Invited critical review

Enzymes in feces: Useful markers of chronic inflammatory bowel disease

Imerio Angriman a,⁎, Marco Scarpa a, Renata D’Incà b, Daniela Basso c, Cesare Ruffolo a, Lino Polese a, Giacomo C. Stumiolo b, Davide F. D’Amico a, Mario Plebanic c

a Clinica Chirurgica I, Dipartimento di Scienze Chirurgiche e Gastroenterologiche, University of Padova, Italy
b Gastroenterologia, Dipartimento di Scienze Chirurgiche e Gastroenterologiche, University of Padova, Italy
c Medicina di Laboratorio, Dipartimento di Scienze Diagnostiche e Terapie Speciali, University of Padova, Italy

Received 22 January 2007; accepted 13 February 2007
Available online 21 February 2007

Abstract

Background: Ulcerative colitis and Crohn’s disease are characterized by a chronic intestinal inflammation. Since the precise etiology is still unknown, current therapies are aimed at reducing or eliminating inflammation.

Methods: Endoscopy and histology on biopsy specimens remain the gold standard methods for detecting and quantifying bowel inflammation. These techniques are expensive, invasive and not well tolerated by patients since the need of repeated examinations affects their quality of life. Although disease activity scores and laboratory inflammatory markers are widely used they showed unreliable relations with endoscopy and histology. Fecal markers have been investigated in inflammatory bowel disease (IBD) by many authors for diagnostic purposes, to assess disease activity and of risk of complications, to predict relapse or recurrence, and to monitor the effect of therapy. Many inflammatory mediators have been detected in the feces such as leukocytes, cytokines and proteins from neutrophil activation. Some of these, particularly lactoferrin and calprotectin, have been demonstrated to be useful in detecting active inflammatory bowel disease, in predicting recurrence of disease after surgery or monitoring the effects of medical therapy. Calprotectin and lactoferrin are remarkably stable and easily detect in stool using ELISA so they appear to be equally recommendable as inflammation markers in the lower gastrointestinal tract especially in IBD patients.

Conclusion: Fecal markers are non-invasive, simple, cheap, sensitive and specific parameters and are useful to detect intestinal inflammation.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Ulcerative colitis; Crohn’s disease; Lactoferrin; Calprotectin; C reactive protein

Contents

1. Introduction ............................................................... 63
2. Fecal markers in chronic inflammatory bowel disease ............................................ 64
3. Neutrophil-derived proteins in the feces in IBD ............................................. 65
4. Fecal enzymes in the clinical management of inflammatory bowel disease ....................... 66
5. Conclusions ............................................................... 66
References .................................................................. 67

1. Introduction

Crohn’s disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel disease (IBD), are chronic illnesses that affect the gastrointestinal tract. The incidence rate
of UC varies between 0.5 and 24.5/105 inhabitants/y, while that of CD varies between 0.1 and 16/105 inhabitants/year, with prevalence rates of IBD reaching up to 396/105 inhabitants. Recent data from South Europe [1–3], East Europe [4] and Asia [5] in the mid-1990s report a rise of the incidence of IBD and in some areas it is already comparable to rates reported in Northern Europe or North America [6]. The anatomic location and degree of inflammation determine the predominant symptoms that include rectal bleeding, diarrhea and abdominal pain. In the absence of rectal bleeding it can be difficult to differentiate between functional disorders of the lower GI tract and therefore, the diagnosis needs invasive and expensive testing such as endoscopy with biopsy for histological examination, small bowel series or barium enema. One of the major problems of clinical management of UC and CD is the lack of univocal diagnostic tool.

Several laboratory markers are used in the diagnosis of IBD and to monitor of IBD disease activity. These include erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), acute phase protein (albumin), and platelets; however, none of these markers is specific for inflammation of the gastrointestinal tract [7].

The pathogenesis of inflammatory bowel diseases implies the loss of the barrier function and the loss of the tolerance against luminal and self antigens and both these phenomenon cause the recruitment of leukocytes in the intestinal wall [8,9]. Activated leucocytes infiltrate the mucosa and can be detected in feces due to shedding in the intestinal lumen [10,11]. The most important leukocyte population in the intestinal wall in IBD are the polymorphonuclear cells. In fact, high fecal neutrophil levels were detected in inflammatory bowel disease patients and may be used as surrogate markers of active disease [12,13]. Nevertheless neutrophil determination in the stools is rather inefficient because of their brief lifetime that implies that the sample should be examined within a few hours of its collection. On the contrary calprotectin, lactoferrin and other proteins are produced in significant amounts by inflammatory cells and both lactoferrin and calprotectin fecal levels have been shown to be as useful as more prolonged intestinal protein loss [24,25]. Random fecal levels have been shown to be as useful as more prolonged collection in measuring CD activity and correlated with several other laboratory measures already known as indicators of CD activity [26]. Fecal α1-antitrypsin clearance is a useful indicator of protein-losing enteropathy [24] and that in patients with inflammatory bowel disease, 72-h fecal clearance of α1-antitrypsin is a useful method for quantization of intestinal protein loss [24,25]. Random fecal α1-antitrypsin levels have been shown to be as useful as more prolonged collection in measuring CD activity and correlated with several other laboratory measures already known as indicators of CD activity [26]. Fecal α1-antitrypsin has been generally accepted as a useful marker of IBD; however, the method is not routinely available and some studies are conflicting about the efficacy of α1-antitrypsin in diagnosis of IBD. Furthermore, data about the efficiency in the follow-up after medical or surgical therapy, such as in case of pouchitis, are lacking [17,27] and other markers were demonstrated more accurate or cost-effective than alpha1-antitrypsin in the stools of such patients [28].

Alpha2-macroglobulin is a serum anti-proteinase and its excretion in the feces is increased in IBD patients. The levels of this protein in the feces seem to correlate with CDAI in CD but not in subjects with UC. There are only a few reports in the literature and the method is not routinely available [17,29].
3. Neutrophil-derived proteins in the feces in IBD

Lysozyme is a polymorphonuclear neutrophil-derived enzyme which catalyses the hydrolysis of Gram-positive bacterial cell walls. Fecal lysozyme was found significantly elevated in patients with active and inactive CD, active UC and non-inflammatory bowel gastrointestinal diseases with diarrhea, compared to healthy controls [30]. Fecal lysozyme correlates with excretion of Indium 111-labeled granulocytes in patients with colonic disease but not in those with small bowel disease [31], therefore it is of limited value in CD patients. Some other studies found that this enzyme is less effective than other markers in diagnosis of IBD [32,33].

Myeloperoxidase is a constituent of neutrophil azure granules. Fecal levels are elevated in active IBD compared with controls and correlate with laboratory parameters and endoscopic grade of inflammation [34]. Fecal lactoferrin correlates with fecal myeloperoxidase but the ratio of fecal lactoferrin and myeloperoxidase is different in IBD compared to infective diarrhea [35]. Maybe because of its short lifetime, other enzymes showed a stronger correlation with the endoscopic classified severity of inflammation and it appears more efficient than myeloperoxidase in the routine use for diagnosis in IBD patients [32].

Granulocytes elastase was shown to correlate with the CDAI in CD and an activity index in UC [36]. Nevertheless, a direct comparison of accuracy and cost effectiveness concluded that lactoferrin was a better neutrophil-derived maker of inflammation than granulocytes elastase, fecal lysozyme and myeloperoxidase [28,37].

Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine produced also by polymorphonuclear neutrophils. Fecal TNF-α has been shown to be a useful marker of disease activity in children with IBD but it needs to be further assessed in adults [38]. The results in children are equivalent to other fecal markers such as calprotectin and the need to keep the stool frozen for risk of degradation makes this technique not useful for routine application [39]. Several studies using fecal tumor necrosis factor alpha have shown promising results, large interindividual variations in both serum and fecal levels have limited its widespread applicability [16].

Lactoferrin is a 76 kDa iron binding glycoprotein that is the major component of the secondary granules of polymorphonuclear neutrophils and is secreted by most mucosal membranes. Lactoferrin is found in many body fluids such as human milk, tears, synovial fluid and serum. The secondary granules are released by polymorphonuclear neutrophils when they degranulate during inflammatory process since lactoferrin has an important role in the innate immunity as a bactericidal [40,41]. Other hematopoietic cells such as monocytes and lymphocytes do not produce lactoferrin [42]. During intestinal inflammation, polymorphonuclear neutrophils infiltrate the mucosa, resulting in an increase in the concentration of lactoferrin in the feces and its presence is proportional to neutrophil translocation to the gastrointestinal tract [43]. The protein is resistant to proteolysis and undamaged by multiple freeze thaws, providing a useful marker in feces as an indicator of intestinal inflammation. Moreover, it is remarkably stable and resistant to degradation when left in room temperatures for extended periods of time rather easy to detect and measure in stool using ELISA [44]. This property makes this marker attractive for clinical use and several studies have been performed to identify the sensitivity and the qualitative assay for lactoferrin patients with IBD.

Lactoferrin was shown to be significantly increased in active UC and CD compared to controls [37]. Several studies on the differential diagnosis of intestinal inflammatory conditions pointed out that fecal lactoferrin is a reliable marker of bowel inflammation with sensitivity of 90% and specificity of 98% [15,40–45]. Buderus S. et al. observed that fecal lactoferrin quickly decreased after clinically successful treatment with infliximab therapy in children with severe CD and they suggested that fecal lactoferrin was a sensitive and specific marker for intestinal inflammation that can be used for monitoring the therapeutic response to infliximab in pediatrics Crohn’s diseased patients [46]. In pouchitis, levels of lactoferrin correlate with the clinical score of disease activity; therefore, fecal lactoferrin was demonstrated to differentiate patients with irritable pouch syndrome from those with pouchitis, cuffitis or CD with a sensitivity of 100% and specificity of 85%. Fecal lactoferrin can serve as a sensitive and non-invasive initial screening test in an algorithm for evaluation of symptomatic patients after restorative proctocolectomy for UC [27]. This marker of bowel inflammation may find a reasonable application on detecting postoperative recurrence of CD [47]. In our cohort of 63 CD patients who had a complete resection of all diseased bowel the mean lactoferrin fecal levels were still significantly higher than normal values after a 3 year follow-up [47]. Lactoferrin showed a reliable threshold value for systemic inflammation and seemed to predict the presence of episodes of clinical recurrence during the post-operative follow-up. In our experience the lactoferrin threshold value for being predictive of systemic inflammation (CRP>6 mg/l) is 11 μg/ml with a sensitivity of 71% and a specificity of 90% [47].

Calprotectin is the most used fecal protein. This protein accounts for up to 50% of the neutrophil cytosolic protein and it is resistant to colonic bacterial degradation. The protein is a heterocomplex protein consisting of 2 heavy (L1H) chains and 1 light (L1L) chain which are non-covalently linked [48,49]. Calprotectin appears to play a regulatory role in the inflammatory process and functions in both an antimicrobial and antiproliferative capacity [50]. Interest in calprotectin as a marker for inflammation in the gut followed the realization that Indium 111-labeled granulocyte scans could be used to both visualize and quantify the acute inflammation in the gut of patients with inflammatory bowel disease [51]. These findings led to the idea that an increased translocation of granulocytes into the intestinal mucosa in conditions of inflammation might give increased levels of proteins from such cells in feces. Some authors have demonstrated that eosinophilic granulocytes are the main cellular source of calprotectin in the normal gut mucosa [52]. However, relatively high levels of calprotectin are found in the stools of normal individuals—about 6 times the plasma levels (which are about 0.5 mg/l). This is compatible with data suggesting that in normal individuals most circulating...
neutrophils migrate through the mucosal membrane of the gut wall and thereby terminate their circulating life [52,53]. Subsequent lyses within the gut lumen and release of cytosolic calprotectin thereby accounts for the median fecal levels of 2.0 mg/l seen in healthy controls [54]. It is easily measured in feces by a commercially available ELISA. The diagnostic use of fecal calprotectin in a broad spectrum of intestinal diseases has been studied by a number of groups with remarkable agreement between the results.

Since the method requires only a single stool sample, extraction and an ELISA, it was used as a screening test to distinguish between patients with IBD and irritable bowel syndrome (IBS) in an outpatient setting. Several studies showed that a cut off of 30 mg/l had a 100% sensitivity and 94% specificity for this purpose [15,55]. Fecal calprotectin concentrations correlate with endoscopic and histological assessment of disease activity in adults and children with IBD [56]. Several other studies collected in a recent review confirmed a close correlation between fecal calprotectin concentrations and disease activity and both endoscopic and histological scores [16]. Some other studies showed that fecal calprotectin appears to be a good predictor of relapse in patients with IBD, although its value may be higher in patients with UC than those in CD [55,57]. In clinical practice fecal calprotectin could be measured at intervals during follow-up, which may allow early detection rather than just prediction of relapses. In our data of 63 patients operated for CD highly significant correlations were evidenced between calprotectin fecal levels and serum CRP (direct), serum iron and serum albumin (inverse) and, as observed for the direct consequence of the inflammatory process itself and

5. Conclusions

Lactoferrin and calprotectin appear to be the most used and useful fecal markers of intestinal inflammation. Many studies have confirmed the accuracy of these markers in the diagnosis and surveillance of patients with IBD. They are inexpensive and easily measured and, therefore, suitable for extensive use. Both tests appear to be useful in detecting bowel inflammation in symptomatic patients, achieving a similar diagnostic accuracy. Different cut-off values are suggested for different patient categories, higher for patients with known inflammatory conditions while lower for screening purposes [14].

The potential clinical implications of detection of calprotectin and lactoferrin in feces are considerable: differential diagnosis at an early stage, with the possibility of early treatment with less side effects, as well as monitoring of new therapeutic strategies to maintain symptomatic remission [16]. In fact, theoretically the monitoring of the treatment with such fecal markers can lead to a dramatic reduction in the frequency and severity of clinical relapses with an improvement in the patient’s quality of life with reduction of invasive tests. More studies are necessary to define
the role of fecal calprotectin and lactoferrin and the specific application of each in different settings of IBD patients.

References


