The use of fecal calprotectin and lactoferrin in patients with IBD. Review

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Abstract

Endoscopy has been the gold standard for diagnosing and following patients with inflammatory bowel disease. However, ileocolonoscopy is still an expensive and invasive method. Secondly we do know that clinical scores for ulcerative colitis and Crohn's disease are subjective which creates several problems. And thirdly, when using the known serological markers such as C-reactive protein, white blood cell count en albumin, one should take into account that these markers are not perfect or superior to the current diagnostic techniques given their low sensitivity and specificity.

Fecal markers may prove to have a greater specificity. Calprotectin can differentiate between active and inactive inflammatory bowel disease and between inflammatory bowel disease and irritable bowel syndrome. It correlates with the severity of symptoms and it may predict relapse especially in ulcerative colitis. Finally it can be used as a surrogate marker for the endoscopic response during treatment given a normal value of calprotectin is a reliable marker for mucosal healing. Lactoferrin also seems to be a sensitive and specific marker for the detection of chronic inflammation and for predicting relapse. The relationship with the endoscopic activity is significant and lactoferrin values are significantly higher in active endoscopic disease as compared to inactive disease. Finally, given the significant correlation with endoscopic activity, lactoferrin can function as an adequate marker for the monitoring of therapy. (Acta gastroenterol. belg., **2013**, 76, **322-328**).

Key words : calprotectin, lactoferrin, fecal markers, IBD.

Introduction

There are no pathognomonic symptoms or signs of Crohn's disease (CD) or ulcerative colitis (UC). Therefore, we need a combination of clinical, radiological, endoscopic and histological data to make a diagnosis, to exclude other diseases with similar clinical signs and to follow up on the course of inflammatory bowel diseases.

Endoscopy remains the gold standard, to confirm the diagnosis as well as to determine disease activity later in the disease course of inflammatory bowel diseases (IBD). Despite optimization of an endoscopic procedure, it remains an expensive, invasive and not well tolerated method, especially when severe lesions are present (1).

Therefore there is a clear need for a simple, rapid, sensitive, specific and inexpensive non-invasive method, especially in the pediatric population as the use of endoscopy in young patients in the follow-up of the disease has been limited due to invasiveness. It is also necessary to achieve objective data, both because patients give assessments of their symptoms and because clinicians are subjective in their assessment of patients.

The aim of this review was to discuss the need for fecal markers, their use in diagnosing IBD and following patients with IBD.

Several studies have investigated the value of non-invasive biological markers and compared them with the gold standard.

Although not all studies are conclusive regarding the sensitivity and specificity of these markers, nor with regard to cut-off values, a number of markers have been identified that can play an important additional role in the diagnosis, in determining the disease activity, in predicting disease progression and in monitoring treatment.

Search methods

A systematic search was performed in Pubmed, Mesh database in September 2009 and in June 2012, using the search terms " Calprotectin + Crohn + Ulcerative Colitis + Inflammatory Bowel Disease", "Lactoferrin + Crohn + Ulcerative Colitis + Inflammatory Bowel Disease", "Serological markers + Crohn + Ulcerative Colitis + Inflammatory Bowel Disease" and "Pediatric + biological markers + Crohn + Ulcerative Colitis + Inflammatory Bowel Disease". Also Embase is used with the terms "Inflammatory bowel disease AND children AND fecal markers". There was searched for reviews, meta-analysis and studies. The restrictions were publications in the "past 25 years" and published in "English".

Serological markers

Serological markers, including C-reactive protein (CRP), sedimentation rate of red blood cells, white blood cells (WBC), albumin and platelets have been extensively studied in inflammatory bowel disease, both for their diagnostic and differential diagnostic value and for determining disease activity and risk of complications.

The most important and most specific and sensitive marker of systemic inflammation is C-reactive protein (CRP). CRP has a short half life, and therefore levels respond rapidly to changes in the inflammatory burden (2).

The difference between CD and UC is important regarding the systemic inflammatory response. Crohn's disease is associated with a significant increase in CRP

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Table 1. — **Advantages en disadvantages in endoscopy, serological markers and fecal markers**

	Advantages	Disadvantages
Endoscopy	– Evaluation of intestinal inflammation and mucosal healing – Histological examination by biopsy – Golden standard for definite diagnosis	– Expensive – Invasive – Time consuming – Bad tolerance
Serological markers (CRP)	– Objective marker of inflammation and active disease – Good correlation with endoscopic and histological activity – Objective marker for the follow up of treatment – Cheap, simple and less invasive	– Low specificity – Detection IBD: Sensitivity up to 50-60% UC* and up to 70-100% CD** – No predictor of disease course – No clear cut-off values
Fecal markers	– Higher specificity for intestinal inflammation/Independent of other extra-intestinal processes. – Good diagnostic precision to distinguish organic en functional diseases. – Stable in faeces in room temperature during 1 week. – Cheap en simple via ELISA technique. – Child friendly	– Not consistent superior in the possibility to reproduce endoscopic inflammation – Variability in different measurements in one sample – No clear cut off values – No differentiation between IBD en infectious of medication enteropathy

* Ulcerative Colitis
** Crohn's disease.

whereas UC is associated with a modest increase. The reason for this difference is still largely unknown although there have been many suggestions in the past. On the one hand, in patients with CD there is a transmural involvement in contrast to UC where inflammation is restricted to the mucosa. On the other hand, the serum concentrations of IL-6, an important trigger of CRP expression, are significantly higher in patients with CD than in patients with UC or control patients (3). The advantages and disadvantages in the use of these serological markers are shown in Table 1.

The use of CRP in the diagnosis has been extensively studied. Studies dating back several decades identified CRP as the best serological marker in differentiating patients with inflammatory bowel disease and control patients (4-6). Depending on the cut-off values used, they identified sensitivity of 100 % in CD and of 50% in UC for the detection of inflammatory bowel disease. With the introduction of the high sensitivity cardio-CRP the cut-off for normal values of CRP in patients with IBD is even less clear. On the other hand there are recent studies by Shoepfer *et al*. showing that CRP is able to differentiate between mild and moderately active endoscopic disease but not between inactive and mildly active endoscopic disease, both in UC and CD. Unlike in CD, CRP is also unable to differentiate between moderately and severely active endoscopic disease in UC. These data underscore the differential value of CRP in CD and UC (7,8). Furthermore, many patients with established CD do not have increased levels of CRP, despite evidence of active disease, so these previous studies probably overestimated the sensitivity of CRP in detecting CD (9).

Second, CRP has also been used to distinguish quiescent from active disease. It is the best and also the only serological marker that correlates significantly with clinical disease activity. This is showed in a study by Petr Ricanek *et al*. Both in UC patients and CD patients, the clinical disease activity at the time of diagnosis was significantly related to the CRP value (95% CI 1.9-5.6 mg/l, $p < 0.001$ and 95% CI 0.0-3.2 mg/l, $p = 0.047$ respectively) (10). However, it is clear from multiple studies that a broad range of CRP values is found in patients with CD, and that there was an overlap between mild, moderate and severe diseases respectively (7,8). Solem *et al*. observed that active disease at ileocolonoscopy was significantly associated with an elevated CRP (OR 3.5 ; 95% CI, 1.4-8.9). This correlation was less strong for UC (11).

In addition, the role of CRP in predicting disease relapse has also been examined. In patients with CD, there are several studies that associated increased levels of CRP with relapse. The hypothesis underlying this predictive value is that elevated CRP values are a surrogate of a subclinical disease (7,8,12,13-16). Data are less clear in UC. A study by Bitton *et al*. showed no association between relapse and increased levels of CRP (17).

Finally, CRP has a role in monitoring the effect of medical therapy on the underlying inflammation. A decrease in CRP during treatment objectively shows the beneficial effect of medication on the gastro-intestinal inflammation even in patients with minimal changes in clinical symptoms. Jürgens *et al*. described an association between CRP levels and the response to Infliximab in patients with CD. A persistent increased CRP indicates failure of the therapy (2,18). In a study by Louis E. *et al*. there was a positive significant association between response to Infliximab and CRP level before treatment. They described a better response to anti-TNF therapy in patients with CRP values > 5 mg/L prior to therapy compared with patients with CRP < 5 mg/L prior to therapy (76% vs. 46% respectively) (19). Colombel *et al*. confirmed this in a randomized clinical trial with Infliximab in patients with active CD (20).

Table 2. — **Calprotectin en Lactoferrin and there use in IBD**

	Calprotectin	Lactoferrin
Diagnosis	– Inflammatory ↔ Non-inflammatory bowel diseases – Active IBD ↔ Inactive IBD – IBD ↔ IBS	– Sensitive and specific marker for detection of chronic inflammation – Active IBD ↔ Inactive IBD – IBD ↔ IBS
Determination of disease activity	– Distinguish inactive, mild, moderate and severe active disease – Higher values in ileocolic localization in CD – Significant correlation with endoscopic and histological activity in UC > CD	– Significant correlation with endoscopic activity in CD – Higher values when colon involved
Disease course	– Significant positive correlation between probability to relapse and basal values	– Significant ↑ in a clinical relapse. – Sensitivity en specificity CD > UC
Therapeutic response	– Normal values are a trustable surrogate marker for endoscopic mucosal healing	– Adequate surrogate marker for endoscopic response in following disease course

IBD : Inflammatory bowel diseases
IBS : Irritable bowel syndrome
CD : Crohn's disease
UC : Ulcerative Colitis.

Fecal markers

The main drawback of the serological markers is their lack of specificity. Biomarkers reflecting the inflammatory burden in the gastrointestinal tract with a high level of specificity are therefore clearly needed. Theoretically, the big advantage of fecal biomarker testing is that these tests measure proteins originating in the intestinal mucosa, which means that they should reflect purely intestinal inflammation. A second important advantage of these markers is that they may eliminate the need for endoscopic evaluation to determine the disease activity. This has to imply that they correlate sufficiently with mucosal lesions and that levels improve with medical therapy. Thirdly, analyzing a stool sample is also less invasive than performing a full colonoscopy (Table 1). Since neutrophil infiltration is a common phenomenon in intestinal mucosal inflammation, a whole series of proteins from neutrophils have been studied including fecal lactoferrin, lysozyme, elastase, myeloperoxidase and calprotectin (1,2). The specific characteristics of Calprotectin en Lactoferrin are shown in Table 2.

Calprotectin

Calprotectin has been most widely studied as a fecal marker of intestinal inflammation (21). It is a 36-kilodalton calcium- and zinc-binding protein that represents 60% of the cytosolic proteins in granulocytes. The concentration of calprotectin in feces is an indirect measure of neutrophil infiltrate in the bowel mucosa. The potential strength of fecal calprotectin assessment is that it is a measure of mucosal inflammatory activity that may be detected at a level insufficient to cause an increase in CRP. Secondly, calprotectin has the advantage of showing excellent stability in feces at room temperature for as long as a week. One of the main disadvantages is the potential lack of specificity as it increases after the use of non-steroidal-anti-inflammatory drugs (aspirin included), probably due to the associated enteropathy (1). Also it had been suggested that calprotectin changes with age. However, one study suggested that the cut-off level for adults (< 50 μg/g) can be used for children aged from 4 to17 years (22). Furthermore, it has been estimated that a bleeding volume of at least 100ml daily, can cause an elevated fecal calprotectin. Therefore, the use of calprotectin is less reliable in a patient with ongoing menstrual or nasal bleeding. Finally, some authors described a considerable variability among measurements in the same fecal sample or different samples from stools of consecutive days of the same patient (1). This day to day variability might be due to changes in diet and physical activity (22). The main advantages en disadvantages of calprotectin are shown in Table 3.

The role of fecal calprotectin in the diagnosis of IBD is still debated. Numerous studies have adressed whether fecal calprotectin could be used to select patients with symptoms suggestive for IBD that warrant endoscopic evaluation. In a meta-analysis of Von Roon *et al*. the precision of fecal calprotectin for the diagnosis of IBD appears to be superior to serological markers such as CRP. Calprotectin has a good diagnostic precision for separating IBD from non-IBD diagnoses overall, providing better results than the classically recommended CRP (23). Van Rheenen *et al*. performed a similar analysis and concluded that using these tests to choose which patients require further testing, reduces the need for endoscopy in a large portion of patients (24). A study by Jost Langhorst also shows that calprotectin can successfully differentiate between active and inactive IBD and between IBD and irritable bowel syndrome (IBS) (25).

The various classification systems of IBDs clinical activity are based, mostly, on subjective criteria and there-

Table 3. — **Advantages en disadvantages in the use of calprotectin**

Advantages	* A measure of mucosal inflammatory activity that may be detected at a level insufficient to cause an increase in CRP * Excellent stability in feces at room temperature for as long as a week
Disadvantages	* Increase with the use of NSAID's (including aspirin) * Age-dependent values * Elevated when there is blood loss with a volume that exceeds 100 ml/day * Within sample and day-to-day

fore less reliable. The correlation between the classic clinical activity indexes and the endoscopic and histological lesions is therefore far from perfect (26). Several studies have confirmed the parallelism between fecal calprotectin levels and degree of IBD activity evaluated with clinical, endoscopic and histological parameters (1). In this evaluation of degree of IBD activity, calprotectin determination seems to better reflect disease activity in UC than in CD. A study by Costa *et al*. found that calprotectin levels > 50 μg/g were better correlated with the UC activity index than the CD activity index (27). On the other hand, other studies have not been able to demonstrate correlation between calprotectin concentrations and UC clinical activity (28). However, in a study by shoepfer et al, in UC fecal calprotectin correlated closest with endoscopic disease activity, followed by Clinical Activity Index, CRP and blood leukocytes. Furthermore, fecal calprotectin was the only marker that reliably discriminated inactive from mild, moderate and highly active disease (7). Also Jun-Ying Xiang *et al*. investigated the possibility and clinical application of fecal calprotectin in determining disease activity of UC. Fecal calprotectin concentrations were significantly higher in patients with active UC than in patients with inactive UC and than in controls. In addition, fecal calprotectin concentration was higher in the patients with inactive UC than in the control population (29). A prospective study in a Southeastern Norway population by P. Ricanek *et al*. investigated disease characteristics in an attempt to improve knowledge regarding factors related to disease activity. The median fecal calprotectin concentration was higher in UC patients with extensive and left-sided colitis compared to patients with proctitis ($p = 0.007$ and $p = 0.009$ respectively). The calprotectin concentrations in feces were significantly related to the clinical activity, the endoscopic grade of inflammation and CRP at diagnosis of UC (10). Studies evaluating the correlation between the CD clinical activity index and fecal calprotectin are scarce. Schoepfer *et al*. evaluated this correlation and demonstrated that fecal calprotectin correlated closest with the CD clinical activity and that it was the only biological marker that reliably discriminated the four subgroups of CD (inactive, mild, moderate and highly active disease). Also fecal calprotectin demonstrated a good correlation with the histological en endoscopic grade of colonic inflammation. Shoepfer *et al*. also described the relationship between fecal calprotectin concentrations and disease location. In summary, ileocolonic CD was associated with significantly higher mean calprotectin compared with CD located in the terminal ileum (8). Jennifer Jones *et al*. showed similar results. Fecal calprotectin concentrations are associated with the endoscopic but not with the clinical activity index (30). Likewise, Sipponem *et al*. made the conclusion that for the evaluation of CD activity , based on endoscopic findings, fecal calprotectin is a more sensitive surrogate marker than is CD clinical activity index. Concentrations were significantly higher in endoscopically active disease than in inactive disease, and were also significantly higher in colonic than in ileal disease (31). In contrast, the prospective study by P. Ricanek *et al*. discussed earlier, showed no relation between fecal calprotectin and the localization of CD. Calprotectin was also not associated with the clinical disease activity but significantly related to the endoscopic grade of inflammation ($p = 0.004$) (10). However all these studies are showing the parallelism between fecal calprotectin levels and degree of IBD activity. There is a new nationwide study by Shoepfer A. *et al*. that investigated by which method gastroenterologists monitor IBD activity in daily practice. The results demonstrate that clinical activity is regarded as the most relevant factor for the assessment of IBD activity by the majority of gastroenterologists. Furthermore, therapeutic decisions are primarily based on the assessment of clinical activity, whereas endoscopic activity and biomarkers measuring inflammation appear to play a minor role (32).

The natural course of IBD is characterized by activity outbreaks and longer or shorter periods of remission. Both for UC and CD these outbreaks are relatively unpredictable. Identifying patients with a significant risk for an activity outbreak, suggests the possibility of targeted treatment of patients according to the existing risk of relapse. Also, the prediction of a relapse could enable early treatment in order to have a faster and greater response with potentially fewer side effects. Thirdly, any maintenance therapy could be stopped when there is a sufficiently low risk of an outbreak (1,33). Gisbert *et al*. informed that 8% of the patients having calprotectin concentrations under 150 μg/g relapsed during follow-up, while this occurred in as many as 30% of the patients with calprotectin concentrations above 150 μg/g at baseline. Therefore, fecal calprotectin's (above 150 μg/g) sensitivity and specificity to predict relapse in IBD were around 70%. A fecal calprotectin concentration > 150 μg/g was associated with an activity outbreak within twelve months after stool collection. Gisbert also demonstrated that early relapse (< three months) was associated with higher calprotectin concentrations than the

values at a later outbreak (> three months) (33). Costa *et al*. reported a 2-and 14-fold greater risk of relapse in patients with CD and UC, respectively, among those subjects with higher concentrations at the time of inclusion in the study. Costa *et al*. considered this marker to offer a more reliable prediction of relapse in UC than in CD. Possibly because this fecal marker could better reflect disease activity in UC than in CD (34). A study by Ho GT al aimed to investigate fecal calprotectin as a biomarker in predicting the clinical course of acute severe UC. Calprotectin values were markedly elevated in severe UC and were significantly higher in patients who have failed medical therapy and requiring urgent colectomy. In contrast, this study hypothesized that in circumstances of very advanced colonic epithelial destruction, fecal calprotectin may not be reliable (12). A prospective study by Valle Garcia-Sanchez *et al*. also evaluated the utility of calprotectin in predicting relapse in patients with IBD. The results showed that patients with IBD in remission and levels in excess of 150 μg/g presented an almost 6-fold greater risk of relapse than those patients with lower concentrations. Patients with UC or colonic or ileocolonic CD and inflammatory pattern exhibiting calprotectin levels in excess of 120 μg/g presented a 5-fold greater risk of relapse than the patients with lower concentrations (35).

Estimation of the response to IBD treatment is generally based on the evaluation of symptoms while endoscopic controls are exceptional. It has been proposed that patients who do not reach intestinal mucosal healing after treatment have higher chances of suffering clinical relapse. Normalization of endoscopic lesions should be the real therapeutic aim for IBD patients. Obviously, routine confirmation of endoscopic and histological healing is not realistic (1). As previously discussed, calprotectin concentrations are correlated with endoscopic and histological activity so response to treatment could be estimated using this fecal marker. The first study that demonstrated that endoscopic healing in IBD patients can be determined by assessment of calprotectin in a simple stool test was made by Roseth *et al*. Also they found a significant difference in median fecal calprotectin levels between UC and CD patients (16 mg/L vs. 35 mg respectively) ($P < 0.05$) but it is uncertain whether this has any clinical relevance (36). A study by Sipponen *et al*. showed that, despite therapy, calprotectin remained abnormal in the majority of endoscopic 'non-responders' or 'partial responders', whereas in all responders calprotectin decreased significantly from his baseline concentration (37). Another prospective study by Sipponen *et al*. showed that fecal calprotectin correlated closely with endoscopic activity during anti-TNF α therapy, and that an elevated concentration was a highly specific surrogate marker of endoscopically active disease (38). Wagner *et al*. also demonstrated that a normalized fecal calprotectin has the potential to be used as a surrogate marker for successful treatment outcome in IBD patients. In both UC and CD, patients with normalized calprotectin levels after 8 weeks of treatment fulfilled predefined criteria of a complete response. Using calprotectin values below the upper limit of normal as a negative predictor of active disease after 8weeks of treatment, revealed a negative predictive value of 100%. So we could suggest that a normalization of calprotectin predicts mucosal healing in patients with IBD. In contrast, using an elevated level of calprotectin as a positive predictor for ongoing active disease of treatment failure after 8 weeks of treatment, there was a positive predictive value of only 38% in UC and 14% in CD (39). A more recent prospective study by De Vos M. *et al*. evaluated the effect of Infliximab induction therapy on calprotectin levels in patients with UC.

He and his colleagues showed that Infliximab induction therapy induced a complete endoscopic remission and a decrease of calprotectin levels to less than 50 mg/kg or at least an 80% decrease from start levels in 58% of UC patients with active disease. The absence of this decrease seems to identify a group of non-responders. Calprotectin < 50 mg/kg is a very good predictor for mucosal healing and can possibly be used as an additional marker for deep remission (40). The role of TNF-α in the pathogenesis of UC has been debated but P. Rutgeerts *et al*. evaluated the efficacy of Infliximab for induction and maintenance therapy in adults with ulcerative colitis. He showed that therapy with Infliximab in patients with moderate-to-severe UC is superior to placebo in achieving clinical response and remission, mucosal healing, and corticosteroid-sparing effects (41).

Lactoferrin

Another marker for intestinal inflammation is lactoferrin. This marker is an iron-binding glycoprotein found in neutrophil granules and serum and is secreted by mucosal membranes. During intestinal inflammation, leukocytes infiltrate the mucosa resulting in an increase in the concentration of lactoferrin in the feces. Since this protein is resistant to proteolysis it can be a useful marker in feces as an indicator of mucosal inflammation (33,42).

Sunanda V. Kane *et al*. determined the sensitivity and specificity of lactoferrin concentrations for IBD or IBS versus healthy controls. They showed that lactoferrin concentrations were significantly higher in patients with active and inactive IBD than in patients with IBS or healthy controls ($p = 0.02$). Furthermore, the sensitivity and specificity for distinguishing active IBD from IBS and healthy controls were 86 and 100% respectively. The sensitivity is better in patients with active IBD compared with those with inactive IBD (86% vs. 56%). Also this study reported higher mean lactoferrin concentrations in UC than in CD ($P = 0.04$) (42). Andrea Vieira *et al*. also evaluated the efficacy of fecal markers as indicators of inflammatory activity. The results were consistent with those found in the study from Sunanda V. Kane *et al*., described earlier. Lactoferrin is a sensitive and specific marker to identify intestinal inflammation in patients with known IBD (43). Langhorst J. en his colleagues

recruited 140 patients with IBD or IBS over an 18-month period. This study carried two messages. First, lactoferrin was able to differentiate active from inactive IBD and IBD from IBS. Secondly, lactoferrin (and also calprotectin) was superior to CRP in his diagnostic accuracy. Furthermore, in UC patients with inflammation, concentrations of lactoferrin were significantly higher than in patients without inflammation ($p < 0.01$). Similarly, in CD patients with inflammation, concentrations of lactoferrin were higher than in CD patients without inflammation ($p < 0.05$) but this was less specific than for UC (25).

As described before, several studies showed the usefulness of fecal markers in evaluating disease activity in IBD. Fecal lactoferrin is less well documented and literature data are mainly limited to patients with CD. Jennifer Jones *et al*. concluded that the lactoferrin concentration, as calprotectin, is associated with endoscopic activity. The correlation with the clinical activity index was less pronounced or even non-existent (30). In addition, Sipponen *et al*. studied the clinical significance of lactoferrin in the assessment of CD activity. They found that lactoferrin concentrations, both in adults and children, were significantly higher in endoscopically active disease than in inactive disease, and were also higher in colonic than in ileal disease. Furthermore in strictly ileal disease, only lactoferrin (and not calprotectin) correlated significantly with the endoscopic activity (31).

The use of fecal lactoferrin in predicting clinical relapse is not yet well described. Although lactoferrin levels may rise significantly prior to clinical relapse, the experience with this fecal marker for this purpose has been very scarce. A prospective study by Gisbert *et al*. determined the role of lactoferrin in the prediction of IBD relapses. A positive lactoferrin test was significantly associated with clinical relapse within 12 months of stool sample collection ($p < 0.05$) (33).

Lactoferrin as a marker for treatment response is mainly studied in CD patients but less well documented in comparison with calprotectin. A prospective study by Sipponem *et al*. showed that despite therapy, lactoferrin remained abnormal in the majority of endoscopic non-responders or partial responder, whereas in all responders lactoferrin (and also calprotectin) decreased significantly from his baseline concentration (37). Another study by Sipponem *et al*. studied the clinical significance of this fecal marker during anti-TNF alfa therapy. Following treatment, both the endoscopic activity score and lactoferrin levels declined from the baseline level significantly. When pre-and post treatment data were combined, the endoscopic activity score correlated significantly with the fecal markers (lactoferrin en calprotectin, both $P < 0.001$) during anti-TNF alfa therapy and their elevated concentration was a highly specific surrogate marker of endoscopically active disease. The concentration of lactoferrin in stool declined in treatment responders significantly from the baseline level and normalized in almost all those who reached endoscopic remission (38).

Conclusion

The lack of specificity of serological markers and the invasive and expensive character of endoscopy has fostered research into fecal markers for IBD. Fecal calprotectin and lactoferrin have a good diagnostic accuracy for distinguishing functional and organic intestinal diseases and provide generally better results than the traditionally identified serological markers. Fecal markers also measure mucosal inflammatory activity at a level insufficient to cause an increase in CRP. They are also able to differentiate between active and inactive IBD. The correlation of calprotectin with the histological and endoscopic activity is significant and normal values appear to be associated with endoscopic mucosal healing. The correlation of fecal lactoferrin with mucosal inflammation is less well documented but it may outperform calprotectin in the assessment of purely ileal disease. No fecal marker is clearly and consistently superior in its ability to reflect endoscopic inflammation, but both calprotectin as lactoferrin are superior to CRP in their diagnostic accuracy. The combination of fecal markers with the CRP and the disease-specific activity indices may increase the diagnostic accuracy. Both calprotectin and lactoferrin are useful markers for the evaluation of therapy in CD and they can discriminate between responders and non-responders to treatment. Also, normalization of calprotectin is a sensitive predictor of mucosal healing during therapy. The main drawback of fecal markers is the lack of specificity to distinguish between mucosal inflammation caused by an IBD disease flare or by infectious or drug induced enteropathy and therefore endoscopic confirmation of IBD related inflammation will not become obsolete any time soon.

References

1. GISBERT J.P., MCNICHOLL A.G. Questions and answers on the role of fecal calprotectin as a biological marker in inflammatory bowel disease. *Dig. Liver Dis*., 2009, **41** (1) : 56-66.
2. VERMEIRE S., VAN ASSCHE G., RUTGEERTS P. Laboratory markers in IBD : useful, magic, or unnecessary toys ? Gut, 2006, 55 : 426-431.
3. VERMEIRE S., VAN ASSCHE G., RUTGEERTS P. The role of C-reactive protein as an inflammatory marker in gastrointestinal diseases. *Nat. Clin. Pract. Gastroenterol. Hepatol*., 2005, **2** : 580-586.
4. SHINE B. *et al*. C-reactive protein as an aid in the differentiation of functional and inflammatory bowel disorders. *Clin. Chim. Acta*, 1985, **148** : 105-109.
5. BEATLLE R.M. *et al*. Indications for investigation of chronic gastrointestinal symptoms. *Arch. Dis. Child*., 1995, **73** : 354-355.
6. POULLIS A.P., ZAR S., SUNDARAM K.K. *et al*. A new, highly sensitive assay for C-reactive protein can aid the differentiation of inflammatory bowel disorders from constipation-and diarrhea-predominant functional bowel disorders. *Eur. J. Gastroenterol. Hepatol*., 2002, **14** : 409-412.
7. SCHOEPFER A.M. *et al*. Ulcerative colitis : Correlation of the Rachmilewitz Endoscopic Activity Index with fecal Calprotectin, Clinical Activity, C-reactive protein, and Blood leukocytes. *Inflamm. Bowel Dis*., 2009, **15** (12) : 1851-1858.
8. SCHOEPFER A.M. *et al*. Fecal calprotectin correlates more closely with de Simple Endoscopic Score for Crohn's Disease (SES-CD) than CRP, Blood leukocytes, and the CDAI. *Am. J. Gastroenterol*., 2010, **105** (1) : 162-169.
9. LEWIS J.D. C-reactive protein : anti-placebo or predictor of response. *Gastroenterology*, 2005, **129** : 1114-1116.

10. RICANEK P. *et al*. Evaluation of disease activity in IBD at the diagnosis by the use of clinical, biochemical, and fecal markers. *Scandinavian Journal of Gastroenterology*, 2011, **46** : 1081-1091.
11. SOLEM C.A. *et al*. Correlation of C-reactive protein (CRP) with clinical, radiographic, and endoscopic activity in inflammatory bowel disease (IBD). *Inflamm. Bowel Dis*., 2005, **11** : 707-712.
12. HO G.T. *et al*. Fecal calprotectin predicts the Clinical Course of Acute Severe Ulcerative Colitis. *Am. J. Gastroenterol*., 2009, **104** : 673-678.
13. CONSIGNY Y., MODIGLIANI R., COLOMBEL J.F. *et al*. A simple biological score for predicting low risk of short-term relapse in Crohn's disease. *Inflamm. Bowel Dis*., 2006, **12** (7) : 551-557.
14. OUSSALAH A., CHEVAUX J.B., FAY R. *et al*. Predictors of infliximab failure after azathioprine withdrawal in Crohn's disease treated with combination therapy. *Am. J. Gastroenterol*., 2010, **105** : 1142-1149.
15. BITTON A., DOBKIN P.L., EDWARDES M.D. et al. Predicting relapse in Crohn's disease : a biopsychosocial model. *Gut*, 2008, **57** : 1386-1392.
16. PAPI C., FESTA V., LEANDRO G. et al. Long-term outcome of Crohn's disease following corticosteroid-induced remission. *Am. J. Gastroenterol*., 2007, **102** : 814-819.
17. BITTON A., PEPPERCORN M.A., ANTONIOLI D.A. et al. Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. *Gastroenterolog*y, 2001, **120** : 13-20.
18. JÜRGENS M., MAHACHIE J. *et al*. Levels of C-reactive protein are associated with response to infliximab therapy in patients with Crohn's disease. *Clin. Gastroenterol. Hepatol*., 2011 May, **9** (5) : 421-427.
19. LOUIS E., VERMEIRE S., RUTGEERTS P. *et al*. A positive response to infliximab in Crohn disease : association with a higher systemic inflammation before treatment but not with -308 TNF gene polymorphism. *Scand. J. Gastroenterol*., 2002, **37** : 818-824.
20. COLOMBEL J.F. *et al*. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med*, 2010 Apr 15, **362** (15) : 1383-1395.
21. ANGRIMAN I. *et al*. 5 Enzymes in faeces : useful markers of chronic inflammatory bowel disease. *Clin. Chim. Acta*, 2007, **381** : 63-68.
22. FAGERBERG U.L. *et al*. Fecal calprotectin levels in children studied with an improved assay. *J. Pediatric Gastroenterol. Nutr*., 2003, **37** : 468-472.
23. VON ROON A.C. *et al*. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. *Am. J. Gastroenterol*., 2007, **102** : 803-813.
24. VAN RHEENEN P.F. *et al*. Fecal calprotectin for screening of patients with suspected inflammatory bowel disease : diagnostic meta-analysis. *BMJ*, 2010, **341** : 3369.
25. LANGHORST J., ELSENBRUCH S., KOELZER J. *et al*. Non-invasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases : Performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP and clinical indices. *Am. J. Gastroenterol*., 2008, **103** : 162-169.
26. POULLIS A. *et al*. Review article : fecal markers in the assessment of activity in inflammatory bowel disease. *Aliment. Pharmacol. Ther*., 2002, **16** : 675-681.
27. COSTA F. *et al*. Role of fecal calprotectin as non-invasive marker of intestinal inflammation. *Dig. Liver Dis*., 2003, **35** : 642-647.
28. MONTALTO M. *et al*. Fecal calprotectin in first-degree relatives of patients with ulcerative colitis. *Am. J. Gastroenterol*., 2007, **102** : 132-136.
29. XIANG J.-Y. *et al*. Clinical value of fecal calprotectin in determining disease activity of ulcerative colitis. *World J. Gastroenterol*., 2008, **14** (1) : 53-57.
30. JONES J., LOFTUS E.V. JR., PANACCIONE R., CHEN L.S., PETERSON S., MC CONNELL J. *et al*. Relationships between disease activity and serum and fecal biomarkers in patients with Crohn's disease. *Clin. Gastroenterol. Hepatol*., 2008, **6** : 1218-1224.
31. SIPPONEN T., SAVILAHTI E., KOLHO K. *et al*. Crohn's disease activity assessed by fecal calprotectin and lactoferrin : correlation with Crohn's disease activity index and endoscopic findings. *Inflamm. Bowel Dis*., 2008, **14** : 40-46.
32. SCHOEPFER A., VAVRICKA S. *et al*. Monitoring inflammatory bowel disease activity : Clinical activity is judged to be more relevant than endoscopic severity or biomarkers. *Journals of Crohn's and Colitis*, 2012, **6** (4) : 412-418.
33. GISBERT J.P. *et al*. Fecal Calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm. Bowel Dis*., 2009, **15** : 1190-1198.
34. COSTA F. *et al*. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut*, 2005, **54** : 364-368.
35. GARCIA-SANCHEZ V. *et al*. Does fecal calprotectin predict relapse in patients with Crohn's diease and ulcerative colitis ? *Journal of Crohn's and colitis*, 2010, **4** : 144-152.
36. ROSETH A.G., AADLAND E., GRZYB K. Normalization of fecal calprotectin : a predictor of mucosal healing in patients with inflammatory bowel disease. *Scand. J. Gastroenterol*., 2004, **39** : 1017-1020.
37. SIPPONEN T. *et al*. Fecal calprotectin and lactoferrin are reliable surrogate markers of endoscopic response during Cohn's disease treatment. *Scand. J. Gastroenterol*., 2009.
38. SIPPONEN T. *et al*. Fecal Calprotectin, Lactoferrin, and Endoscopic Disease Activity in Monitoring Anti-TNF-alpha Therapy for Crohn's Disease. *Inflamm. Bowel Dis*., 2008.
39. WAGNER M., PETERSON CG., RIDEFELT P., SANGFELT P., CARLSON M. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. *World J. Gastroenterol*., 2008, **14** : 5584-5589.
40. DE VOS M., DEWIT O. *et al*. Fast and sharp decrease in calprotectin predicts remission by Infliximab in anti-TNF naïve patients with ulcerative colitis. *Journal of Crohn's and colitis*, 2012, **6** : 557-562.
41. RUTGEERTS P., SANDBORN W.J. *et al*. Infliximab for induction and maintenance therapy for ulcerative colitis. *N. Engl. J. Med*., 2005, **353** : 2462-2476.
42. KANE S.V. *et al*. Fecal lactoferrin is a sensitive and specific marker in identifying intestinal inflammation. *Am. J. Gastroenterol*., 2003, **98** : 1309-1314.
43. VIEIRA A., FANG C.B. *et al*. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin : correlation with laboratory parameters, clinical, endoscopic and histological indexes. *BCD Research Notes*, 2009, **2** : 221.