Fecal calprotectin use in inflammatory bowel disease and beyond: A mini-review

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B Alibrahim, MI Aljasser, B Salh. Fecal calprotectin use in inflammatory bowel disease and beyond: A mini-review. Can J Gastroenterol Hepatol 2015;29(3):157-163.

Given the number of inflammatory disorders affecting the gastrointestinal tract directly and indirectly, coupled with the considerable overlap with functional disorders, it is evident that more useful noninvasive diagnostic tests are required to aid with diagnosis. If these tests can also have some utility for individual patient follow-up in terms of disease activity and response to treatment, as well as providing forewarning of disease relapse, it would be extremely useful information for the clinician. One recently described test that may fulfill several of these attributes is based on leakage of a mononuclear cell cytoplasmic protein, calprotectin, along the intestinal tract, which can then be quantified in feces. This has been used to distinguish patients exhibiting symptoms of irritable bowel syndrome from patients with inflammatory bowel disease, with a measure of success greater than with currently used techniques. The present article summarizes the experience with this test used in inflammatory bowel disease, as well as a variety of gastrointestinal disorders.

Key Words: *Calprotectin; Fecal; Gastrointestinal disorders; Inflammatory bowel disease*

L'utilisation de la calprotectine fécale en cas de maladie inflammatoire de l'intestin et d'autres affections : une mini-analyse

Étant donné le nombre de troubles inflammatoires de l'intestin qui touchent directement et indirectement le tube digestif, de même que leur chevauchement considérable avec les troubles fonctionnels, il est évident qu'il faut plus de tests non effractifs utiles pour contribuer au diagnostic. Les cliniciens trouveraient également très utile que ces tests aient également une certaine utilité lors du suivi des patients, pour déterminer l'activité pathologique et la réponse au traitement, et pour de prévoir les récidives. Un test décrit récemment, qui respecterait plusieurs de ces caractéristiques, repose sur la fuite de la calpoprectine, une protéine cytoplasmique des cellules mononucléées située le long du tube digestif, qui peut ensuite être quantifié dans les selles. Elle a été utilisée pour distinguer les patients ayant des symptômes de syndrome du côlon irritable de ceux atteints d'une maladie inflammatoire de l'intestin, et la mesure de son succès est plus élevée que celle des techniques actuelles. Le présent article résume l'expérience de ce test chez les personnes atteintes d'une maladie inflammatoire de l'intestin, de même que de divers autres troubles gastro-intestinaux.

Gastrointestinal (GI) symptoms, such as abdominal pain and diarrhea, are common presenting symptoms in the general population. Determining the underlying cause of these symptoms is often challenging. The development of noninvasive diagnostic tools is useful to differentiate organic from functional bowel diseases and may reduce the need for unnecessary invasive procedures such as colonoscopy. One of these diagnostic tools is the measurement of fecal calprotectin (CPN), a major cytoplasmic protein in neutrophils (1).

Fecal CPN has gained popularity because of its high sensitivity and specificity in the diagnosis of inflammatory bowel disease (IBD) (2). In addition, it is a relatively simple and inexpensive test. However, it should be noted that many conditions have been shown to be associated with fecal CPN elevation, and that intestinal inflammation is a common feature in those conditions. The degree of elevation varies according to the cause, which may interfere with the diagnostic value of the test. Therefore, a broad knowledge of these conditions is essential for understanding the clinical utility of fecal CPN. The present review is aimed at highlighting the different causes associated with fecal CPN elevation.

HISTORY

The first description of CPN in the literature was in 1980 by Fagerhol et al (3) when it was named 'L1 protein'. Thereafter, it has been mentioned in the literature under different names such as MRP-8/14, calgranulin and cystic fibrosis antigen. In 1992, Roseth et al (4) developed the first method for isolating and quantifying CPN in stool using ELISA and rabbit anti-CPN. Many years later, an improved, commercially available and validated ELISA was developed, which measures CPN concentration in mg/kg rather than mg/L as in the original assay (5). Over the past two decades, fecal CPN was described to be a useful marker of several GI diseases. These include gastric cancer, colorectal adenoma or cancer, Crohn disease (CD) and ulcerative colitis (UC) (6).

FECAL CPN

CPN is a 36 kDa calcium-binding heterocomplex protein consisting of two heavy chains and one light chain. It belongs to the S-100 protein family and is derived predominantly from neutrophils and monocytes. CPN and its subunits appear to have mainly regulatory functions in inflammatory processes, as well as antimicrobial and antiproliferative activities (1). Being resistant to enzymatic degradation, it can be easily measured in stools with a commercially available ELISA immunoassay. Due to its high sensitivity and specificity, relative simplicity, quick turnaround time and long stability at room temperature (up to seven days), it has been increasingly used in the diagnostic process for IBD (4). The test can be performed on 50 mg to 100 mg of random stool samples that can be sent to the laboratory by mail because the protein is remarkably stable in stool. It costs approximately USD$100 per assay.

Fecal CPN concentrations in healthy individuals have been established in several studies. In the original study, the median stool CPN concentration in healthy adult was 2 mg/L, and the suggested cut-off for a positive test was 10 mg/L. In the newer assay, the suggested upper

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Received for publication November 15, 2014. Accepted December 5, 2014

TABLE 1
Conditions associated with fecal calprotectin elevation

Inflammatory
Inflammatory bowel disease
Pouchitis
Graft rejection following intestinal transplant
Collagenous colitis
Graft versus host disease
Ankylosing spondylitis
Systemic sclerosis
Diverticular disease
Celiac disease
Pancreatitis
Infectious
HIV
Infectious diarrhea
Neoplastic
Colon cancer
Intestinal polyposis
Pancreatic cancer
Iatrogenic
Nonsteroidal anti-inflammatory drugs
Proton pump inhibitors
Radiotherapy
Miscellaneous
Liver cirrhosis
Food allergy
Gastroesophageal reflux disease
Cystic fibrosis
Young age

limit of normal has been increased by a factor of five, to 50 µg/g (7). However, the test appears to have better diagnostic precision for IBD at a cut-off of 100 µg/g than at 50 µg/g (2).

Levels of fecal CPN has been found to correlate well with radiolabelled white cell scanning, as well as histopathological and endoscopic features used in assessing IBD activity (8,9) (Box 1) .

CONDITIONS ASSOCIATED WITH ELEVATION OF FECAL CPN

Many conditions have been linked to an elevation of fecal CPN (Table 1). However, the current clinical use of fecal CPN is focused on differentiating IBD from irritable bowel syndrome (IBS), monitoring disease activity for relapse and detecting disease recurrence postoperatively. The evidence for fecal CPN elevation in most of the other conditions listed is limited to case series and small observational studies.

IBD

Distinguishing between IBD and IBS: Fecal CPN has been shown to be of diagnostic value in IBD. The major systematic reviews (10,11) and meta-analyses (2,12) are shown in Table 2. In a meta-analysis of 30 studies including 5983 patients who underwent fecal CPN testing, Von Roon et al (2) found that the mean fecal CPN levels in patients with IBD were higher by 219.2 µg/g compared with control subjects (P<0.001). The pooled sensitivity and specificity of fecal CPN for distinguishing IBD from non-IBD patients from nine studies of adult and pediatric populations at a 50 µg/g cut-off was calculated to be 89% and 81%, respectively.

Disease activity and treatment response in IBD: Notably, fecal CPN has been shown to be a reliable marker for assessment of disease activity in IBD patients (13-17). In a study of patients with CD who underwent 140 ileocolonoscopies (13), fecal CPN correlated well with the simple endoscopic score for CD (Spearman rank correlation coefficient r=0.75). This correlation with endoscopic assessment was superior to that of C-reactive protein, blood leukocyte levels and CD activity index. Additionally, fecal CPN discriminated among the various subgroups of endoscopic activity index (inactive, mild, moderate and high activity). The sensitivity and specificity of fecal CPN for detecting endoscopic active disease (simple endoscopic score for CD ≥4) was 89% and 72%, respectively, at a cut-off value of 70 µg/g.

Similarly, a study of 134 patients with UC showed a significant correlation with the Rachmilewitz endoscopic activity index (r=0.834) (18), which was superior to the Rachmilewitz clinical activity index, C-reactive protein and blood leukocyte for the detection of disease activity. Also, fecal CPN was able to discriminate among various groups of endoscopic activity indices. The sensitivity and specificity of fecal CPN for detection of endoscopic active disease (Rachmilewitz endoscopic activity index ≥4) was 93% and 71%, respectively, at a cut-off of 50 µg/g.

Fecal CPN has been investigated in assessing IBD patients' clinical response to treatment. A study of 38 patients (27 UC, 11 CD) presenting with disease relapse had fecal CPN measured at presentation, and at week 4 and week 8 after treatment (19). Treatment of relapse was individualized according to standard recommendations for management of IBD. UC patients showed a significant correlation of fecal CPN with the clinical score at week 4 after treatment (r=0.0424, P<0.01), while CD patients showed a significant correlation with the clinical score (Harvey-Bradshaw index) at week 8 after treatment (r=0.7804, P<0.01).

Predicting relapse in IBD: Several studies have indicated the value of CPN in predicting relapse in IBD, and have been the subject of a meta-analysis (20) (Table 3). This included a total of 672 patients of whom 318 had UC and 354 had CD. For CPN to predict relapse there was a pooled sensitivity and specificity of 78% and 73%, respectively. The data were comparable for both UC and colonic/ileocolonic CD.

Underscoring the value of regular monitoring of CPN, De Vos et al (21) determined levels every four weeks on patients with UC receiving infliximab, and found that the best predictor of a flare were two consecutive CPN levels >300 mg/kg. Those patients considered to be in deep remission (partial Mayo score <3 at all times and endoscopic Mayo score 0 at week 52) had consistently low CPN levels (<40 mg/kg).

Cost: With respect to cost, Waugh et al (10) conducted a comprehensive review regarding the economic value of fecal CPN in distinguishing IBD from IBS. They found that by reducing the number of unnecessary colonoscopies/gastroenterologist referrals, the use of fecal CPN test could lead to cost savings, especially in the secondary care setting. Furthermore, a recent cost-effectiveness study found that fecal CPN screening for a patient suspected to have IBD saved USD$417 per patient screened (22) (Box 2).

Subclinical disease: Interestingly, fecal CPN has been used to demonstrate the presence of subclinical intestinal inflammation in the first-degree relatives of CD patients (23). This particular study included 49 patients, 16 spouses and 151 first-degree relatives of CD patients. There was a statistically significant higher fecal CPN in the patients and first-degree relatives compared with the spouses and the control group (P<0.0001).

BOX 1
Fecal calprotectin is a useful test for organic causes of gastrointestinal symptoms, especially IBD. Its high sensitivity and simplicity make it a very useful screening tool in the primary care setting.

BOX 2
Proper use of fecal calprotectin has the potential to substantially reduce the numbers of unnecessary colonoscopies and the overall cost of care of patients with gastrointestinal symptoms.

TABLE 2
Studies evaluating the performance of fecal calprotectin (CPN) in distinguishing inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS)

Author/year	Study design	Subjects/studies, n/n	Sensitivity, %	Specificity, %	Cut-off value	Comments
Waugh et al, 2013 (10)	Systematic review	730/7	93	94	50 µg/g	Included many studies of different GI conditions. The seven studies included in this table specifically compared IBD with IBS
Jellema et al, 2011 (11)	Systematic review (primary care)	863/9	64–100	70–100	Multiple cut-offs ranging from 10 mg/L to 170 µg/g	When compared with other blood and fecal tests (CRP, ESR, IgG, ANCA, lactoferrin), CPN performed the best
Van Rheenen et al, 2010 (12)	Meta-analysis	670/6 (adults)	93	97	50 µg/g (Most studies)	CPN theoretically has the potential of reducing the number adults requiring colonoscopy by 67%.
Von Roon et al, 2007 (2)	Meta-analysis	5983/30	95	91	50 µg/g	IBD patients were compared with a heterogeneous group of patients with other GI conditions and showed a statistically significant higher level of CPN

ANCA Antineutrophil cytoplasmic antibody; CRP C-reactive protein; ESR Erythrocyte sedimentation rate; GI Gastrointestinal; IgG Immunoglobulin G

TABLE 3
Summary of the major studies correlating fecal calprotectin (CPN) with inflammatory bowel disease activity/relapse in adults

Author/year	Patients, n	Sensitivity, %	Specificity, %	Comments
Mooiweer et al, 2014 (17)	164 (83 CD, 74 UC, 7 unclassified)	86	72	The study used a cut-off of 140 mg/kg, and used the Mayo endoscopic activity score to assess mucosal inflammation
Faubion et al, 2013 (16)	264 (157 CD, 107 UC)	78	54	The sensitivity and specificity are for CPN at cut-off of 100 µg/g and SES-CD ≥4. The sensitivity and specificity in UC was not mentioned. However, there was a strong association with Mayo endoscopic score
Lobatón et al, 2013 (15)	123 UC	73.5	89.7	These values are for fecal CPN <250 µg/g to predict Mayo endoscopic score ≤1
Schoepfer et al, 2013 (14)	228 UC	91	90	The study used a cut-off of >57 µg/g to predict endoscopic active disease using modified Baron Score ≥2 points
Mao et al, 2012 (20)*	672 (meta-analysis of 6 studies; 318 UC, 354 CD)	78	73	This study had multiple CPN cut-off ranges, from 50 µg/g tp 340 µg/g. Identification of relapse was based on clinical activity indices or endoscopic findings.
Schoepfer et al, 2010 (13)	122 CD	89	72	These results are for CPN ≥70 µg/g correlated with SES-CD. The study also shows superior diagnostic accuracy compared with C-reactive protein, blood leukocyte and CD activity index

**This meta-analysis includes several earlier studies. CD Crohn disease, SES-CD Simple endoscopic score for CD; UC Ulcerative colitis*

Pouchitis

Patients undergoing restorative proctocolectomy for UC have a 40% lifetime risk of developing pouchitis (24), which is an inflammatory condition of the pouch with neutrophilic infiltration of the mucosa (25). The role of fecal CPN as a biomarker of this condition was assessed by a study of 54 patients who had undergone restorative proctocolectomy for UC and familial adenomatous polyposis (26). Six of 46 UC patients had pouchitis and pre-pouch ileitis, 13 had pouchitis alone and 27 were uninflamed. Of the eight familial adenomatous polyposis patients, one had pouchitis and pre-pouch ileitis, and seven had pouchitis alone. Fecal CPN >50 µg/g correlated significantly with high endoscopic inflammatory scores (scale of 0 to 6, depending on the number of acute macroscopic inflammatory features seen) (P<0.001). In addition, fecal CPN >50 µg/g correlated significantly with higher histological inflammation score (modification of the Moskowitz 12-point scoring system) (P<0.001).

Having established that fecal CPN levels are elevated in IBD, there are still a number of disorders that present with diarrhea that may impact on the interpretation of results. These are discussed below (Box 3).

Collagenous/microscopic colitis

Collagenous colitis is a disorder characterized by watery diarrhea, microscopic mucosal inflammation and deposition of collagen below the surface epithelium of colonic mucosa (27). There are generally no endoscopic abnormalities and colonic biopsy is needed to establish the diagnosis.

BOX 3
Inflammatory bowel disease is the only well-studied indication for fecal calprotectin test. However, because this test is a marker of gastrointestinal inflammation, it may have other potential uses.

The value of fecal CPN as a biomarker of collagenous colitis was evaluated by a study that involved 21 patients with active collagenous colitis, 12 patients with collagenous colitis in remission and 13 healthy controls (28). The median fecal CPN level in the active disease group was 80 µg/g compared with 26 µg/g in the disease remission group (P=0.025), and 6.25 µg/g in the control group (active versus control P=0.002).

In a recent study of 78 patients with chronic nonbloody diarrhea, 15 were found to have microscopic colitis (29). However, there was no correlation between this and CPN levels, indicating the importance of colonic biopsy for making this specific diagnosis.

Graft-versus-host disease

Diarrhea is common after hematopoietic stem cell transplant. The differential diagnosis includes infectious causes, chemo- or radiotherapy-associated toxicity, and GI graft-versus-host disease (GI-GVHD). Endoscopy remains the gold standard for the diagnosis of GI-GVHD.

However, the invasive nature of this procedure and the fact that this procedure may yield nonspecific findings, has led to a search for non-invasive markers. Fecal CPN may be a good marker due to the inflammatory nature of GVHD. This hypothesis was prospectively tested by Rodriguez-Otero et al (30), who recruited a total of 72 GVHD patients, of whom 51 had GI-GVHD. There were no statistically significant differences in fecal CPN levels between the GI-GVHD and non-GI-GVHD groups. The sensitivity of fecal CPN was only 30%. However, in this study, 21 of the 51 GI-GVHD patients had stage 1 GVHD, which has less inflammation than more advanced stages of GI-GVHD. Notably, higher concentrations of fecal CPN were strongly associated with steroid-resistant GVHD (P=0.00001).

A recent study of 59 patients with GVHD showed that mean fecal CPN in the patient with GI-GVHD was 500 mg/kg compared with 95 mg/kg in non GI-GVHD patients (P=0003) (31). Sensitivity and specificity were 100% and 81.8%, respectively at a cut-off of 160 mg/kg. However, the authors did not specify the stage of GVHD. These results were consistent with a prospective study of 23 post-stem cell transplant patients presenting with diarrhea, 11 of whom had colonoscopy-proven GI-GVHD (32). The fecal CPN level in patients with GI-GVHD was significantly higher than in patients with non-GI-GVHD diarrhea (P<0.001). The calculated sensitivity and specificity of fecal CPN were 83.3% and 90.9%, respectively, at a cut-off of 250 mg/kg. In this study, most of the patients presented with more advanced stages of GI-GVHD.

In an intriguing study hinting at the role of mucosal immunity in the development of acute GVHD (33), 24 pediatric allogeneic hematopoietic cell transplant recipients had their fecal CPNs measured on days 0, +5, +10 and +15. Surprisingly, a value of <424 mg/kg on day +10 was associated with a 77.8% incidence of acute GVHD.

In conclusion, fecal CPN is a good marker for advanced stages of GI-GVHD as well as steroid-resistant GI-GVHD, and reduced levels early on may predict the onset of acute GVHD.

Ankylosing spondylitis

Ankylosing spondylitis (AS) and IBD are known to share some clinicopathological features. These include chronic inflammation of unknown etiology, genetic overlap, and similar microscopic and macroscopic findings in gut inflammation (34-37). In addition, 5% to 10% of patients with AS have concurrent IBD (38).

A pilot prospective study of 39 patients with AS and 42 healthy controls showed a significant difference in the median fecal CPN in the AS group compared with the control group (P<0.001) (39). Another study showed that 68% of 210 AS patients had positive fecal CPN without associated GI symptoms (40). However, patients taking nonsteroidal anti-inflammatory drugs (NSAIDs), which can cause fecal CPN elevation, were not excluded. In fact, fecal CPN levels were found to be higher in the latter group of patients (40).

Systemic sclerosis

GI involvement in systemic sclerosis is known to have an inflammatory component (41). Therefore, a study of 81 systemic sclerosis patients was conducted to evaluate the role of fecal CPN as a biomarker of GI involvement of this disease (42). The study showed that 62 of 81 (76%) patients had a positive fecal CPN test using a cut-off of 50 µg/g. The mean CPN level was 174 µg/g.

Furthermore, mean fecal CPN was significantly higher in patients with pathological cineradiography results compared with patients with normal cineradiography (P=0.013).

Diverticular disease

Given the therapeutically beneficial effect of 5-aminosalicylic acid and the histologically inflammatory nature of diverticular disease, the potential use of fecal CPN as a marker for this disease was investigated (43). Tursi et al (44) used a semiquantitative test to assess fecal CPN elevation in 48 patients with newly diagnosed diverticular disease, 16 healthy controls and 16 patients with IBS. There was a significant fecal CPN elevation in patients with symptomatic uncomplicated diverticular disease and acute diverticulitis compared with the other groups. Fecal CPN levels normalized after treatment with mesalazine and rifaximin, in both symptomatic uncomplicated diverticular disease and acute diverticulitis.

Celiac disease

Celiac disease is a chronic immune-mediated disorder that primarily affects the GI tract. Histologically, celiac disease is characterized by villous atrophy and crypt cell hyperplasia, with both lamina propria and epithelial infiltration by lymphocytes, macrophages and plasma cells. One adult and two pediatric studies have correlated fecal CPN level with the inflammation in celiac disease.

In a study of 28 untreated adult celiac patients and 30 healthy volunteers (45), the mean fecal CPN levels were 45 µg/g and 36 µg/g, respectively. Although this difference was not statistically significant, it revealed a trend toward higher fecal CPN in celiac patients. Capone et al (46) assessed the correlation between CPN levels above a cut-off of 75 µg/g with symptoms, histology (Marsh grade) and tissue transglutaminase in 50 patients (46). They came to the conclusion that there was no signal for subclinical inflammation in celiac disease.

Fecal CPN was found to be significantly higher in 31 children with untreated celiac disease compared with 33 treated celiac disease patients and 34 normal controls (47). The mean fecal CPN was 117.2 µg/g in celiac patients compared with 3.7 µg/g for celiac children on a gluten-free diet and 9.6 µg/g in the control group (P=0.001). The second study included 29 children with newly diagnosed celiac disease and 10 healthy children (48). The mean fecal CPN was 13.4 mg/L in celiac patients compared with 4.3 mg/L in the controls (P<0.004).

Although there is no convincing evidence to support the use of fecal CPN as a diagnostic marker for celiac disease in the adult population, it may be considered as one of the possible causes of fecal CPN elevation in pediatric cases.

Pancreatitis

Pancreatic enzymes play a major role in digestion and absorption, and alterations of the normal secretion of these enzymes has been shown to have significant effect on intestinal ecology including bacterial overgrowth, change of bile acid absorption and modification of intestinal permeability (49-51). These changes may be expected to be associated with intestinal inflammation.

A study of 90 patients with different pancreatic diseases aimed to determine the association between pancreatic diseases and intestinal inflammation (52). This study included 20 patients with chronic pancreatitis and 11 patients with a history of recent acute pancreatitis. It was found that 55% and 40% of those patients, respectively, had high fecal CPN compared with only 4.3% of the normal control group. In this study, a semiquantitative method was used with a cut-off of only 15 µg/g, indicating that there is unlikely to be any confusion between IBD and pancreatic disorders.

Infectious diarrhea

Stool culture is considered to be the gold standard for the diagnosis of infectious diarrhea. However, the time required to obtain results (typically 48 h) and the relatively high cost of this test has led to a search for alternative diagnostic methods. Understandably, many stool biomarkers have been tested, of which fecal CPN showed the most promising results. A large multicentre study of 2383 patients with acute diarrhea showed a sensitivity and specificity of 83% and 87%, respectively, for fecal CPN in the diagnosis of acute bacterial diarrhea (53). The mean fecal CPN level for patients with culture-proven bacterial diarrhea was 142.84 mg/L compared with 16.8 mg/L in culture-negative patients. However, this study did not test patients for viral or parasitic infections.

Three viral infections were tested in a pediatric study that assessed fecal CPN elevation in patients with bacterial and viral diarrhea (54). These infections were rotavirus (52 patients), adenovirus (eight patients) and norovirus (31 patients). The mean fecal CPN levels were 89 µg/g, 95 µg/g and 93 µg/g, respectively, compared

with 43 µg/g in the control group. The mean fecal CPN level for bacterial (*Salmonella*, *Campylobacter*) diarrhea patients was 754 µg/g, indicating a clear separation from viral diarrhea patients.

HIV

Immune activation is an important mechanism of CD4+ lymphocyte depletion in HIV infection, and is hypothesized to be due to gut flora translocation (55). A possible cause of gut flora translocation is damage of the mucosal barrier by intestinal inflammation and alteration of intestinal flora. To verify this, a study of 58 HIV-positive asymptomatic antiretroviral-naive individuals measured fecal CPN and the alteration of gut microbiota (56). The study showed that approximately one-half of the subjects (27 of 58) had a positive fecal CPN test at a cut-off 50 µg/g. Moreover, 34% (18 of 53) of the patients had levels >100 µg/g. It will be of interest to see how fecal CPN is affected by diarrhea from various causes in this patient population.

NEOPLASTIC CONDITIONS

Colorectal cancer and adenomatous polyps

One of the earliest areas of interest regarding fecal CPN was colorectal malignancy. A pilot study in 1993 by Roseth et al (57) found that fecal CPN was increased in stools in 50 of 53 patients with colorectal cancer (CRC) and in 32 of 40 patients with colorectal polyps.

However, data from the Norwegian Colorectal Cancer Prevention trial including 2321 patients (58) suggested a poorer sensitivity and specificity of fecal CPN compared with fecal occult blood test. Similarly, in a meta-analysis, Von Roon et al (2) found that the mean fecal CPN was higher by 132 µg/g in patients with CRC compared with those without CRC (P=0.18). They concluded that fecal CPN cannot be recommended as a screening test for CRC in the general population because of low-pooled sensitivity and specificity (36% and 71%, respectively) (Box 4).

Although fecal CPN is not recommended as a screening tool for CRC due to the availability of better screening tests, colorectal neoplasms should be considered as one of the causes of fecal CPN elevation.

Pancreatic cancer

The same mechanism of pancreatic enzyme insufficiency inducing intestinal inflammation may apply to pancreatic cancer. In the aforementioned study on pancreatitis (52), eight of 15 (53.3%) patients with pancreatic cancer had a positive fecal CPN level using a semi-quantitative method; 15 µg/g was used as the cut-off, making it of very limited value in this condition.

IATROGENIC

NSAIDs

NSAIDs have been shown to induce enteropathy at different levels of the GI tract. Some studies that assessed the inflammatory process associated with NSAIDs used fecal CPN to demonstrate this effect. In a study involving 312 patients taking different types of NSAIDs, 44% had a positive fecal CPN at a cut-off of 8.9 mg/L (59). The median CPN level was 7.3 mg/L compared with 2 mg/L in the control group (P<0.001). There were no significant differences between the different NSAIDs in the amplitude of CPN elevation. Another study demonstrated that the effect of NSAIDs on fecal CPN might be seen as early as a few days after the initiation of these medications (60).

Proton pump inhibitors

Although the exact mechanism is not clear, an incidental finding from a study involving 230 subjects to assess the normal range of fecal CPN in a late middle-aged population suggested that the use of proton pump inhibitors (PPIs) was associated with a higher level of fecal CPN (61). The mean fecal CPN level for those on PPIs was 78.16 µg/g compared with 30.9 µg/g for those who were not on PPIs (P<0.001); this finding appeared to be independent of the presence of reported dyspepsia.

BOX 4

Successful use of the fecal calprotectin test may rely not only on knowledge of potential causes of fecal CPN elevation, but also on disease and treatment-related changes within each individual patient.

Radiotherapy

Therapeutic radiotherapy to the pelvis induces acute GI symptoms in >90% of patients (62). Currently, there is no noninvasive method to measure radiotherapy-induced damage in the GI tract.

A study involving 59 patients who underwent pelvic radiotherapy measured fecal CPN at baseline and five weeks following radiotherapy (63). The mean fecal CPN at base line was 37.7 µg/g versus 62.9 µg/g at the fifth week post radiotherapy (P=0.01). However, this is unlikely to be any value in the management of these patients.

MISCELLANEOUS

Liver cirrhosis

In liver cirrhosis, numerous alterations in intestinal flora, mucosal barrier and immunological defense mechanisms have been described (64). The gut flora and bacterial translocation also play a role in hepatic encephalopathy (65). The role of fecal CPN as a marker of complications of liver cirrhosis was evaluated in a study of 61 patients with liver cirrhosis and 42 controls (66). The mean fecal CPN in the former group was 65.7 µg/g compared with a mean of 17.5 µg/g in the control group (P<0.001). Furthermore, this study showed that the degree of fecal CPN elevation is directly proportional to the severity of liver disease as assessed by the Child-Pugh score and the Model for End-stage Liver Disease scores, as well as hepatic encephalopathy and spontaneous bacterial peritonitis.

Food allergy

Food allergy is defined as an immune-mediated adverse reaction following the ingestion of an allergen; therefore, fecal CPN might be used as a marker of mucosal inflammation. A pediatric study of 281 children referred with GI symptoms other than diarrhea or abdominal pain included 49 patients with allergic colitis (67). This study showed that these patients had significantly higher fecal CPN compared with controls and patients with functional bowel disorders (P<0.001). Additionally, a reducing pattern toward normal fecal CPN level occurred after at least four weeks of exclusion diet (P<0.001).

Another study (68), conducted to determine the diagnostic accuracy of fecal CPN in the diagnosis of organic causes of diarrhea, suggested that food allergies have an association with high fecal CPN level. This study included 20 children diagnosed with chronic diarrhea attributed to cow's milk allergy or multiple food allergies, 17 (85%) of whom had elevated CPN levels.

Gastroesophageal reflux disease

Fecal CPN may also be a marker of mucosal inflammation of the upper GI tract. The aforementioned pediatric study of 281 children presented with different GI disorders (67) included 17 patients diagnosed with gastroesophageal reflux disease. The median fecal CPN value was 138 µg/g in the gastroesophageal reflux disease group compared with a median of 28 µg/g in the healthy control group (P<0.001).

Cystic fibrosis

There is some evidence of intestinal inflammation in cystic fibrosis (CF) supported by studies using gut lavage and endoscopic biopsy techniques (69,70). A study of 30 children with CF, 30 healthy controls and 15 IBD patients attempted to establish the incidence of intestinal inflammation in CF patients using fecal CPN and rectal nitric oxide production (71). All the CF patients were on pancreatic enzyme replacement before the study. The mean fecal CPN in CF patients was 219 µg/g versus 46 µg/g in the control group (P<0.01). The mean fecal CPN in the CF group was significantly lower than the mean value of the IBD group (309 µg/g) (P<0.001).

Graft rejection following intestinal transplant

Graft surveillance after intestinal transplant is difficult because of the need of serial invasive procedures and biopsies for the diagnosis of graft rejection. The ability to diagnose rejection noninvasively and early will be a major advance in the care of intestinal transplant patients. To find a better method of graft surveillance, a study collected serial stool samples for CPN level from 72 postintestinal transplant patients (64 children, 10 adults, two children underwent transplant twice) (72). The patients were divided into groups based on the biopsy results (normal, rejection, viral enteritis, bacterial enteritis, nonspecific enteritis, indeterminate rejection and total mucosal loss). Fecal CPN level was significantly higher in the rejection, indeterminate rejection and total mucosal loss groups ($P<0.05$). However, the three stool samples taken at days 4, 7 and 14 before the index biopsies showing rejection showed no statistically significant different compared with the day of the biopsy. The study concluded that the exact role of fecal CPN in monitoring of graft rejection was unclear, and more frequent prospective sampling could perhaps demonstrate an advantage as an earlier indication of rejection.

Young age (<5 years)

A tendency toward a higher fecal CPN levels in infants and young children has been demonstrated. One study analyzed fecal CPN level of 115 presumably healthy children visiting local community centres for a routine examination (73). The children were divided into six age groups. The study showed abnormal mean fecal CPN levels for infants and young children up to five years of age (considering 50 µg/g as the upper limit of normal). The groups younger than one year of age had the highest mean fecal CPN levels (Box 5).

BOX 5

The noninvasive, inexpensive nature of the fecal CPN test, together with its excellent performance as a marker of inflammation, makes it a useful tool for intestinal inflammatory diseases research.

CONCLUSION

Although there are many conditions that may contribute to the elevation of fecal CPN level, the best available evidence currently only supports the use of fecal CPN as a screening and monitoring tool for IBD. We believe there may be an under-appreciated potential of other uses for fecal CPN as a diagnostic and follow-up tool for other GI conditions, where the individual patient acts as his/her own control. The simplicity and cost effectiveness of this test might help in decreasing the use of more invasive procedures and better planning for further investigation. However, more studies are clearly still needed to validate the utilization of fecal CPN use in different medical and surgical conditions.

DISCLOSURES: The authors have no financial disclosures or conflicts of interest to declare.

REFERENCES

1. Johne B, Fagerhol MK, Lyberg T, et al. Functional and clinical aspects of the myelomonocyte protein calprotectin. Mol Pathol 1997;50:113-23.
2. von Roon AC, Karamountzos L, Purkayastha S, et al. Diagnostic precision of fecal calprotectin for inflammatory bowel disease and colorectal malignancy. Am J Gastroenterol 2007;102:803-13.
3. Fagerhol MK, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. Bull Eur Physiopathol Respir 1980;16(Suppl):273-82.
4. Roseth AG, Fagerhol MK, Aadland E, et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol 1992;27:793-8.
5. Ton H, Brandsnes, Dale S, et al. Improved assay for fecal calprotectin. Clin Chim Acta 2000;292:41-54.
6. Aadland E, Fagerhol MK. Faecal calprotectin: A marker of inflammation throughout the intestinal tract. Eur J Gastroenterol Hepatol 2002;14:823-5.
7. Konikoff MR, Denson LA. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. Inflamm Bowel Dis 2006;12:524-34.
8. Roseth AG, Aadland E, Jahnsen J, et al. Assessment of disease activity in ulcerative colitis by faecal calprotectin, a novel granulocyte marker protein. Digestion 1997;58:176-80.
9. Roseth AG, Schmidt PN, Fagerhol MK. Correlation between faecal excretion of indium-111-labelled granulocytes and calprotectin, a granulocyte marker protein, in patients with inflammatory bowel disease. Scand J Gastroenterol 1999;34:50-4.
10. Waugh N, Cummins E, Royle P, et al. Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: Systematic review and economic evaluation. Health Technol Assess 2013;17:xv-xix, 1-211.
11. Jellema P, van Tulder MW, van der Horst HE, et al. Inflammatory bowel disease: A systematic review on the value of diagnostic testing in primary care. Colorectal Dis 2011;13:239-54.
12. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: Diagnostic meta-analysis. BMJ 2010;341:c3369.
13. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin correlates more closely with the Simple Endoscopic Score for Crohn's disease (SES-CD) than CRP, blood leukocytes, and the CDAI. Am J Gastroenterol 2010;105:162-9.
14. Schoepfer AM, Beglinger C, Straumann A, et al. Fecal calprotectin more accurately reflects endoscopic activity of ulcerative colitis than the Lichtiger Index, C-reactive protein, platelets, hemoglobin, and blood leukocytes. Inflamm Bowel Dis 2013;19:332-41.
15. Lobaton T, Rodriguez-Moranta F, Lopez A, et al. A new rapid quantitative test for fecal calprotectin predicts endoscopic activity in ulcerative colitis. Inflamm Bowel Dis 2013;19:1034-42.
16. Faubion WA Jr, Fletcher JG, O'Byrne S, et al. EMerging BiomARKers in Inflammatory Bowel Disease (EMBARK) study identifies fecal calprotectin, serum MMP9, and serum IL-22 as a novel combination of biomarkers for Crohn's disease activity: Role of cross-sectional imaging. Am J Gastroenterol 2013;108:1891-900.
17. Mooiweer E, Fidder HH, Siersema PD, et al. Fecal hemoglobin and calprotectin are equally effective in identifying patients with inflammatory bowel disease with active endoscopic inflammation. Inflamm Bowel Dis 2014;20:307-14.
18. Schoepfer AM, Beglinger C, Straumann A, 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;15:1851-8.
19. Wagner M, Peterson CG, Ridefelt P, et al. Fecal markers of inflammation used as surrogate markers for treatment outcome in relapsing inflammatory bowel disease. World J Gastroenterol 2008;14:5584-9.
20. Mao R, Xiao YL, Gao X, et al. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: A meta-analysis of prospective studies. Inflamm Bowel Dis 2012;18:1894-9.
21. De Vos M, Louis EJ, Jahnsen J, et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis 2013;19:2111-7.
22. Yang Z, Clark N, Park KT. Effectiveness and cost-effectiveness of measuring fecal calprotectin in diagnosis of inflammatory bowel disease in adults and children. Clin Gastroenterol Hepatol 2014;12:253-62, e2.
23. Thjodleifsson B, Sigthorsson G, Cariglia N, et al. Subclinical intestinal inflammation: An inherited abnormality in Crohn's disease relatives? Gastroenterology 2003;124:1728-37.
24. Moskowitz RL, Shepherd NA, Nicholls RJ. An assessment of inflammation in the reservoir after restorative proctocolectomy with ileoanal ileal reservoir. Int J Colorectal Dis 1986;1:167-74.
25. Shepherd NA, Jass JR, Duval I, et al. Restorative proctocolectomy with ileal reservoir: Pathological and histochemical study of mucosal biopsy specimens. J Clin Pathol 1987;40:601-7.
26. Johnson MW, Maestranzi S, Duffy AM, et al. Faecal calprotectin: A noninvasive diagnostic tool and marker of severity in pouchitis. Eur J Gastroenterol Hepatol 2008;20:174-9.
27. Offner FA, Jao RV, Lewin KJ, et al. Collagenous colitis: A study of the distribution of morphological abnormalities and their histological detection. Hum Pathol 1999;30:451-7.

28. Wildt S, Nordgaard-Lassen I, Bendtsen F, et al. Metabolic and inflammatory faecal markers in collagenous colitis. Eur J Gastroenterol Hepatol 2007;19:567-74.
29. Larsson JK, Sjoberg K, Vigren L, et al. Chronic non-bloody diarrhoea: A prospective study in Malmo, Sweden, with focus on microscopic colitis. BMC Res Notes 2014;7:236.
30. Rodriguez-Otero P, Porcher R, Peffault de Latour R, et al. Fecal calprotectin and alpha-1 antitrypsin predict severity and response to corticosteroids in gastrointestinal graft-versus-host disease. Blood 2012;119:5909-17.
31. Chiusolo P, Metafuni E, Giammarco S, et al. Role of fecal calprotectin as biomarker of gastrointestinal GVHD after allogeneic stem cell transplantation. Blood 2012;120:4443-4.
32. Bastos Oreiro M, Castilla-Llorente C, de la Guia AL, et al. Fecal calprotectin in allogeneic stem cell transplantation for the diagnosis of acute intestinal graft versus host disease. Bone Marrow Transplant 2012;47:1241-2.
33. August KJ, Chiang KY, Qayed M, et al. Relative defects in mucosal immunity predict acute graft-versus-host disease. Biol Blood Marrow Transplant 2014;20:1056-9.
34. De Keyser F, Elewaut D, De Vos M, et al. Bowel inflammation and the spondyloarthropathies. Rheum Dis Clin North Am 1998;24:785-813, ix-x.
35. Leirisalo-Repo M, Turunen U, Stenman S, et al. High frequency of silent inflammatory bowel disease in spondylarthropathy. Arthritis Rheum 1994;37:23-31.
36. Taurog JD. The role of HLA-B27 in spondyloarthritis. J Rheumatol 2010;37:2606-16.
37. Salvarani C, Fries W. Clinical features and epidemiology of spondyloarthritides associated with inflammatory bowel disease. World J Gastroenterol 2009;15:2449-55.
38. Rosenbaum J, Chandran V. Management of comorbidities in ankylosing spondylitis. Am J Med Sci 2012;343:364-6.
39. Matzkies FG, Targan SR, Berel D, et al. Markers of intestinal inflammation in patients with ankylosing spondylitis: A pilot study. Arthritis Res Ther 2012;14:R261.
40. Klingberg E, Carlsten H, Hilme E, et al. Calprotectin in ankylosing spondylitis – frequently elevated in feces, but normal in serum. Scand J Gastroenterol 2012;47:435-44.
41. Manetti M, Neumann E, Muller A, et al. Endothelial/lymphocyte activation leads to prominent CD4+ T cell infiltration in the gastric mucosa of patients with systemic sclerosis. Arthritis Rheum 2008;58:2866-73.
42. Andreasson K, Scheja A, Saxne T, et al. Faecal calprotectin: A biomarker of gastrointestinal disease in systemic sclerosis. J Intern Med 2011;270:50-7.
43. Tursi A, Brandimarte G, Elisei W, et al. Assessment and grading of mucosal inflammation in colonic diverticular disease. J Clin Gastroenterol 2008;42:699-703.
44. Tursi A, Brandimarte G, Elisei W, et al. Faecal calprotectin in colonic diverticular disease: A case-control study. Int J Colorectal Dis 2009;24:49-55.
45. Montalto M, Santoro L, Curigliano V, et al. Faecal calprotectin concentrations in untreated coeliac patients. Scand J Gastroenterol 2007;42:957-61.
46. Capone P, Rispo A, Imperatore N, et al. Fecal calprotectin in coeliac disease. World J Gastroenterol 2014;20:611-2.
47. Balamtekin N, Baysoy G, Uslu N, et al. Fecal calprotectin concentration is increased in children with celiac disease: Relation with histopathological findings. Turk J Gastroenterol 2012;23:503-8.
48. Ertekin V, Selimoglu MA, Turgut A, et al. Fecal calprotectin concentration in celiac disease. J Clin Gastroenterol 2010;44:544-6.
49. Trespi E, Ferrieri A. Intestinal bacterial overgrowth during chronic pancreatitis. Curr Med Res Opin 1999;15:47-52.
50. Casellas F, Guarner L, Vaquero E, et al. Hydrogen breath test with glucose in exocrine pancreatic insufficiency. Pancreas 1998;16:481-6.
51. Madsen JL, Graff J, Philipsen EK, et al. Bile acid malabsorption or disturbed intestinal permeability in patients treated with enzyme substitution for exocrine pancreatic insufficiency is not caused by bacterial overgrowth. Pancreas 2003;26:130-3.
52. Pezzilli R, Barassi A, Morselli-Labate AM, et al. Fecal calprotectin and elastase 1 determinations in patients with pancreatic diseases: A possible link between pancreatic insufficiency and intestinal inflammation. J Gastroenterol 2007;42:754-60.
53. Shastri YM, Bergis D, Povse N, et al. Prospective multicenter study evaluating fecal calprotectin in adult acute bacterial diarrhea. Am J Med 2008;121:1099-106.
54. Chen CC, Huang JL, Chang CJ, et al. Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. J Pediatr Gastroenterol Nutr 2012;55:541-7.
55. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12:1365-71.
56. Gori A, Tincati C, Rizzardini G, et al. Early impairment of gut function and gut flora supporting a role for alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis. J Clin Microbiol 2008;46:757-8.
57. Roseth AG, Kristinsson J, Fagerhol MK, et al. Faecal calprotectin: A novel test for the diagnosis of colorectal cancer? Scand J Gastroenterol 1993;28:1073-6.
58. Hoff G, Grotmol T, Thiis-Evensen E, et al. Testing for faecal calprotectin (PhiCal) in the Norwegian Colorectal Cancer Prevention trial on flexible sigmoidoscopy screening: Comparison with an immunochemical test for occult blood (FlexSure OBT). Gut 2004;53:1329-33.
59. Tibble JA, Sigthorsson G, Foster R, et al. High prevalence of NSAID enteropathy as shown by a simple faecal test. Gut 1999;45:362-6.
60. Meling TR, Aabakken L, Roseth A, et al. Faecal calprotectin shedding after short-term treatment with non-steroidal anti-inflammatory drugs. Scand J Gastroenterol 1996;31:339-44.
61. Poullis A, Foster R, Mendall MA, et al. Proton pump inhibitors are associated with elevation of faecal calprotectin and may affect specificity. Eur J Gastroenterol Hepatol 2003;15:573-4.
62. Khalid U, McGough C, Hackett C, et al. A modified inflammatory bowel disease questionnaire and the Vaizey Incontinence questionnaire are more sensitive measures of acute gastrointestinal toxicity during pelvic radiotherapy than RTOG grading. Int J Radiat Oncol Biol Phys 2006;64:1432-41.
63. Wedlake L, McGough C, Hackett C, et al. Can biological markers act as non-invasive, sensitive indicators of radiation-induced effects in the gastrointestinal mucosa? Aliment Pharmacol Ther 2008;27:980-7.
64. Garcia-Tsao G, Wiest R. Gut microflora in the pathogenesis of the complications of cirrhosis. Best Pract Res Clin Gastroenterol 2004;18:353-72.
65. Gupta A, Dhiman RK, Kumari S, et al. Role of small intestinal bacterial overgrowth and delayed gastrointestinal transit time in cirrhotic patients with minimal hepatic encephalopathy. J Hepatol 2010;53:849-55.
66. Gundling F, Schmidtler F, Hapfelmeier A, et al. Fecal calprotectin is a useful screening parameter for hepatic encephalopathy and spontaneous bacterial peritonitis in cirrhosis. Liver Int 2011;31:1406-15.
67. Berni Canani R, Rapacciuolo L, Romano MT, et al. Diagnostic value of faecal calprotectin in paediatric gastroenterology clinical practice. Dig Liver Dis 2004;36:467-70.
68. Carroccio A, Iacono G, Cottone M, et al. Diagnostic accuracy of fecal calprotectin assay in distinguishing organic causes of chronic diarrhea from irritable bowel syndrome: A prospective study in adults and children. Clin Chem 2003;49:861-7.
69. Smyth RL, Croft NM, O'Hea U, et al. Intestinal inflammation in cystic fibrosis. Arch Dis Child 2000;82:394-9.
70. Raia V, Maiuri L, de Ritis G, et al. Evidence of chronic inflammation in morphologically normal small intestine of cystic fibrosis patients. Pediatr Res 2000;47:344-50.
71. Bruzzese E, Raia V, Gaudiello G, et al. Intestinal inflammation is a frequent feature of cystic fibrosis and is reduced by probiotic administration. Aliment Pharmacol Ther 2004;20:813-9.
72. Mercer DF, Vargas L, Sun Y, et al. Stool calprotectin monitoring after small intestine transplantation. Transplantation 2011;91:1166-71.
73. Rugtveit J, Fagerhol MK. Age-dependent variations in fecal calprotectin concentrations in children. J Pediatr Gastroenterol Nutr 2002;34:323-4.