Gaya *et al.* 2005; Jones *et al.* 2008; Sipponen *et al.* 2008; Schoepfer *et al.* 2010]. In contrast, it has been established that calprotectin concentrations correlate well with endoscopic and histological IBD activity. The correlation could be demonstrated for both UC [Roseth *et al.* 1997; Bunn *et al.* 2001; Schoepfer *et al.* 2008, 2013] and CD [Roseth *et al.* 2004; Sipponen *et al.* 2008; Langhorst *et al.* 2008]. Furthermore, calprotectin was able to discriminate endoscopically inactive from mild, moderate and severely active disease [Schoepfer *et al.* 2013]. The correlation of calprotectin with endoscopic and histological disease activity could also be demonstrated in children [Bunn *et al.* 2001; Canani *et al.* 2008; Fagerberg *et al.* 2005, 2007]. Of note, faecal calprotectin mirrored the inflammatory status in children after proctocolectomy [Pakarinen *et al.* 2010], but there was no correlation between calprotectin concentrations and the pediatric Crohn's disease activity index [Shaoul *et al.* 2012]. Finally, preliminary evidence suggests that at least in children, calprotectin

correlates better with histological than endoscopic findings [Bunn *et al.* 2001].

Role of colonoscopy

Colonoscopy is the gold standard for the diagnosis and assessment of IBD. It should be performed in all patients with symptoms suggestive of IBD not only to enable diagnosis, but also to determine the extent, location, and severity of the disease and to differentiate CD from UC. However, many patients with suspected IBD will have functional disorders and negative findings on endoscopy. Among these patients, colonoscopy represents an unnecessary risk. Moreover, endoscopy is invasive, expensive, time consuming, not always available and often not very well accepted by patients. Endoscopic assessment is especially difficult in paediatric populations. Children will need hospital or day-care admission, and often experience problems with fasting and bowel preparation [Henderson *et al.* 2014].

It has been examined whether selecting patients for colonoscopy based on increased calprotectin levels instead of clinical symptoms would reduce the number of negative endoscopies. In the meta-analysis by van Rheeën and colleagues, the efficacy of calprotectin in reducing unnecessary endoscopic procedures was examined [van Rheeën *et al.* 2010]. A prior screening for calprotectin would result in a 67% reduction in the number of adults and of 35% in children requiring endoscopy, but also lead to delayed diagnosis of IBD in 6% of patients because of a false negative calprotectin test. Thus, the price of delayed diagnosis must be balanced against the number of unnecessary colonoscopies. In clinical practice, all patients with alarming symptoms such as bleeding, weight loss or iron deficiency anaemia should be endoscopically examined, irrespective of their individual calprotectin level.

Mucosal healing

In modern IBD treatment, mucosal healing (MH) rather than clinical symptom control is increasingly becoming the primary therapeutic target [Pineton de Chambrun *et al.* 2010]. In both UC and CD, MH has been associated with sustained clinical remission, reduced rates of hospitalization and operations, as well as a lower incidence of colorectal cancer [Peyrin-Biroulet *et al.* 2011]. However, no clinically valid definition of MH exists so far.

To assess MH, either repetitive endoscopic examinations or use of surrogate markers are necessary. Frequent assessment of disease activity by colonoscopy is not applicable and would negatively affect patient's quality of life. As alternative, faecal calprotectin has been suggested as surrogate marker for MH. Data on calprotectin as a surrogate marker of MH are emerging, but the evidence is not yet conclusive. So far, only few and small studies have been performed. Roseth and colleagues performed colonoscopies in patients with IBD in clinical remission. In 38 of 45 cases, patients with normal calprotectin values also had normal histology [Roseth *et al.* 2004]. D'Haens and colleagues reported in patients with CD that calprotectin below 250 µg/g predicted MH with a 94% sensitivity, a 62% specificity, and a PPV and NPV of 48% and 96%, respectively [D'Haens *et al.* 2012]. Use of a noninvasive objective marker to reliably assess MH would be of paramount clinical importance. However, larger studies are warranted before calprotectin or any other test could be introduced in daily clinical practice.

Monitoring of treatment

Traditionally, monitoring of treatment response in IBD has been based on a combination of symptoms, activity scores such as the Crohn's Disease Activity Index (CDAI), the Harvey-Bradshaw Index and the Rachmilewitz Index, nonspecific serum markers and occasionally endoscopy. The activity scores, which are calculated from a combination of symptoms, clinical examination data and laboratory values, have significant limitations: the scores depend on the subjective patients reporting; they represent more the patient's well-being than the degree of mucosal inflammation; the collection of data is tedious. For these reasons, the activity indices are mainly used in studies, but not in daily clinical practice.

Stool markers such as calprotectin or lactoferrin have been suggested for assessment of treatment success and their use in therapy monitoring is increasing. Calprotectin can be used to differentiate between clinically active and inactive IBD [Langhorst *et al.* 2008]. In several clinical trials of different CD therapies, normalization of faecal markers appeared to be a useful and reliable surrogate for mucosal improvement and healing [Roseth *et al.* 2004; Sipponen *et al.* 2008, 2010]. Similar results were shown for UC, with infliximab inducing a fast and significant decrease in calprotectin concentrations [de Vos *et al.* 2012]. In a recently

published study of 60 patients with IBD (34 CD, 26 UC), normalization of calprotectin after infliximab or adalimumab predicted sustained clinical remission in the majority of patients [Molander *et al.* 2012]. In acute severe UC, calprotectin may be able to predict the need for colectomy. In the study by Ho and colleagues, calprotectin concentrations remained significantly higher in patients requiring colectomy than those who responded to medical treatment [Ho *et al.* 2009]. Similarly, Theede and colleagues repeatedly measured faecal calprotectin in 16 patients with active UC requiring high-dose steroid treatment [Theede *et al.* 2014]. Although median calprotectin levels dramatically decreased after 4 and 27 days of treatment, respectively, they were not predictive of sustained clinical remission after 12 months.

The role of calprotectin in assessing the treatment response in active IBD has also been examined in children. In 15 paediatric patients treated with corticoids [Kolho *et al.* 2006], calprotectin decreased in line with clinical improvement, but rarely normalized, suggesting incomplete MH. Discontinuation of therapy resulted in an immediate increase of faecal calprotectin. In a recent study of 76 children treated with infliximab, low calprotectin levels at weeks 2 and 6 were associated with a sustained therapeutic response [Kolho *et al.* 2014].

Our own experience suggests that faecal calprotectin could be used for guiding medical treatment in active UC. In a series of 41 patients with UC, calprotectin was more reliable than clinical scores to predict therapy intensification (unpublished data). Faecal calprotectin may increase even before the onset of clinical symptoms, suggesting that treating presymptomatic patients primarily on the basis of elevated calprotectin concentrations could be advantageous. We agree with the recommendation that inflammatory markers should be assessed to confirm active disease before starting or switching IBD therapy [Colombel *et al.* 2010].

Noninvasive monitoring of disease activity requires repeated calprotectin measurements. It is likely that serial measurements would be more accurate than a single test in predicting disease activity and treatment outcome. Data on serial FC measurements are limited [Roseth *et al.* 2004; Casellas *et al.* 2007]. In the study of Roseth, one patient was sequentially monitored from onset of active disease until remission. The levels of faecal calprotectin decreased with ongoing treatment and corresponded with clinical, endoscopic and

histological improvement. The recent introduction of a rapid test for faecal calprotectin measurement has opened doors for better guiding IBD therapy [Kolho *et al.* 2012].

Risk of relapse

Most patients with clinically successful treatment seem to have some degree of residual mucosal inflammation. Persisting inflammation in the absence of gastrointestinal symptoms may lead to progressive bowel damage, complications and risk of relapse. In several clinical studies with both adults and children, elevated calprotectin concentrations were found despite clinical remission [Tibble *et al.* 2000a; Costa *et al.* 2003; Thjodleifsson *et al.* 2003; Walkiewicz *et al.* 2008]. It appears that faecal calprotectin can detect subclinical mucosal inflammation and thus may identify patients at risk for relapse. During the early relapse stage, clinical symptoms are usually not present. The development of symptoms reflects the progressive inflammatory process. Being able to reliably identify patients at pending risk of relapse may allow treatment adjustment and prevention of clinical relapse.

The ability of calprotectin to predict clinical relapse in quiescent IBD has been examined in several studies. Elevated calprotectin levels in patients with clinical remission were associated with a significantly increased risk of disease relapse within a 12-month follow-up period [Tibble *et al.* 2000a; Costa *et al.* 2005; D'Incà *et al.* 2008; Walkiewicz *et al.* 2008; Diamanti *et al.* 2008; Sipponen and Kolho, 2010]. As shown in the STORI study, elevated calprotectin seemed to predict relapse after stopping infliximab therapy in patients with CD patients. A calprotectin concentration of over 300 µg/g was an independent risk factor associated with disease relapse [Louis *et al.* 2012]. A recently published meta-analysis of six prospective studies showed a pooled sensitivity of 78% and a specificity of 73% for calprotectin in predicting IBD relapse [Mao *et al.* 2012]. The test results were comparable between UC and CD. Faecal calprotectin appears to be less useful in predicting relapse in patients with ileal CD compared with UC or colonic/ileocolonic CD [D'Incà *et al.* 2008; García-Sánchez *et al.* 2010].

Postoperative recurrence of CD

More than 80% of patients with CD require surgery within 10 years of diagnosis [Bernell *et al.* 2000]. After resection of the terminal ileum in

CD, up to 80% have endoscopic evidence of recurrence within 12 months [Rutgeerts *et al.* 1990] and clinical relapse within 3–5 years.

Calprotectin and lactoferrin may have a role in predicting recurrence after ileocecal resection [Scarpa *et al.* 2007; Sorrentino *et al.* 2010]. Lamb and colleagues showed that calprotectin values dropped significantly after surgery and normalized in patients with uncomplicated course [Lamb *et al.* 2009]. Calprotectin remained elevated in a high proportion of postoperative patients with CD with severe clinical activity, whereas low levels indicated clinically inactive disease. Scarpa and colleagues found that calprotectin after ileocolonic resection in 63 patients with CD remained elevated even in patients with clinical remission [Scarpa *et al.* 2007], suggesting ongoing inflammation. The surgical resection did not appear to remove all of the inflammatory process.

In a clinical study of 50 consecutive patients with ileocecal resection, Orlando and colleagues found a calprotectin concentration greater than 200 µg/g to be predictive of endoscopic recurrence after 12 months [Orlando *et al.* 2006]. Lobaton and colleagues reported similar results [Lobaton *et al.* 2013]. Calprotectin had a sensitivity of 63% and a specificity of 75% in predicting endoscopic recurrence. The authors proposed to measure faecal calprotectin 2 or 3 months after the operation. Patients with increased calprotectin levels should be examined endoscopically for recurrence [Papay *et al.* 2013].

At the recent meeting of the European Crohn's and Colitis Organisation, preliminary results of a large multicentre trial, the Post-Operative Crohn's Endoscopic Recurrence study, were presented. The data so far show that faecal calprotectin might be a reliable marker for endoscopic postoperative recurrence with a high sensitivity and NPV. Calprotectin was also superior to C-reactive protein (CRP) and other faecal markers (lactoferrin, S100A12) and allowed 41% of patients to avoid colonoscopy. The final results of this study are eagerly awaited by clinicians worldwide.

Monitoring in patients with pouch

Patients undergoing restorative proctocolectomy for severe UC have a high risk of developing pouchitis during the following years. Pouchitis is an inflammatory disease with significant associated morbidity. The diagnosis of pouchitis

requires both endoscopic and histological evidence of inflammation. Only sparse data are available about the role of faecal markers in monitoring patients with pouchitis. Faecal calprotectin and lactoferrin have been shown to reliably distinguish between inflamed and noninflamed pouches and to correlate with the severity of pouchitis. In the study by Thomas and colleagues, all patients with endoscopic and histological signs of pouchitis had elevated calprotectin levels compared with only 2 of 15 patients with noninflamed pouches [Thomas *et al.* 2000]. In the study by Johnson and colleagues, calprotectin levels were significantly increased in patients with pouchitis. In addition, calprotectin correlated with the Pouch Disease Activity Index as well as the endoscopic and histological inflammatory score [Johnson *et al.* 2008]. Faecal calprotectin measurement reflected the inflammatory status in children after proctocolectomy [Pakarinen *et al.* 2010]. Parsi and colleagues reported that faecal lactoferrin levels correlated with pouchitis disease activity index scores and had a sensitivity and specificity of 100% and 85% respectively in diagnosing pouchitis [Parsi *et al.* 2004]. The results are in line with the study of Lim and colleagues. They calculated that lactoferrin had a sensitivity of 100% and a specificity of 86%, with a PPV of 76% in predicting inflammation of the pouch [Lim *et al.* 2008].

Despite considerable consistency of these test results, available data are still insufficient to draw final conclusions. Studies have been performed in only a small number of patients. If these preliminary data are confirmed in larger studies, stool markers such as calprotectin and lactoferrin could reduce the need for endoscopy.

Calprotectin versus blood tests

For the assessment of mucosal inflammation, faecal calprotectin has been consistently shown to be superior to clinical indices or serum parameters such as CRP, erythrocyte sedimentation rate or leucocytes. Blood tests have a low sensitivity and specificity to detect IBD and their correlation with clinical parameters is poor [Tibble *et al.* 2000a; Schoepfer *et al.* 2008]. The same findings apply to children, as recently shown in a comprehensive case-control study [Henderson *et al.* 2012].

Calprotectin: different ELISA tests

Calprotectin can be measured in faeces by quantitative enzyme-linked immunosorbent assay

(ELISA) technique. Roseth and colleagues described the original faecal calprotectin ELISA test [Roseth *et al.* 1992]. In the meantime, several different ELISA test kits are available. The diagnostic accuracy of faecal calprotectin depends on the individual test used. In a recently published study, we found, that monoclonal testing of faecal calprotectin was superior to polyclonal testing in identifying symptomatic patients with organic intestinal disease [Burri *et al.* 2013].

Recently, bedside tests for faecal calprotectin have been developed, which may be more suitable in primary care. These tests can be performed in less than 30 min and show a good correlation with the traditional laboratory ELISA tests [Wassell *et al.* 2012; Kok *et al.* 2012; Sydora *et al.* 2012; Kolho *et al.* 2012].

Limitations of stool biomarkers

- (1) Patients may be reluctant to handle faecal material. So far, this concern cannot be confirmed. In a recently published study, our group found a compliance rate of 89.5% with only 6 out of 57 patients denying a second stool test [Lehmann *et al.* 2014]. This is in the same range as the compliance rate of 96% in 602 patients referred for colonoscopy described by Tibble and colleagues [Tibble *et al.* 2002]. For serial measurements of stool tests, the compliance rate might be lower.
- (2) Stool markers are not specific for IBD. Faecal calprotectin may be increased in different organic diseases such as colorectal carcinoma, bacterial or viral gastroenteritis, diverticulitis, food intolerance, nonsteroidal enteropathy, and after pelvic radiation. In an observational study on 575 patients, we demonstrated that faecal calprotectin is increased in organic diseases of the upper gastrointestinal tract as well [Manz *et al.* 2012].
- (3) Calprotectin concentration depends on different physiological factors, for example, age, clinical comorbidities, and may also have considerable day-to-day variation. The distribution of calprotectin within a stool sample seems to be homogeneous [Røseth *et al.* 1992]. In a study by Naismith and colleagues in 143 consecutive patients with quiescent CD, the interclass correlation of consecutive calprotectin samples was high (0.84) and

only a minority of patients (16%) showed within-patient variability across three samples [Naismith *et al.* 2013]. However, considerable day-to-day variability of faecal calprotectin may exist [Husebye *et al.* 2001; Moum *et al.* 2010]. In a recent well controlled study [Lasson *et al.* 2014] the coefficient of variation suggested a marked intraindividual variability of faecal calprotectin values but it is important to note that the variability was greatest in patients with high concentrations of calprotectin, questioning the clinical relevance of this finding.

- (4) No validated cutoff exists to define active disease and clinical remission. The optimal cutoff threshold has not yet been established and may depend on the specific assay used as well as on the clinical situation. It is conceivable that a higher cutoff might be suitable for patients with known IBD and a lower threshold for screening purposes [D'Incà *et al.* 2007]. Most studies use a cutoff of 50 µg/g as suggested by the manufacturer. Tibble and colleagues reported that a cutoff of as low as 30 µg/g had the best sensitivity for discriminating IBS from IBD [Tibble *et al.* 2000b]. The lower the cutoff, the lower the number of false-positive patients referred for unnecessary endoscopies.

The lack of validated cutoff levels applies not only to distinguish IBD from IBS, but also to predict clinical remission, MH and assessment of treatment response. For predicting endoscopic remission, a broad range of cutoffs (57–274 µg/g) has been suggested. The decision of which cutoff to use depends on different parameters, including recommendations of the manufacturer, published data, receiver operating characteristic (ROC) analyses or 95% percentiles of healthy volunteers as discussed by Burri and Beglinger [Burri and Beglinger, 2012]. Results from the different studies are not directly comparable because different assays and different cutoffs were used. For clinical use, a calprotectin level of less than 50 µg/g is negative, 50–100 weakly positive, and over 100 positive.

- (5) There is considerable overlap in calprotectin levels between organic gastrointestinal disease and IBS. It is currently unclear, whether patients with calprotectin levels from 50 to 150 µg/g should be referred for colonoscopy or not. It is believed that most

of these patients, at least in a primary care setting, do not have organic disease. Repeat testing may be appropriate before deciding on endoscopy.

- (6) Calprotectin and lactoferrin may be normal in patients with clinically and endoscopically active CD, particularly in ileal disease [Sipponen *et al.* 2008]. So far, no validated definition of MH exists and thus calprotectin levels indicating endoscopically quiescent IBD are still under debate.
- (7) Calprotectin does not help to differentiate between CD and UC.
- (8) Calprotectin cannot be used for localization of IBD.

Only sparse and controversial data exist as to whether calprotectin levels are correlated to specific disease locations. Sipponen and colleagues found higher calprotectin concentrations in colonic compared with ileal CD [Sipponen *et al.* 2008]. In contrast, Jensen and Bremner found similar calprotectin levels in patients with CD, irrespective of ileal, ileocolonic or colonic disease [Jensen *et al.* 2011; Bremner *et al.* 2005]. In UC, Ricanek and colleagues reported that the median calprotectin concentrations were higher in extensive and left-sided disease than in exclusive proctitis [Ricanek *et al.* 2011].

New markers

A variety of different stool markers have been examined for their role in diagnosis and monitoring of active IBD. Most studies have been performed with the neutrophil-derived calprotectin. Other biomarkers including lactoferrin and S100A12 have yielded comparable results to calprotectin, but are not yet sufficiently characterized or validated. The results from other stool markers have to be considered preliminary.

Lactoferrin

Faecal lactoferrin is an iron-binding protein, which is mainly neutrophil derived, but may also be secreted by several mucosal epithelial cell types. Lactoferrin is stable in stool for at least 48 h and can be measured by quantitative ELISA.

Like calprotectin, lactoferrin is nonspecific. Besides in active IBD, it can be increased in several other disorders such as infective diarrhoea, colon cancer or after the use of nonsteroidal anti-inflammatory drugs.

Several studies demonstrated significantly increased lactoferrin levels in active UC and CD. In distinguishing IBD from IBS, lactoferrin had a sensitivity of 56–100%, a specificity of 61–100%, a PPV of 59–100% and a NPV of 78–99% [D'Incà *et al.* 2007; Schoepfer *et al.* 2008; Schroeder *et al.* 2007; Kane *et al.* 2003; Foell *et al.* 2009]. Even in patients with inactive IBD, lactoferrin concentrations were significantly higher than in healthy controls or in patients with IBS [Sidhu *et al.* 2010].

Lactoferrin has been shown to correlate well with clinical, endoscopic and histological grading of IBD disease activity. Patients with UC and CD with active inflammation had significantly higher levels of lactoferrin than those with inactive disease [Kane *et al.* 2003; Uchida *et al.* 1994; Silberer *et al.* 2005; Walker *et al.* 2007; Parsi *et al.* 2004; Dai *et al.* 2007; Langhorst *et al.* 2005, 2008; Schoepfer *et al.* 2007, 2008; Sipponen *et al.* 2008; Jones *et al.* 2008].

Lactoferrin may be useful in monitoring IBD therapy, although the studies performed so far are limited. In an observational study of five children with severe CD, lactoferrin concentrations decreased following infliximab therapy [Buderus *et al.* 2004]. Similar results were reported from Sipponen and Masoodi. In the study of Sipponen, lactoferrin normalized in all five patients with CD who achieved MH [Sipponen *et al.* 2008]. In the study by Masoodi and colleagues, the decline of lactoferrin correlated with a decrease in Mayo Score [Masoodi *et al.* 2009].

Patients with IBD experiencing clinical relapse within the next 2 months show significantly higher lactoferrin levels [Sipponen *et al.* 2010]. In the study by Gisbert and colleagues, lactoferrin had a 46% sensitivity and 61% specificity to predict relapses in UC and of 77% sensitivity and 68% specificity in CD [Gisbert *et al.* 2009].

In studies designed to compare patients with IBD with patients with IBS or healthy controls, calprotectin and lactoferrin showed comparable diagnostic accuracy. In the study by D'Incà and colleagues, calprotectin and lactoferrin had similar sensitivity (78% *versus* 80%), specificity (83% *versus* 85%), PPV (86% *versus* 87%) and diagnostic accuracy (80% *versus* 81%) [D'Incà *et al.* 2007].

It is controversial whether lactoferrin may be able to differentiate between UC and CD. Scarpa and

colleagues described significantly higher lactoferrin concentrations in UC [Scarpa *et al.* 2007]. However, these findings could not be confirmed [Dai *et al.* 2007]. As for calprotectin, lactoferrin levels appear not to correlate with disease location.

S100A12

S100A12 is a specific neutrophilic protein, which is strongly upregulated during active IBD. Its release from intestinal mucosal specimens correlates with the activity of inflammation [Foell *et al.* 2008]. In the study by de Jong and colleagues, faecal S100A12 had a sensitivity of 96% and a specificity of 92% when distinguishing between healthy controls and PIBD [de Jong *et al.* 2006]. Current data suggest that S100A12 may be a more accurate marker of intestinal inflammation than calprotectin [Foell *et al.* 2009].

Kaiser and colleagues calculated the correlation between faecal S100A12 and endoscopic as well as histological activity in both CD and UC. They found that S100A12 was superior to calprotectin in separating active IBD from IBS [Kaiser *et al.* 2007]. Sidler and colleagues confirmed the higher diagnostic accuracy of S100A12 [Sidler *et al.* 2008]. The sensitivity and specificity of faecal S100A12 for the diagnosis of paediatric IBS were both 97%, whereas calprotectin had a sensitivity of 100% but a specificity of only 67%. The better diagnostic performance of S100A12 was attributed to its specific expression in neutrophils. However, data on S100A12 are limited and the results have not been confirmed by others [Manolakis *et al.* 2010].

Neopterin

Neopterin, an intermediate metabolite of biop-
terin, is released from activated macrophages. In clinically and endoscopically active IBD, faecal neopterin is significantly higher than in inactive disease [Nancey *et al.* 2013]. Neopterin concentrations correlated well with the severity of mucosal lesions. The diagnostic accuracy of neopterin and calprotectin was comparable and both markers performed better in UC. In line with calprotectin and lactoferrin, the increased release of neopterin is nonspecific and may also be triggered by other disorders such as viral infections. It has been postulated that the release of neopterin as a marker of cell-mediated immune response might occur earlier in the inflammatory process than calprotectin.

Haemoglobin

In the study by Mooiweer and colleagues, faecal haemoglobin and calprotectin had comparable diagnostic accuracy in detecting IBD. Before introducing faecal haemoglobin in the diagnostic and therapeutic management of patients with IBD, data need to be confirmed in future studies [Mooiweer *et al.* 2014].

Metalloproteinases

Metalloproteinases (MMPs) belong to a family of zinc-dependent endopeptidases, which are secreted by various cell types. In IBD, MMPs such as MMP-9 are released from activated neutrophils. In biopsies of patients with UC, MMP-1, -2, -3 and -9 were detected in significantly higher concentrations than in controls [Baugh *et al.* 1999]. Annahazi and colleagues examined faecal MMP-9 concentrations in 47 adult patients with active UC [Annahazi *et al.* 2013]. They found that MMP-9 levels were significantly higher in active UC than in IBS or healthy controls. Sensitivity and specificity of MMP-9 in distinguishing between UC and IBS was 85% and 100%, respectively. Faecal MMP-9 concentrations correlated significantly with calprotectin levels as well as with endoscopically assessed disease activity. These findings could be confirmed in 110 patients with PIBD. In the study by Kolho and colleagues, MMP-9 and calprotectin showed comparable diagnostic accuracy [Kolho *et al.* 2014].

Myeloperoxidases

Myeloperoxidases (MPOs) are lysosomal proteins which are released by activated neutrophils during inflammation. Only a few authors examined the role of MPOs as a diagnostic marker of IBD. Silberer and colleagues found that the diagnostic accuracy of MPOs to detect patients with IBD was inferior to calprotectin and PMN elastase [Silberer *et al.* 2005].

Polymorphonuclear elastase

PMN elastase is released from activated neutrophils. It is stable for at least 4 days at room temperature. Patients with active IBD have significantly higher concentrations of faecal PMN elastase than those with IBS or inactive IBD [Langhorst *et al.* 2008]. Sensitivity, specificity, PPV and NPV of faecal PMN elastase in distinguishing between IBD and IBS were 84%, 87%, 91% and 79% respectively and increased to 96%,

100%, 100% and 94% respectively when combined with calprotectin and lactoferrin [Schroeder *et al.* 2007]. In the study by Silberer and colleagues, faecal PMN elastase and calprotectin correlated with the endoscopically assessed severity of intestinal inflammation [Silberer *et al.* 2005].

M2-pyruvate kinase

M2-pyruvate kinase (M2PK) is a multifunctional protein, which is present in undifferentiated and proliferating tissues. Several studies clearly showed that M2PK is increased in active IBD [Czub *et al.* 2007; Turner *et al.* 2010]. Faecal M2PK has been shown to differentiate reliably between IBD and IBS [Chung-Faye *et al.* 2007]. In the same study, a significant correlation of calprotectin and M2PK has been described. The ability of M2PK to predict steroid refractoriness in severe paediatric UC was superior to calprotectin, lactoferrin and S100 A12 [Turner *et al.* 2010].

Other stool markers

Many other stool tests including $\alpha 1$ antitrypsin, TNF α , lysozyme, eosinophilic protein X or human β -defensin-2 have been suggested as potential markers of intestinal inflammation. Most of them have only a low diagnostic performance or have not been sufficiently examined and their clinical role remains unclear.

Combination of several stool markers

Most studies suggest that the combination of calprotectin with additional stool markers such as neopterin, lactoferrin or S100A12 does not result in higher diagnostic accuracy. However, in the study by Schroeder and colleagues the combination of PMN elastase with calprotectin and lactoferrin was associated with a higher sensitivity, specificity, PPV and NPV compared with PMN elastase alone [Schroeder *et al.* 2007]. There are currently not sufficient data to support the clinical use of more than one stool test at the same time. Using more than one stool test in clinical practice would lead to higher costs without a corresponding increase in diagnostic performance.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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