

Noninvasive Markers of Disease Activity in Inflammatory Bowel Disease

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Abstract: It is often difficult to assess disease activity in inflammatory bowel disease (IBD). Noninvasive biomarkers are a means of quantifying often nebulous symptoms without subjecting patients to endoscopy or radiation. This paper highlights markers present in feces, serum, or urine that have all been compared with the gold standard, histologic analysis of endoscopically collected specimens. Two categories of markers are featured: well-researched markers of mucosal inflammation with high sensitivity and specificity (calprotectin, lactoferrin, and S100A12) and novel promising markers, some of which are already clinically employed for reasons unrelated to IBD (interleukin [IL]-17, IL-33/ST2, adenosine deaminase, polymorphonuclear elastase, matrix metalloproteinase-9, neopterin, serum M30, and fecal immunohistochemistry). The data pertaining to the more-established markers are intended to highlight recent clinical applications for these markers (ie, assessing disease outside of the colon or in the pediatric population as well as being a cost-saving alternative to colonoscopy to screen for IBD). As there is no evidence to date that a specific marker will accurately be able to represent the entire IBD patient population, it is likely that a combination of the existing markers will be most clinically relevant to the practicing gastroenterologist attempting to evaluate disease severity in a specific patient. Familiarity with the most promising emerging markers will allow a better understanding of new studies and their impact on patient care.

Keywords

C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, lactoferrin, inflammatory bowel disease

Inflammatory bowel disease (IBD) is an idiopathic chronic inflammatory disorder that encompasses Crohn's disease and ulcerative colitis. The 2 diseases are quite different in that Crohn's disease can involve the entire digestive tract and all 3 mucosal layers, whereas ulcerative colitis affects only the colon and the mucosa. Inherent in the etiology of each disease type might be the answer to why these diseases have different responses to therapy, as systemic treatment with anti-tumor necrosis factor (anti-TNF) agents appears to work better in Crohn's disease, whereas local treatment with concentrated

delivery of mesalamine to the colon appears to work better in ulcerative colitis. However, the clinical presentation of the 2 diseases is often similar, with diarrhea, abdominal discomfort, and extraintestinal manifestations involving skin, joints, and eyes.

Determining disease activity in IBD is difficult, as patients might have a concurrent source of gastrointestinal symptoms, such as irritable bowel syndrome (IBS) or infection. Attributing certain clinical symptoms to IBD has traditionally been accomplished either by examining biopsy specimens or by using radiologic imaging. However, these methods are not without risks,^{1,2} and there is much interest in assessing disease severity in a noninvasive fashion. The pathophysiologic process that drives IBD has as its endpoint an invasion of the intestinal tissue by inflammatory cells. The gold standard for assessing intestinal damage is fecal excretion of ¹¹¹indium-labeled leukocytes,³ but because this process involves patient exposure to radiation as well as prolonged collection of feces, it is rarely used in clinical practice.

This paper focuses on markers of current disease activity rather than prognostic information. Therefore, the discussion does not include antibodies or genes, as these are not currently used to assess inflammation in real time. Also excluded are biomarkers present in intestinal tissue specimens, as patients and doctors alike are focusing on minimally invasive testing using only blood, fecal, or urine samples.

Current disease activity closely parallels mucosal healing and has been demonstrated to reflect useful clinical parameters such as response to treatment and potential to wean off medications. Noninvasive markers of disease activity are becoming important criteria for enrollment in clinical trials, and the limitations of the Crohn's Disease Activity Index (CDAI) as an accurate gauge of disease activity were noted most visibly with the SONIC (Study of Biologic and Immunomodulator-Naive Patients in Crohn's Disease) trial.⁴ Additionally, elevated marker levels have the potential to lead to better understanding of other inflammatory pathways and subsequent new drug development. This direct application to patient care makes noninvasive determination of disease activity in IBD a vital topic for the practicing gastroenterologist.

Initial attempts to noninvasively gauge disease activity had employed serologic markers, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). These markers have been more recently thought to be less sensitive and specific than fecal markers, as reviewed in detail elsewhere.⁵

The majority of literature on this topic focuses on fecal calprotectin and lactoferrin. These 2 components of neutrophils, when excreted in feces, have been proven to reflect disease activity with good sensitivity and specific-

ity (calprotectin, 70%-100% sensitivity and 44%-100% specificity; lactoferrin, 66%-80% sensitivity and 67%-100% specificity).⁶

There is also growing evidence for newer biomarkers. The literature on this topic is extensive, with novel indicators being compared with either more-established ones or activity indices (eg, CDAI, Harvey-Bradshaw Index, and Pediatric CDAI). The markers that have been assessed with concomitant histologic or endoscopic data are most clinically relevant, as by design they would be most suited to replace endoscopy. They are, therefore, highlighted here. The markers discussed were selected from the plethora of possible markers because, apart from having confirmatory endoscopic data, a plausible mechanism for contribution to epithelial inflammation was described for each.

Calprotectin

Calprotectin, a heterodimer of 2 calcium-binding proteins (S100A8 and S100A9), is present in the cytosol of neutrophils (where it makes up almost 60% of the proteins present) as well as in macrophages and monocytes. It is homogeneously distributed in feces and is stable for at least 48 hours and, occasionally, as long as 1 week at room temperature. Its presence in feces directly correlates with the amount of inflammation present in the colon. Especially compelling have been the data demonstrating that calprotectin excretion from colonocytes mirrors fecal excretion of ¹¹¹indium-labeled leukocytes.⁷

A recent meta-analysis of 13 studies (6 in adults and 7 in children and adolescents) compared fecal calprotectin levels to biopsy samples for detecting intestinal inflammation and included all studies published in Medline and Embase on this topic through October 2009.⁸ The sensitivity of calprotectin to determine disease activity in adults was 0.93 (95% CI, 0.85-0.97), and the specificity was 0.96 (95% CI, 0.79-0.99). The sensitivity in children was comparable at 0.92 (95% CI, 0.84-0.96), but the specificity was lower at 0.76 (95% CI, 0.62-0.86). The authors advanced the innovative suggestion, which has not been unanimously adopted at this point, that adult patients should be screened for IBD using fecal calprotectin levels, as this would decrease the number of patients requiring endoscopy to diagnose IBD by 67%.

A prior meta-analysis by von Roon and colleagues with almost 6000 patients demonstrated that calprotectin levels in children should have a higher cutoff than in adults (100 µg/g vs 50 µg/g); with this higher cutoff, the sensitivity and specificity of this marker are 95% and 91%, respectively.⁹

There is utility in checking calprotectin levels to determine disease severity, response to treatment, mucosal healing, relapse, and ability to withdraw from treatment.¹⁰⁻¹² Because the role of calprotectin in reflect-

ing disease severity is now well established, the recent literature has focused on its role in special populations, such as children and patients with predominantly small bowel disease. A second focus has been on the use of this biomarker as a substitute for endoscopic procedures, both in making the diagnosis of IBD and in determining its severity. Other studies have looked at the various testing methods for calprotectin to determine which would best combine accuracy with fast turnaround. Perhaps most important, in this era of accountable medical care, are data focused on the cost-effectiveness of using calprotectin levels as a screening tool prior to colonoscopy.

Calprotectin in the Pediatric Population

Three recent studies have focused on the role of calprotectin in determining disease activity in children. Aomatsu and colleagues compared 35 pediatric patients with IBD (17 with ulcerative colitis and 18 with Crohn's disease) to 28 healthy controls.¹³ The median fecal calprotectin level was more than 1 order of magnitude higher in active than in inactive ulcerative colitis (1562.5 $\mu\text{g/g}$ vs 38.9 $\mu\text{g/g}$) and almost 2 orders of magnitude higher than in control subjects without IBD (19.9 $\mu\text{g/g}$). This degree of difference was similar in patients with Crohn's disease, in whom fecal calprotectin levels during active disease averaged 2037.5 $\mu\text{g/g}$, as opposed to 172.5 $\mu\text{g/g}$ in quiescent disease and 19.9 $\mu\text{g/g}$ in healthy control subjects. It is noteworthy that this marker is able to differentiate quiescent disease from both lack of disease and active disease, as this is an important clinical distinction and one that is often not easy to make.

Henderson and colleagues compared 91 patients with IBD and 99 pediatric control subjects without IBD for whom fecal calprotectin data were available prior to a colonoscopy.¹⁴ The median fecal calprotectin level in patients with IBD was significantly more elevated than in the non-IBD control group (1265 $\mu\text{g/g}$ vs 65 $\mu\text{g/g}$; $P < .001$). The area under the curve (AUC) for fecal calprotectin was 0.93 (95% CI, 0.89-0.97), which was better than for less-specific markers, such as white blood cell count and CRP.

Van de Vijver and colleagues studied 117 pediatric patients with a clinical suspicion of IBD by checking fecal calprotectin levels but blinding the pediatricians to these values.¹⁵ Using clinical parameters, 68 children were sent to endoscopy by their pediatricians, but only 54 would have undergone the procedure if a calprotectin cutoff of 50 $\mu\text{g/g}$ had been used to screen for the procedure. Forty-two patients were confirmed to have IBD, giving the fecal calprotectin level a sensitivity of 78%, as opposed to 62% for clinical judgment.

Calprotectin as a Substitute for Endoscopy

Manz and colleagues conducted a study similar to the one conducted by Van de Vijver and colleagues, but in adults.¹⁶

The goal was to determine the role of calprotectin in evaluating abdominal pain. A total of 575 consecutive patients presenting with abdominal discomfort that prompted endoscopy (405 colonoscopies and 170 endoscopies) were studied. Calprotectin levels were obtained within a day of the endoscopy. A cutoff of 50 $\mu\text{g/g}$ resulted in a sensitivity of 73% and a specificity of 93% for positive findings on endoscopy. The patients with positive findings had significantly higher calprotectin levels than those with normal findings (97 $\mu\text{g/g}$ vs 10 $\mu\text{g/g}$; $P < .001$). Related specifically to IBD, there were 16 patients with ulcerative colitis and 10 patients with Crohn's disease with mean calprotectin levels elevated over baseline for both diseases, although more elevated for ulcerative colitis than for Crohn's disease (152 $\mu\text{g/g}$ vs 69 $\mu\text{g/g}$).

D'Haens and colleagues compared 126 patients with IBD and 32 patients with IBS by having them all provide a stool sample prior to undergoing a colonoscopy.¹⁷ Using a fecal calprotectin cutoff value of 250 $\mu\text{g/g}$ resulted in a sensitivity of 60.4% and a specificity of 79.5% for predicting active disease in patients with Crohn's disease as well as a sensitivity of 71% and a specificity of 100% for predicting active disease in patients with ulcerative colitis.

Ricanek and colleagues took a population of 109 patients with IBD who were admitted to the hospital and collected stool samples as well as biopsy data.¹⁸ The calprotectin concentration correlated well with disease activity in both patients with Crohn's disease ($P = .004$) and patients with ulcerative colitis ($P = .031$).

Schoepfer and colleagues took a group of 228 patients with ulcerative colitis and compared them with 52 healthy control subjects.¹⁹ These researchers demonstrated that fecal calprotectin correlated better (Spearman rank correlation coefficient $r = 0.821$) with endoscopic disease activity than any of the other clinical parameters measured (Lichtiger Index, $r = 0.682$; CRP, $r = 0.556$; platelet count, $r = 0.488$; blood leukocyte count, $r = 0.401$; and hemoglobin level, $r = -0.388$).

Calprotectin in Small Bowel Disease

A study by Jensen and colleagues determined that there is a role for calprotectin in evaluating small bowel Crohn's disease.²⁰ This is important because calprotectin has traditionally been thought of as being excreted by the inflamed epithelial lining of the colon exclusively.^{21,22} Thirteen of 40 patients with Crohn's disease had exclusively small bowel disease as determined by endoscopic or imaging criteria, and their calprotectin levels were in the same range as levels in patients with colonic disease (890 $\mu\text{g/g}$ vs 830 $\mu\text{g/g}$, respectively). Using the cutoff value of 50 $\mu\text{g/g}$ resulted in a sensitivity of 92% for detecting small bowel disease, which compared favorably with the equivalent value for colonic disease (94%).²⁰

Calprotectin Testing Methods

The current methods for determining fecal calprotectin levels include point-of-care testing, enzyme-linked immunosorbent assays (ELISAs), and immunohistochemical assays. Hessels and colleagues sought to test the reliability of 2 rapid tests for measuring calprotectin compared with a time-resolved fluorometric assay, which is considered more precise but is also more time-consuming.²³ The 2 types of rapid tests used were Prevent ID CalDetect (Preventis) and Quantum Blue (Bhülmann). Eighty-five patients with lower abdominal complaints had stool collected. Quantum Blue had the better correlation with the time-resolved fluorometric assay (k , 0.77 vs 0.46 for Prevent ID CalDetect).

Sydora and colleagues conducted a similar study to assess the accuracy of Quantum Blue testing vs ELISA for calprotectin in differentiating IBD from IBS.²⁴ Fifty patients with Crohn's disease, ulcerative colitis, or IBS and healthy control subjects provided samples. The values looked the same for the control subjects, patients with IBS, and patients with IBD recently after surgery (presumably, the inflammation was at least transiently gone). Excluding these patients, the study had 100% specificity for Crohn's disease and ulcerative colitis. The accuracy rate was 1.00 in Crohn's disease and 0.89 in ulcerative colitis in receiver operating characteristics analysis.

Lobatón and colleagues compared Quantum Blue and ELISA measurements of calprotectin level to endoscopic data.²⁵ A total of 123 patients with ulcerative colitis underwent 146 colonoscopies. Using a cutoff of less than 280 $\mu\text{g/g}$ for Quantum Blue and less than 250 $\mu\text{g/g}$ for ELISA compared with a Mayo endoscopic score of 1 or less resulted in AUC values of 0.906 and 0.924, respectively, and an intraclass correlation of 0.904 ($P < .001$). The cutoffs were more accurate in demonstrating remission than active disease.

Naismith and colleagues sought to determine whether calprotectin's day-to-day variability in the same patient is relatively low or whether multiple measurements need to be carried out to establish a patient's inflammatory status.²⁶ Ninety-eight patients with Crohn's disease in clinical remission (by CDAI) provided calprotectin samples on 3 consecutive days. The intraclass correlation was 0.84 (95% CI, 0.79-0.89), where the maximum value is 1 (indicating that there is no daily variation at all in the 3 samples provided by the same patient). Using a cutoff of 50 $\mu\text{g/g}$ resulted in a K statistic of 0.648 (0.511-0.769) for how well the samples agreed beyond chance, which indicates substantial agreement between the 3 samples provided, as greater than 0.8 is considered to be almost perfect agreement. This study suggests that a 1-time calprotectin measurement is adequate to gauge disease activity.

An abstract presented at Digestive Disease Week 2013 evaluated 8 different tests for accuracy in determining calprotectin levels.²⁷ Thirty-three patients with sus-

pected IBD and 31 with confirmed IBD were studied with 3 point-of-care tests (Quantum Blue; Calfast, Eurospital; and Certest, Biotest), 4 ELISAs (Bhülmann, Eurospital, Calpro, and Calprolab), and 1 automated immunoassay (Phadia). The authors concluded that the point-of-care tests can safely replace the ELISAs, as all of the studies had equivalent sensitivities and specificities compared with an endoscopic scoring system. The authors did note that there were interest disagreements in 18% of cases (usually in mild vs no disease), such that the same patient might have different results based on the test used.

Cost-Effectiveness of Calprotectin

Mindemark and colleagues determined the expenses that could have been avoided in terms of colonoscopy fees if 3639 patients had been evaluated for this procedure using 2 fecal calprotectin cutoffs.²⁸ The cost analysis compared colonoscopy with initial testing of fecal calprotectin, followed by scoping patients with calprotectin values that were over either 50 $\mu\text{g/g}$ or 100 $\mu\text{g/g}$. The results indicated that a reduction of 50% and 67%, respectively, in the number of procedures could be achieved using a calprotectin screening strategy. The study was performed in Sweden, where the cost avoidance would be €1,569,989 to €2,131,669 depending on the calprotectin cutoff used.

A limitation of using calprotectin levels as a marker of disease activity is that they are not specific for IBD, as they are also elevated in celiac disease, diverticulitis, microscopic colitis, infections, and neoplastic conditions of the colon as well as with exposure to medications (eg, nonsteroidal anti-inflammatory drugs and proton pump inhibitors). Historically, there has been some doubt regarding the reproducibility of calprotectin levels in the same patient,²⁹ although less so in more recent literature.^{24,26} Other limitations are that these levels appear to be a better predictor of relapse in ulcerative colitis than Crohn's disease^{30,31} and that optimal threshold parameters are still not fully defined.^{32,33}

Lactoferrin

Lactoferrin is an 80-kDa, single-polypeptide, iron-binding, neutrophil-derived protein present over most mucosal surfaces, where it is secreted. It is found in many body fluids, including serum, tears, synovial fluid, and breast milk.³⁴ It is a major component of secondary granules of neutrophils,³⁵ and it is stable at room temperature for up to 4 days.³⁶⁻³⁸ Its function appears to be both pro- and anti-inflammatory, and it promotes iron uptake and is antimicrobial.³⁵ Kane and colleagues authored the first study to demonstrate that this marker can be used to distinguish IBS from IBD.³⁹ Lactoferrin has a role in monitoring pediatric IBD and in determining response

to therapy.³⁶ More recently, lactoferrin was demonstrated to be a good marker of pouchitis, compared with direct examination, in 85 patients with an ileal pouch, with a sensitivity of 100% and a specificity of 92%.⁴⁰

A 2009 meta-analysis determined that the sensitivity and specificity of lactoferrin for detecting active inflammation are 80% and 82%, respectively.⁴¹ There is a stronger correlation between lactoferrin levels and ulcerative colitis than between lactoferrin levels and Crohn's disease. There has been a dearth of more recent lactoferrin studies, perhaps due to the general lower sensitivity of this marker compared with calprotectin's sensitivity⁶ or, alternatively, due to its shorter half-life at room temperature.

Limitations of lactoferrin include that it might not merely be a reporter protein but might have a direct anti-inflammatory role when released, that it might be produced not only by neutrophils but also by epithelial cells⁴² (ie, it does not appear to be able to differentiate active from quiescent disease³⁶), and that the level is elevated in patients with an ileoanal pouch in the absence of visible inflammation on endoscopy.^{43,44} Lactoferrin is nonspecific for IBD, and levels can be elevated by other infectious processes, such as those caused by *Salmonella* spp and *Clostridium difficile*, other forms of colitis, colon cancer, or polyposis syndromes.³⁶ Levels are also markedly elevated in breast-fed infants and, therefore, cannot be used to determine intestinal inflammation in this patient population.⁴⁵ The ability of lactoferrin to evaluate ileal disease is uncertain, as there are data reflecting poor correlation between endoscopic findings and lactoferrin level,⁴⁶ but there are also data indicating good correlation with capsule endoscopy.⁴⁷ This is an area that warrants future investigation.

The S100 Family of Proteins

This family has 25 members involved in diverse functions ranging from degenerative diseases (eg, Alzheimer disease and heart failure) to inflammatory diseases (eg, vasculitis and rheumatoid arthritis) and neoplastic diseases.⁴⁸ Calprotectin, to date the most studied and widely used marker, is a heterodimer composed of two S100 proteins. Apart from calprotectin, another important member of this family in terms of disease activity in IBD is S100A12.

The 3 components of the family involved in IBD are also referred to as calgranulins, due to their ability to bind calcium and be secreted in granulocytes.⁴⁸ (S100A8 is calgranulin A, S100A9 is calgranulin B, and S100A12 is calgranulin C.)

Unlike other potential IBD biomarkers—CRP, for example, which is produced by the liver in response to systemic inflammation—calgranulins are produced by the inflamed tissue itself, making them an inherently more specific marker of colonic inflammation. Calgranulins

have extracellular functions related to inflammation (although whether they actually participate in the inflammation or help control it is currently unclear) and have recently been considered damage-associated molecular pattern proteins (DAMPs). DAMPs interact with receptors such as toll-like receptors and stimulate acute or chronic inflammation cascades through reactive oxygen species, inflammatory cytokines, and nitric oxide.⁴⁹

S100A12

S100A12 is a calcium-binding cytoplasmic protein expressed by granulocytes and secreted by activated neutrophils.⁵⁰ It is also known as calgranulin C, EN-RAGE (extracellular newly identified RAGE—a binding protein, where RAGE stands for receptor for advanced glycation end products), or cystic fibrosis-associated antigen. S100A12 is thought to have proinflammatory properties through activation of nuclear factor kappa B after binding to RAGE. S100A12 is thought to also be part of a feed-forward loop, which activates TNF- κ , which in turn activates S100A12 release from neutrophils. S100A12 is a potent chemoattractant of monocytes, macrophages, and, to a lesser extent, neutrophils. S100A12 is resistant to degradation by fecal bacteria⁵¹ and is stable for 7 to 10 days at room temperature.

Manolakis and colleagues and van de Logt and colleagues summarized the recent literature on S100A12.^{48,52} Manolakis and colleagues quote a 96% to 97% sensitivity and a 92% to 100% specificity for S100A12 to differentiate IBD from normal gut as well as slightly lower numbers (86%-97% sensitivity and 92%-97% specificity) to differentiate IBD from IBS.⁴⁸ Endoscopically based studies would bring these numbers to a lower range, with sensitivities of 24% to 97% and specificities of 94% to 97% for distinguishing IBD from non-IBD in colonic disease, as described in further detail below.

To date, 5 biopsy-matched studies have evaluated the role of S100A12 in determining disease activity (Table 1).⁵³⁻⁵⁷ There is growing consensus regarding cutoff values for active IBD vs no IBD, with 75 ng/mL to 82 ng/mL in serum or 0.06 mg/kg to 1.2 mg/kg in stool as the upper limit of normal. The cutoff values for active vs inactive IBD, although more clinically relevant, are less well established. There is also substantial agreement between the studies that S100A12 is a good marker for colonic disease, although perhaps less so for small bowel inflammation.

Foell and colleagues studied simultaneous endoscopic and serologic data on 12 patients, although the group studied was much larger.⁵³ In this small subgroup, the researchers noted that $r=0.72$ for correlation between S100A12 and endoscopic findings and $r=0.83$ for the correlation between S100A12 and histologic findings (which is better than the correlations with ESR or CDAI).

Table 1. Studies of S100A12

Study	N	Disease Group	Median Disease Group Calprotectin Values	Comparison Group	Median Comparison Group Calprotectin Values	Sensitivity	Specificity	Gold Standard
Foell et al ⁵³ (serum S100A12)	119 adults	40 CD, 34 UC	Active CD, 470 ng/mL; inactive CD, 215 ng/mL; (<i>P</i> <.01 active vs inactive CD) Active UC, 400 ng/mL; inactive UC, 115 ng/mL; (<i>P</i> <.001 active vs inactive UC)	15 severe bacterial infection, 30 HC	HC, 75 ng/mL (<i>P</i> <.001 active CD vs HC; <i>P</i> <.05 inactive CD vs HC; <i>P</i> <.001 UC vs HC)	N/A	N/A	Endoscopy
Leach et al ⁵⁴ (serum S100A12)	88 children	39 IBD (29 CD, 4 UC, 6 IBDU)	IBD, 196 ng/mL (27-14,810 ng/mL); (<i>P</i> <.01 IBD vs non-IBD) CD, 239 ng/mL (27-14,810 ng/mL); (<i>P</i> <.01 CD vs non-IBD) UC, 750 ng/mL (247-1391 ng/mL); (<i>P</i> <.01 UC vs non-IBD) IBDU, 94 ng/mL (40-294 ng/mL); (<i>P</i> >.05 IBDU vs non-IBD)	33 non-IBD, 16 celiac disease	Non-IBD, 82 ng/mL (15-4242 ng/mL) Celiac disease, 75 ng/mL (17-1707 ng/mL)	24%	94%	Endoscopy, calprotectin
Kaiser et al ⁵⁵ (feces S100A12)	171 adults	59 IBD (32 CD, 27 UC)	2.45±1.15 mg/kg	24 IBS, 88 infection (65 bacterial, 23 viral), 24 HC	HC, 0.06±0.03 mg/kg (<i>P</i> <.001) IBS, 0.05±0.11 mg/kg (<i>P</i> <.001)	86% IBD vs HC 86% IBD vs IBS	100% IBD vs HC 96% IBD from IBS	Endoscopy
Sidler et al ⁵⁶ (feces S100A12)	61 children	31 IBD	55.2 mg/kg (8.9-500 mg/kg); cutoff, 10 mg/kg	30 HC	1.2 mg/kg (0.5-28.3 mg/kg); (<i>P</i> <.0001)	97%	97%	Endoscopy, calprotectin
Sipponen et al ⁵⁷ (feces S100A12)	84 adults	14 CD	0.087 µg/g (0.008-0.896 µg/g); (<i>P</i> =.166 for distinguishing CD from non-CD)	70 non-CD	0.06 µg/g	59%	66%	Capsule endoscopy, calprotectin

CD, Crohn's disease; HC, healthy controls; IBD, inflammatory bowel disease; IBDU, IBD type unclassified; IBS, irritable bowel syndrome; UC, ulcerative colitis.

Table 2. Novel Markers of Disease Activity

Marker	Cutoff	Type of Disease Activity Detected	Sensitivity	Specificity
IL-17 ⁵⁸	N/A	Active vs inactive UC	N/A	N/A
IL-33/ST2 ⁵⁹	74.87 pg/ml	Active vs inactive UC	83.33%	83.33%
PMN elastase ⁶⁰	0.062 µg/mL	IBD vs IBS Active vs inactive IBD	76.7%	77.2%
Adenosine deaminase ⁶¹	9.45 U/L	Active vs inactive UC	83.3%	84.2%
MMP-9 ⁶²	0.245 ng/mL	Active vs inactive UC	85.1%	99.99%
Neopterin ^{63,64}	98.4-200 pmol/g	Active vs inactive IBD	74%-87.5%	73%-100%
M30 ⁶⁵	176.455 U/L	Active vs inactive UC	N/A	N/A
FIT (hemoglobin) ⁶⁶	1.45 mg/g	Active vs inactive UC	77%	88%

FIT, fecal immunochemical test; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IL, interleukin; MMP-9, matrix metalloproteinase-9; PMN, polymorphonuclear; UC, ulcerative colitis.

Interesting histologic data have also been presented, demonstrating that S100A12 is preferentially detected at sites of active inflammation (eg, Crohn's disease granulomas and ulcerative colitis crypt abscesses).⁵³

Leach and colleagues evaluated serum and mucosal S100A12 levels in a pediatric population.⁵⁴ Serum levels of calprotectin and S100A12 correlated well with each other ($r=0.746$; $P<.0001$), but the mucosal levels did not. On a histologic level, this protein was not only expressed in the lamina propria of noninflamed tissue but was also abundant in the epithelium of inflamed specimens. Serum S100A12 was also found to be the most specific of the markers measured in this study but had lower sensitivity. (Calprotectin, platelets, CRP, ESR, and albumin measures were all more sensitive.)

Kaiser and colleagues demonstrated that fecal S100A12 was the most accurate marker of inflammation of all the markers employed in the study (S100A12, CRP level, ESR, platelet count, white blood cell count, and hemoglobin level).⁵⁵ The authors found similarly low fecal S100A12 values in patients with IBS and healthy control subjects and equally elevated levels in patients with Crohn's disease and those with ulcerative colitis. Values were also elevated in active vs inactive disease. In adults, the fecal S100A12 values did correlate in a weak but statistically significant fashion with other markers of inflammation, such as histology inflammation score ($r=0.44$; $P<.01$), ESR ($r=0.77$; $P<.01$), CRP level ($r=0.396$; $P<.01$), platelet count ($r=0.418$; $P<.01$), white blood cell count ($r=0.287$; $P<.05$), and hemoglobin level ($r=-0.512$; $P<.001$).

Sidler and colleagues studied S100A12 in a pediatric population and were able to demonstrate that, in this cohort, fecal S100A12 level had a positive predictive value of 97% and a negative predictive value of 97% and was, therefore, more specific than fecal calprotectin for detecting active disease (97% vs 67%, respectively).⁵⁶ This marker did not correlate with calprotectin level, Pediatric

CDAI score, ESR, CRP level, or platelet count and only weakly correlated with albumin level ($r=0.3917$; $P=.03$), perhaps indicating its unique role in paralleling disease activity in this patient group.

Sipponen and colleagues demonstrated poor correlation of S100A12 with capsule endoscopy in terms of detecting ileal disease, with a positive predictive value of 38% and a better negative predictive value of 82%.⁵⁷ These findings might be expected, as fecal markers in general have been thought of as more illustrative of colonic disease, although there are some encouraging data for calprotectin as a marker of small bowel Crohn's disease, as mentioned earlier.²⁰

A strength of this marker is its high specificity for active disease (especially compared with other markers) as well as the fact that it can be measured in both serum and feces. Limitations include that S100A12 is nonspecific to IBD—with levels also being elevated due to other causes, such as infection (viral or bacterial, including diverticulitis), polyposis (colon cancer and adenomas), other autoimmune disorders (celiac disease and immunodeficiency), increased age, obesity, and physical inactivity—and that S100A12 is decreased with more fiber consumption. Another limitation is the weak ability of S100A12 to measure small bowel disease, according to current data.⁴⁸

Novel Biomarkers

The novel markers can be organized into 3 groups: serum cytokines driving inflammation, such as interleukin (IL)-17 and IL-33/ST2; enzymes involved in inflammation at the tissue level, such as adenosine deaminase, polymorphonuclear elastase, and matrix metalloproteinase-9; and breakdown products of the inflammatory process, such as neopterin, serum M30, and fecal hemoglobin. Table 2 reviews the recent studies exploring these agents in further detail.⁵⁸⁻⁶⁶

IL-17 is particularly interesting as a marker of disease activity because a clinical trial focused on antibody inhibi-

tion of this cytokine has recently been conducted.⁶⁷ The antibody to the IL-17 cytokine proved to be ineffective at controlling disease activity, but the cytokine itself remains an intriguing parameter for assessing inflammation.

Fecal immunohistochemistry testing for fecal hemoglobin merits special attention as well, as it is the only agent among the novel markers for which an immunoassay is commercially available (InSure FIT, Enterix).

Conclusion

It is likely that there is no one marker that will reliably measure disease activity in IBD. However, much work is being done on a multitude of potential markers, which, when combined, would likely be more accurate and sensitive than any one alone. Until then, it is important to acquire a growing familiarity with the potential candidates because, apart from gauging current disease activity, they might also guide treatment avenues in the future. Other potential markers of disease activity to explore include proinflammatory chemokines and cytokines found in serum and tissue using serum Luminex profiling, serum N6:N3 polyunsaturated fatty acid ratio to determine ulcerative colitis disease activity, and neutrophil Fc- κ receptor 1 as an activity marker in pediatric IBD.⁶⁸⁻⁷⁰

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