

ADVANCES IN IBD

Current Developments in the Treatment of Inflammatory Bowel Disease

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The Use of Fecal Calprotectin in Inflammatory Bowel Disease



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G&H What is fecal calprotectin?

IB Calprotectin is a calcium- and zinc-binding protein, which for practical purposes can be considered to be neutrophil-specific, although low levels are found in other phagocytic cells. Calprotectin accounts for approximately 60% of total soluble proteins in the cytosol fraction of neutrophils. Neutrophils are the common effector cells that define acute inflammation in response to a number of factors. Once the neutrophil migrates to a site of chemoattraction, the contact sets off a cascade of events leading to a respiratory burst, oxygen radical generation, and disintegration of the neutrophil with the release of its cytosolic granules (and calprotectin), which contain a variety of hydrolytic and proteolytic enzymes. In this way, the neutrophil deals with the chemoattractant but at the same time causes indiscriminate damage to its surroundings.

The amount of calprotectin reflects the number of participating neutrophils in this inflammation. This has been amply confirmed in intestinal inflammatory diseases by the significant correlation between fecal calprotectin levels and other measures of acute inflammation, be it with ¹¹¹indium-labeled white cell excretion or quantitative histopathologic assessment of inflammation in colonic biopsies in controls and individuals with ulcerative colitis. The correlation with histology in Crohn's disease is somewhat less satisfactory, not because of a problem with calprotectin, but due to the patchy nature of Crohn's disease and the fact that the small bowel is not assessed histologically. The crucial points with calprotectin are that it is highly resistant to degradation by intestinal pancreatic secretions, intestinal proteases, and bacterial degradation

and that it is stable in feces at room temperature for at least a week. In short, the amount of calprotectin in feces provides a noninvasive quantitative measure of neutrophil flux to the intestine.

G&H How is fecal calprotectin measured?

IB The most common method of fecal sampling is with a universal tube that contains a small spoon on its lid. Calprotectin is then assessed by enzyme-linked immunosorbent assay (ELISA) in an extract made from a stool sample of less than 1 g. It is important to note that there are numerous manufacturers of ELISA kits, and all use different antibodies and extraction mediums, which results in different normal ranges and other differences that may involve the sensitivity of the test. ELISA kits usually use 80 to 100 cells for measurement, including for quality controls, and all samples are analyzed in duplicate. Even if clinicians fail to acknowledge the strengths and weaknesses of the different assays and the reliability of the various kits that are commercially available, the kits undergo rigorous quality control measures by laboratory technicians.

It is important to note that the normal ranges that were reported with the use of the very first calprotectin kit have by and large been extrapolated to the newer kits, which may cause confusion, as kits from different manufacturers all differ and the normal ranges of calprotectin will differ accordingly. There are times when the batch has to be discarded because of quality control issues. However, quality control is so rigorous that false-positive or -negative results are not usually a problem. If such results

do occur, they are most likely due to biologic variation in inflammatory intensity or the lack of mixing of the calprotectin with stool.

A recent effort has been made to commercialize home-based spot tests, allowing patients, including those with inflammatory bowel disease (IBD), the ability to extract the sample at home with the use of a simple sampling device. Results can be obtained within hours if the patients have the application on their smartphones so that the sample can be analyzed centrally. This allows patients to avoid a trip to the hospital, and because the results are available immediately, they can step up treatment whenever required. However, the reliability of this method is dependent upon the manufacturer.

G&H What are considered to be abnormal concentrations of fecal calprotectin?

IB When quantitative tests are used, most studies report the normal range to be 10 to 50 or 60 $\mu\text{g}/\text{mg}$. However, as mentioned above, many doctors use the historical data that were obtained with the first calprotectin kit, which come from developed countries. In contrast, the normal range of calprotectin in developing countries with poor sanitation and frequent intestinal infections (which may be perceived as normal within these communities) may be much higher, thus limiting the use of the test. Values over 50 to 60 $\mu\text{g}/\text{mg}$ (depending upon which kit is used) are generally viewed as abnormal, although values as high as 100 $\mu\text{g}/\text{mg}$ may be normal with some kits. For example, for screening purposes in London, values between 50 and 200 $\mu\text{g}/\text{mg}$ are often considered normal, especially in people of African-Caribbean descent, who seem to have a higher normal limit to the test. At this range, doctors often suggest that individuals undergo a repeat test several weeks later and that there is no need to investigate the elevation. If a patient's calprotectin level is persistently slightly elevated, there is still no rush for investigation, as the likelihood of abnormal pathology is low. However, levels over 200 $\mu\text{g}/\text{mg}$ have a higher positive predictive value for pathology, and values of 500 to 600 $\mu\text{g}/\text{mg}$ nearly guarantee pathology findings.

It is important to keep in mind that fecal calprotectin is inflammation- and not disease-specific. Almost every colonic disease and many small bowel diseases are associated with inflammation and, hence, test positive for calprotectin. However, most of these diseases are associated with low-grade inflammation, such as non-steroidal anti-inflammatory drug (NSAID) enteropathy, although calprotectin values over 500 to 600 $\mu\text{g}/\text{mg}$ are extremely predictive of IBD or food infections. However, there are no fixed rules for calprotectin values. As clinicians use the calprotectin test more often, they improve

in judging the test results in relation to the symptoms of patients.

G&H What is the sensitivity and specificity of fecal calprotectin for diagnosing IBD?

IB As previously mentioned, the fecal calprotectin test is a functional quantitative measure of intestinal inflammation, and it is inflammation- and not disease-specific. It is a test that usually supplements endoscopy clinically and, at times, can even replace the procedure. Fecal calprotectin certainly has the potential to serve as a diagnostic screening test (as opposed to merely a diagnostic test). An abnormal test result simply indicates intestinal inflammation of any cause. There are numerous intestinal diseases and drugs (eg, NSAIDs, alcohol) associated with low-grade intestinal inflammation with average calprotectin levels between 50 and 300 $\mu\text{g}/\text{mg}$. However, only untreated IBD and certain food infections are associated with very high levels. Given a degree of clinical disease activity in, for example, Crohn's colitis and small bowel Crohn's disease, it is noteworthy that calprotectin is somewhat lower in the latter. This is in keeping with the fact that the small bowel bacterial load (the main neutrophil chemoattractant) is far less than in the colon and, hence, reflected by a less intense inflammatory response. This is also reflected by histology.

The common clinical scenario in which general practitioners (and gastroenterologists) use fecal calprotectin is in a young person presenting with gastrointestinal symptoms that may indicate either irritable bowel syndrome (IBS) or IBD. No doctor wants to miss the latter diagnosis because it has life-long implications for the patient and requires targeted treatment. Nearly 99% of patients who have active IBD have elevated fecal calprotectin levels. At the same time, 15% to 20% of patients with IBS have mildly elevated calprotectin levels. (It is important to note that patients with postinfectious or postdiverticulitis IBS-like symptoms may have been included in these studies, and these diseases differ from conventional IBS.) The sensitivity and specificity of the calprotectin test for the identification of IBS or IBD can be calculated at different degrees of inflammation by conventional means, but this information is not particularly helpful clinically. Rather, the take-home message is that a normal calprotectin level is much more likely to represent IBS. Hence, the message to general practitioners is not to refer these patients to gastroenterologists, who often perform endoscopy, but to treat the IBS, and for gastroenterologists who see these patients not to always proceed to endoscopy.

G&H When can the fecal calprotectin test replace endoscopy in patients with IBD?

IB There are a number of situations in which fecal calprotectin is useful in established cases of IBD, where it usually provides data complementary to endoscopy and occasionally provides data that endoscopy cannot. The most obvious case of the use of fecal calprotectin is with patients with increasing clinical symptoms compatible with clinical relapse of IBD. Tools such as colonoscopy or capsule enteroscopy can be used in most of these cases, but an ulcer can be present without much inflammation (ie, a solitary rectal ulcer). However, it is also clear that clinical symptoms, and in particular clinical relapse of disease, relate to intestinal inflammatory activity, which is precisely the information that the calprotectin test provides. Given that a patient in this scenario has a very high calprotectin level, there is little justification for instead using an invasive procedure, and acute treatment can be given safely in the vast majority of cases. It is only if this treatment does not work that it is necessary to look for alternative causes for the symptoms.

However, if a symptomatic IBD patient has a normal or near-normal calprotectin level, is it safe to assume that the symptoms are not due to IBD in most cases? On average, patients with a very limited proctitis (where there is little mixing of the inflammatory exudate with the stool) and some patients with small bowel Crohn's disease have somewhat lower calprotectin levels than patients with IBD colitis with comparable clinical disease activity. In addition, patients with Crohn's disease may have alternative explanations for their symptoms, such as strictures and fistulas. As with any test, the usual advice is not to rely on any single clinical or laboratory measure, but to consider the overall clinical picture.

G&H Can fecal calprotectin predict clinical relapse of IBD?

IB Calprotectin predicts imminent clinical relapse with an 80% sensitivity and accuracy in patients with established, relatively asymptomatic IBD (ulcerative colitis or Crohn's disease). A patient with asymptomatic IBD with a high calprotectin level has an 80% chance of a clinical relapse in the next 6 months, whereas only 20% of patients with a low calprotectin level will experience a clinical relapse. The precise cutoff value for the distinction between high and low calprotectin levels in this context is variable. Data from my colleagues and I suggest a cutoff point of 250 $\mu\text{g}/\text{mg}$, but other investigators have suggested somewhat lower cutoff points using receiver operating characteristic curve analyses. One study using leukocyte aphaeresis showed that treatment at this asymptomatic stage could avert clinical relapse. It is also possible that simpler treatment, such as dose escalation of 5-aminosalicylates or a short sharp course of corticosteroids, would have the same

effect, but this has yet to be studied. Avoiding clinical relapse is an important issue in IBD because of associated morbidity and deterioration of quality of life.

G&H Does fecal calprotectin also have a role for predicting mucosal healing?

IB New therapeutic agents for treating problematic IBD have created a wave of enthusiasm, not only because of their efficacy and acceptable side effects, but because of the possibility of achieving mucosal healing. The definition of mucosal healing differs between different studies, and there is no universally accepted definition. Most clinical trials define mucosal healing on the basis of endoscopies and biopsy specimens. This definition works for trials and gastroenterologists who perform colonoscopies, but it is impractical in clinical practices with limited resources. If mucosal healing is defined as the absence of significant acute inflammation, then this can be assessed by the fecal calprotectin test, whose result represents the entire intestine rather than small biopsy samples that may not reflect the rest of the intestine.

G&H How else can fecal calprotectin predict response to treatment?

IB The usefulness of measuring fecal calprotectin is evident in its increasing use in clinical trials, but the primary endpoints are largely still symptom-based. Gastroenterologists should follow the example of rheumatologists, who alter treatment according to inflammatory markers rather than symptoms of patients. This has resulted in a beneficial change in the natural treated history of disease.

In a similar vein, measuring calprotectin levels during treatment of acute relapse of the disease is reassuring that the treatment is working and not simply masking the symptoms. This may not be necessary in most patients, but there are good data showing that normal calprotectin levels just before routine administration of a biologic agent may predict whether the treatment can be delayed or stopped. However, much more research needs to be done before a firm decision can be made as to whether this approach is safe.

In a patient with symptomatic stricturing Crohn's disease, the obstructive symptoms can be due to a fibrous stricture or an inflammatory component. An elevated fecal calprotectin level will help determine treatment in this situation: surgery for the former cause but aggressive medical treatment for the latter cause. Again, there is a lack of clinical data on this issue, but common sense should prevail until research is performed.

In addition, patients with IBD are often put on long-term 5-aminosalicylate therapy, which has been shown

to reduce the frequency and severity of clinical relapse. Many patients, especially those with ulcerative colitis, take a fixed daily dose. Fecal calprotectin may identify those patients with normal calprotectin levels, who may not require maintenance treatment. Likewise, as above, patients with high levels may benefit from an increase in therapeutic dose.

G&H Can postsurgical calprotectin levels predict the recurrence of Crohn's disease?

IB This is an issue especially after a right hemicolectomy for small bowel Crohn's disease in which the surgeon might think that the bulk, if not all, of the disease has been removed. At present, recurrence is usually defined as anastomotic disease in symptomatic individuals. However, recurrence of Crohn's disease needs a better definition than that. My colleagues and I measure calprotectin levels several months after the operation and then every 4 to 6 months, looking for a significant increase, which is suggestive of new disease. It is very important to perform a capsule enteroscopy shortly after the operation, as this procedure will also help to decide whether there is residual disease. This may affect the prognosis of the patient and might be controlled medically (not studied) in order to alter the natural history of the disease, whereas normal calprotectin levels and capsule images may be prognostically favorable indicators (also not studied). Such studies would require multicenter collaboration and highlight the potential use of calprotectin in IBD.

G&H Does fecal calprotectin have a role in pouchitis?

IB Fecal calprotectin is also useful for the detection of the acute inflammatory component in pouchitis. However, there are few, if any, studies that show that the

calprotectin level correlates well with clinical symptoms, although there is usually significant correlation with the histopathologic assessment of acute inflammation. The problem is that patients with symptomatic pouches may have very localized inflammation that contributes to the urgency and frequency of bowel openings, and the calprotectin test may be inferior to pouchoscopy under these circumstances.

G&H How cost-effective is the use of fecal calprotectin in IBD?

IB There has not been any formal cost-effectiveness analysis to date, but we know that the cost savings are significant, both for general practices as well as hospitals. The number of false-negative results in patients with IBD is negligible, and only very elderly patients seem to have problems with sample collection. Moreover, the fecal calprotectin test occasionally allows patients to avoid endoscopic procedures, which are more expensive than the test.

Dr Bjarnason has no conflicts of interest in relation to any of the companies that manufacture or market calprotectin kits.

Suggested Reading

- Bunn SK, Bisset WM, Main MJ, Gray ES, Olson S, Golden BE. Fecal calprotectin: validation as a noninvasive measure of bowel inflammation in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr.* 2001;33(1):14-22.
- Johnson MW, Mastranzi S, Duffy AM, et al. Faecal calprotectin: a noninvasive diagnostic tool and marker of severity in pouchitis. *Eur J Gastroenterol Hepatol.* 2008;20(3):174-179.
- Pavlidis P, Gulati S, Dubois P, et al. Early change in faecal calprotectin predicts primary non-response to anti-TNF α therapy in Crohn's disease. *Scand J Gastroenterol.* 2016;51(12):1447-1452.
- Smith LA, Gaya DR. Utility of faecal calprotectin analysis in adult inflammatory bowel disease. *World J Gastroenterol.* 2012;18(46):6782-6789.
- Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. *Clin Exp Gastroenterol.* 2016;9:21-29.