REVIEW ARTICLE

The role of fecal markers in the investigation of patients with chronic diarrhea

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ABSTRACT

Chronic diarrhea often presents a dilemma for a practicing clinician as to whether an endoscopic evaluation would be necessary. The current application of colonoscopy-for-most approach increases the burden on the endoscopy services and is associated with higher costs. Therefore, there is a need for newer tools which are cost-effective, less invasive, and easily accessible, in order to triage patients referred for endoscopic evaluation. In this context, fecal markers are becoming considered as triage tools in clinical practice. Emerging evidence in support of their high performance will certainly influence future practice.

Introduction The investigation and management of chronic diarrhea can be challenging, owing to its wide spectrum of etiological factors. Recent guidelines by the British Society of Gastroenterology (BSG) set out a detailed approach on the investigation of chronic diarrhea based on clinical differential diagnoses rather than a hierarchical investigative algorithm.[1]

Chronic diarrhea is generally defined as a persistent change in bowel frequency and consistency of type 5 and above according to the Bristol Stool Chart lasting for 4 or more weeks.[2] Diarrhea can be defined in terms of stool frequency, consistency, volume, or weight. Moreover, the way diarrhea is described by the patients in lay terms and its medical definition vary considerably. Patients' descriptions are usually based on stool consistency.[3]

Although chronic diarrhea can result from numerous different conditions, it is usually indicative of noninfectious etiology, and warrants further investigation, with chronic infections remaining as a differential.

Clinical assessment, understood as the collection of detailed medical history and examination, is the mainstay of initial evaluation. A history of diarrhea not exceeding 3 months with nocturnal symptoms and significant weight loss would suggest an organic pathology. Further investigation often becomes necessary, as it can be difficult to determine etiology on clinical grounds alone. Significant bowel pathologies, for example, neoplasia, can manifest with nonspecific symptoms.

Fecal markers are emerging as important primary investigative tools for chronic diarrhea. Their cost-effectiveness, wide availability, and noninvasiveness, supported by good scientific data reporting their high-performance, make them attractive options for investigation. In this study, we describe the diagnostic role that fecal markers play in the common conditions encountered in gastroenterology clinics.

Functional bowel disease The majority of our patients with bowel symptoms who are referred to specialized gastroenterology services typically have subsequent colonic examinations. It is crucial not to overlook any treatable conditions, such as inflammatory bowel disease or cancerous lesions, and therefore most patients undergo endoscopy examinations. A reliable, cost effective, and noninvasive investigation that could differentiate patients with normal colon from those with pathologies prior to conducting an endoscopic evaluation would be a preferred choice.

The National Institute of Clinical Excellence (NICE) recommends the use of fecal calprotectin (FC) as one of such options when differentiating inflammatory bowel pathologies from functional bowel conditions in patients who do not exhibit

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symptoms of colorectal cancer (DG11 diagnostic guidance).[4] Fecal calprotectin is a calcium-binding heterodimer that is present in large quantities in the cytoplasm of neutrophils. The BSG guidelines on chronic diarrhea explain the role that FC plays in each of the aforementioned scenarios (inflammation, cancer, etc.).[1] Dhaliwal et al[5] assessed 311 fecal samples from patients with both inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) for different FC cutoff levels. A FC level of 50 μg/g of feces was found to be sensitive for gut inflammation. For FC cutoff of 50 μg/g of feces, the sensitivity and specificity were 88% and 78%, respectively (area under the receiver operator curve [AUROC], 0.84; 95% confidence interval [CI], 0.78–0.90). The positive predictive value was 79% and the negative predictive value was 92%. When a FC cutoff of 100 μg/g was applied, the sensitivity increased to 97% and the specificity decreased to 76%, and negative predictive value and positive predictive value were 97% and 75%, respectively (AUROC, 0.88; 95% CI, 0.82–0.92). These findings imply that the diagnosis of IBD is less likely in low levels of FC.[5,6] Another retrospective study compared FC levels with findings from colonoscopy and histologic examination from 119 patients with chronic diarrhea. In this study, the FC level of less than 8 μg/g of feces excluded colonic inflammation regardless of etiology with a negative predictive value of 100%, but with low specificity of 51%.[7] The role of FC in inflammatory bowel conditions is discussed below.

Fecal volatile compounds have also been shown to be useful in differentiating functional bowel pathology from inflammatory conditions. In a case-control study by Ahmed et al[8] that included 30 patients with IBS and diarrhea, 62 patients with active Crohn disease, and 48 patients with active ulcerative colitis, it was established that positive and negative likelihood ratios in differentiating IBS from active IBD were 4.83 (95% CI, 3.36–7.14) and 0.04 (95% CI, 0.01–0.21), respectively, on receiver operating characteristic curve.

Inflammation **Infection** It is prudent to rule out infection as a potential cause whenever a patient presents with chronic diarrhea. The fecal tests for *Clostridium difficile* are most widely used in clinical practice and consist of nucleic acid amplification test or glutamate dehydrogenase (GDH) enzyme immunoassay (EIA) in combination with tests to detect toxins A and B. These require a wet feces sample. The European Society of Clinical Microbiology and Infectious Diseases recommends a 2-step algorithm in diagnosing *C. difficile* infection: nucleic acid amplification test or GDH EIA as the first step and EIA for toxins A and B in the positive samples as the next step. Alternatively, the samples can be screened with both a GDH and EIA for toxins A and B simultaneously.[9]

Other available bacterial fecal toxin assays include those for *Salmonella*, *Shigella*, *Klebsiella*, and *Escherichia coli*. Testing for opportunistic infections should always be considered in immunocompromised populations.

There are various other integrated multiplex molecular stool tests, which have been cited in literature recently. These tests can simultaneously detect and identify nucleic acids from up to 22 viruses, parasites, and bacteria that cause gastroenteritis, and some of them do not require fresh feces sample. However, these tests lack reference standards. Their clinical relevance in the management and outcome of patients needs to be evaluated in further studies.[10]

Inflammatory bowel disease Fecal inflammatory markers are routinely used when an inflammatory etiology of chronic diarrhea is suspected. Serum based biomarkers have poor accuracy, both in their detection and monitoring of IBD, and therefore their role is limited.

Fecal calprotectin Fecal calprotectin levels of more than 250 μg/g of feces suggest active inflammation, which correlates with findings from colonoscopy and histologic examinations. This cutoff level has a sensitivity of 90% and specificity of 76% (AUROC, 0.93; 95% CI, 0.89–0.97). The values above the cutoff point also have similar accuracies for disease monitoring in patients with quiescent colitis.[5, 11] However, there are other causes, such as infection and nonsteroidal anti-inflammatory drugs, that could equally result in high FC levels, thus, it could be misleading in the diagnosis.[12] It is important to exclude infectious etiology prior to attributing high FC levels to inflammatory bowel pathologies. Elevated FC levels have also been observed in patients with colorectal cancer, as the neoplastic cells trigger an inflammatory process in the surrounding tissue.[13]

Indeterminate levels of FC (50–249 μg/g of feces) can pose a problem for the clinician. In a retrospective study by McFarlane et al,[14] 41 patients with FC range of 50 to 249 μg/g of feces were followed over a period of 12 months. They found that over the follow-up period, 19% of patients were newly diagnosed with IBD (either Crohn disease or ulcerative colitis), as compared with 1% whose FC levels were less than 50 μg/g of feces. The odds ratio for patients with a new diagnosis of IBD with intermediate levels of FC, compared with those with normal levels of FC (<50 μg/g of feces), was 26.6. Repeating the FC evaluation in 6 to 8 weeks in this group of patients may help determine whether the inflammation is improving.[15] Colonoscopy may be offered where history is suggestive of IBD along with rising FC.[16]

Although FC levels are widely used to aid the diagnosis and monitoring of the response to treatment, its role in small bowel inflammation without colonic involvement remains unclear. One study reported good correlation between FC levels and mucosal healing in Crohn disease, where lesions were only in the small bowel (AUROC, 0.753; 95% CI, 0.557–0.950, $P = 0.035$).[17]

Age is another important factor to bear in mind when interpreting FC levels in the context of chronic diarrhea. NICE does not currently recommend any age cutoff for interpreting FC levels, although there is wide variation in local guidelines across the United Kingdom. The 2016 BSG guidance on FC suggests that FC should not be used in patients above 40 or 50 years old (depending on local fast-track pathways) with a new change in bowel habit. Age of more than 50 years is considered a red flag in patients with changes in bowel habits. In these patients, normal FC levels should not be used alone to rule out organic pathology because of the rising incidence of colorectal cancer.

Fecal lactoferrin Fecal lactoferrin is an iron binding glycoprotein expressed by activated neutrophils. The role of fecal lactoferrin in differentiating IBD from functional bowel pathologies has been studied widely in literature with varying results. Sidhu et al[18] reported higher median fecal lactoferrin levels in patients with IBD in comparison with those with irritable bowel syndrome (P <0.001). The sensitivity and specificity of lactoferrin in patients with active IBD as compared with patients with IBS or healthy controls were 67% and 96%, respectively.

Fecal neopterin Neopterin is a metabolite of biopterin released from activated macrophages. This is another fecal marker of accuracy similar to FC. A study by Nancey et al[19] assessed FC and fecal neopterin concentrations in 78 patients with Crohn disease and 55 patients with ulcerative colitis and compared those with endoscopic findings from the patients. For the fecal neopterin cutoff value of 200 pmol/g of feces, the overall accuracy in predicting endoscopic activity in Crohn disease and ulcerative colitis was 74% and 90%, respectively. This was similar to the FC cutoff value of 250 μg/g of feces. This suggests fecal neopterin can be used not only as a surrogate biomarker but also as an alternative to FC with similar accuracy in the diagnosis and monitoring of IBD.

Other fecal markers Other fecal markers reported in literature are S100A12, M2-PK, metalloproteinases, hemoglobin, myeloperoxidase, lysozyme, polymorphonuclear elastase, and nitric oxide, all of which have variable performance indices, and are mainly used in research.

Amongst the currently available fecal markers for inflammation, FC remains the preferred and widely used one because of its relatively high-performance and easy accessibility in comparison with others.

Microscopic colitis Raised FC levels can also be seen in microscopic colitis. A small study by Wildt et al[20] including 46 patients compared patients with active collagenous colitis with those with quiescent collagenous colitis and healthy controls. This showed that FC levels were elevated in patients with collagenous colitis as compared with healthy controls. However, this finding was not universal, since some patients with active microscopic colitis had normal FC levels and there was a wide variation in FC levels among these patients. Another prospective study by von Arnim et al[21] including 23 patients with microscopic colitis reported difference in median FC concentrations between active microscopic colitis group (median, 48 μg/g; range, 23–1060) and IBS group (median, 2 μg/g; range, 1–111.83; P = 0.0001). Based on the limited data available, it can be concluded that FC may have the potential to become a biomarker in microscopic colitis with uncertain performance indices. Well-designed, larger studies are necessary to assess this further.

Colorectal Cancer **Fecal occult blood test** Detection of hemoglobin in feces has been used as a screening test for colorectal cancer. The older fecal occult blood test in a symptomatic population has a sensitivity and specificity of 69% and 73%, respectively.[22,23] The current European guidelines recommend the use of fecal immunochemical test (FIT) for colorectal cancer screening, and NICE recommends it in low risk symptomatic individuals (DG30 diagnostic guidance).

Fecal immunochemical test Fecal immunochemical test uses immunoassay methods to quantify human hemoglobin concentrations in feces. Unlike fecal occult blood test, it is not affected by diet, seasonal variations, and drugs. A meta-analysis reported that pooled sensitivity and specificity of FITs for colorectal cancer in asymptomatic average-risk adults were 0.79 (95% CI, 0.69–0.86) and 0.94 (95% CI, 0.92–0.95) respectively, positive likelihood ratio and negative likelihood ratio were 13.10 (95% CI, 10.49–16.35) and 0.23 (95% CI, 0.15–0.33) respectively, with an overall diagnostic accuracy of 95% (95% CI, 93%–97%).[24]

In symptomatic population, the sensitivity and specificity of FIT were 84% and 93%, respectively (AUROC, 0.94; 95% CI, 0.88–1.0).[18] Its higher negative predictive value (0.99) for the cutoff value of 7 to 10 μg/g of feces means that it can be useful as a test to exclude colorectal cancer in patients with suggestive lower gastrointestinal symptoms.[13,25,26]

A recent updated systematic review of FIT within symptomatic population (number of studies, 9; population, 6755) showed that the overall pooled sensitivity and specificity of FIT for colorectal cancer were 0.90 (95% CI, 0.87–0.92) and 0.87 (95% CI, 0.83–0.90), respectively.[27] Furthermore, when the receiver operator curve was produced for all studies of FIT over multiple cutoff values for colorectal cancer (hemoglobin concentrations 7–50 μg/g of feces), the area under the curve was calculated as 0.94 (95% CI, 0.92–0.96). Using the average prevalence of colorectal cancer of 5.1%, pooled sensitivity of 0.90, and pooled specificity of 0.86, it can be calculated

that 81.6% of colonoscopies could be considered as unnecessary for exclusion of cancer but not for other enteric diseases.

New studies are emerging with a focus on improving the sensitivity of FIT and using other noninvasive tests in conjunction with FIT, for example, urinary volatile compounds.[28]

Multi-target stool DNA test Multi-target stool DNA test (mt-sDNA) is a relatively new tool. There are limited data on screening outcomes as well as on its performance against already established, stool-based screening tests. Colorectal malignancies are known to develop characteristic epigenetic and genetic mutations as they progress, which serve as the basis for stool molecular DNA testing. This includes an immunochemical assay for hemoglobin and assays for methylated *BMP3*, *NDRG*, and *NDRG4*, mutated K-ras, and β-actin from neoplastic cells. A large study funded by a manufacturer that was conducted in an asymptomatic population with average risk for colorectal cancer reported a sensitivity of 92% for mt-sDNA (95% CI, 83.0–97.5) compared with a sensitivity of 73.8% for FIT. The specificity of the mt-sDNA was lower than that of FIT (89.8% and 96.4 %, respectively), and thus showed higher false positive rates.[29] The American Cancer Society guidelines recommend mt-sDNA as one of the stool-based screening tests for colorectal cancer.[30]

Fecal volatile compounds Recently, the utility of fecal volatile compounds as a screening test for the detection of colorectal cancer has also been studied increasingly. A recent systematic review by Bosch et al[31] reported potentially improved test performance in early detection of both colorectal cancer and advanced adenoma.

Malabsorption **Bile acid diarrhea** Bile acid diarrhea (BAD) is underdiagnosed as an etiology for chronic diarrhea, largely because it usually coexists with other bowel pathologies. Smith et al[32] reported that one-third of patients with diarrhea-predominant IBS had BAD. The BSG guidelines describe various tests used in the diagnosis of BAD.[1]

Bile acids can be measured in the feces using chromatography or enzymatic assay methods. This requires a 48-hour fecal sample collection to minimize the effect of diurnal variation on the results and ensure the majority of the isotope is excreted.[33,34] It is inconvenient and not widely available commercially. The bile acid content of more than 2300 μmol/48 hours is indicative of BAD. The use of this test in diagnosing bile acid diarrhea is largely replaced by a more user-friendly SeHCAT (tauroselcholic [selenium 75] acid) scan.[35]

A recent retrospective analysis by Vijayvargiya et al[36] in a population with diarrhea-predominant IBS reported that the percentage of primary bile acids in fecal samples (48-hour collection) can be an alternative to total fecal bile acids in diagnosing BAD. This is further supported by our own data (unpublished), which shows that the percentage of primary bile acid in single fecal sample can detect patients with BAD, as diagnosed by SeHCAT. Such test certainly has advantages over a 48-hour stool collection on a defined diet.

Pancreatic exocrine deficiency Fecal elastase-1 Fecal elastase-1 is a protease secreted solely by pancreas that is stable throughout the intestinal transit and correlates well with the duodenal levels of lipase and bicarbonate. It is more sensitive and specific than previously used fecal chymotrypsin, especially in severe pancreatic exocrine insufficiency (PEI). A study by Löser et al[37] reported that at the elastase cutoff of 200 μg/g of feces, the overall sensitivity and specificity was 93%. The sensitivities for mild, moderate, and severe PEI at the above cutoff were 63%, 100%, and 100%, respectively. False positives can occur in several comorbid diseases, such as diabetes,[38] celiac disease, and short-bowel syndrome.[39] A recent meta-analysis suggested that a normal fecal elastase level of less than 200 μg/g of feces could be used to exclude PEI in low-risk patients (false negative rate of 1.1% and false positive rate of 11%).[40]

Usefulness of fecal chymotrypsin is limited because it is biodegraded by proteolytic enzymes, and the assay cannot differentiate the exogenous chymotrypsin found in pancreatic enzyme supplements.[41]

Fecal fat The coefficient of fat absorption using a 72-hour fecal fat content test was considered the gold standard in diagnosing fat malabsorption. However, it has practical difficulties as it requires a strict diet of 100 g of fat daily for 5 days and stool collection over the last 3 days.[42] Therefore, it is not often the preferred initial test in clinical practice.

Both fecal elastase and fat quantification are indirect tests for pancreatic exocrine function. Direct hormone stimulated pancreatic function tests are the current gold standard in the diagnostic workup of PEI. These specialized tests can detect early pancreatic insufficiency with high accuracy, although they are expensive, invasive, and technically challenging.[43]

Conclusions It is paramount that clinicians adopt a pragmatic approach when investigating chronic diarrhea. Thorough, detailed history taking and clinical examination still remain the most important steps in the initial assessment. Fecal markers play a role as a noninvasive next step in the assessment of chronic diarrhea. To date, no single fecal marker is diagnostic in chronic diarrhea. It is because of their presence in various bowel pathologies and poor tissue specificity. Future research should aim to address those issues.

Fecal markers are useful individually or in conjunction with other tests in arriving at a diagnosis. The choice of an appropriate test depends on the initial clinical assessment, local availability, and patient's preference. It is also equally

important that these fecal markers are correctly interpreted in the appropriate context.

A significant proportion of fecal tests are not widely available. Further studies are needed to assess the practicality and economic impact of fecal tests on health services, which in turn will help reduce the pressure on colonoscopy services.

ARTICLE INFORMATION

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