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Biomarkers of Inflammation in Inflammatory Bowel Disease

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Recent observations suggest that subjective measures of disease activity in inflammatory bowel disease (IBD) are often misleading. Objective measures of inflammation are more closely associated with important long-term outcomes, but often depend upon invasive and costly procedures such as ileocolonoscopy and cross-sectional imaging by computed tomography or magnetic resonance imaging. Noninvasive, accurate, and inexpensive measures of intestinal inflammation would allow clinicians to adopt widely the paradigm of adjusting therapies with a goal of controlling inflammation. Blood, stool, and urine markers have all been explored as indicators of intestinal inflammation in IBD, and although none has been universally adopted, some have been well-characterized, and others hold great promise. Serum C-reactive protein and fecal calprotectin are among the best-studied noninvasive biomarkers of inflammation in IBD, and their test characteristics have been described in the setting of differentiating IBD from irritable bowel syndrome, for grading inflammation, to describe the response to therapy, and in demonstrating recurrent inflammation after medical or surgically induced remission. High-throughput research platforms, including gene expression arrays, metabolomics and proteomics, are also being applied to the discovery of novel biomarkers of inflammation. It is certain that biomarkers of inflammation will attain growing importance in the clinic as we strive for more effective and cost-effective strategies to treat patients with IBD.

Keywords: Calprotectin; C-Reactive Protein; Crohn's Disease; Ulcerative Colitis; Biomarkers.

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are characterized by mucosal inflammation that may flare and remit over time. An important advance in recent years is the understanding that outcomes may be improved by directing treatment to the state of mucosal inflammation rather than to symptoms. It is now recognized that symptoms do not consistently reflect the presence or severity of mucosal inflammation, and evidence suggests that relying upon symptoms to guide therapy will lead to two important errors in management. First, symptoms may be present when mucosal inflammation is not substantially active. This may occur in as many as 20% of patients with CD[1] or UC[2] who have no significant mucosal inflammation, potentially leading to abandonment of effective therapy or unnecessary escalation. Conversely, patients with active inflammation may fail to report symptoms, leading to under-treatment of the disease, with progression to complications in CD, and higher likelihood of symptomatic recurrence in both CD and UC.

With this renewed focus on the state of bowel inflammation as a guide to therapy has come a resurgent interest in biomarkers of inflammation. Ileocolonoscopy and cross-sectional imaging are important tests that can inform the clinician about the current state of disease, such as anatomic extent and location, presence and severity of inflammation, and the occurrence of disease complications. However, while widely accepted as representing the state of bowel inflammation, endoscopy and imaging have notable limitations of cost, inconvenience, and invasiveness, making these modalities unsuitable for frequent monitoring of patients with IBD. Laboratory testing that is rapid, convenient, noninvasive, inexpensive, standardized, reproducible, and accurate in reflecting the state of bowel inflammation in IBD would greatly assist in the management of these patients (Table 1).

Although many noninvasive biomarkers of IBD disease activity have been described (Table 2), primarily blood and stool biomarkers, each has notable limitations with regard to the ideal characteristics described above. None have been fully validated for each of the clinical scenarios for which biomarkers of disease activity are needed. The validity and limitations of inflammation biomarkers used in IBD are summarized below. In addition, a description of investigational approaches to discovering biomarkers follows.

Serum and Blood Markers

The intense bowel inflammation of IBD is accompanied by an acute phase response detectable in the serum and blood. The acute phase response is characterized by

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Table 1. Desirable Attributes of Biomarkers of Inflammation in IBD

Attribute	Rationale
Noninvasive	Increased patient acceptance; superior safety and cost compared to endoscopy or imaging
Convenient	Increased patient acceptance
Rapid	Rapid turnaround time from test to result accelerates clinical decision-making
Inexpensive	Test helps to minimize cost of care. Cost of test should be balanced against benefit in ultimate patient outcomes (cost-effective)
Reproducible	Low variation in the result with repetition of the test using the same process/instrumentation over time
Specificity for the disease	Result is specific to intestinal inflammation, and preferably to IBD, as opposed to other bowel condiitions
Accurate	Test maintains good balance between test performance characteristics of sensitivity and specificity. Note that different thresholds for the same test may be desirable, depending upon the test scenario
Precise	Repeated testing of a sample clusters tightly around the same mean. Note that precision does not imply accuracy, which is the ability of a test to cluster around the true value for the analyte
Standardized	Test results for an analyte are comparable across assays, or can be converted to a single standard
Available	Wide availability increases adoption and utilization
Stable	Analyte is stable for storage and transport after acquistion, without degradation that would interfere with accurate measurement
Dynamic range	Tests with wider dynamic range have the potential to more easily distinguish between different degrees of inflammation
Defined thresholds	Cutoff values that differentiate between states where inflammation is absent or present, or between different grades of inflammation (eg, mild, moderate, severe)
Responsive	Test result changes rapidly in concordance with change in the state of inflammation

IBD, inflammatory bowel disease.

increased elaboration of proteins involved in coagulation and fibrinolysis, such as fibrinogen, plasminogen, Factor VIII, and prothrombin; complement system components such as C1 inhibitor, C1s, C2, C3, C4, C5 and C9; proteinase inhibitors, including α-1-antitrypsin, α1 anti-chymotrypsin; transport proteins such as haptoglobin and ceruloplasmin; and a variety of other proteins such as C-reactive protein (CRP), serum amyloid A (SAA), ferritin, fibronectin and orosomucoid.[3] Other serum proteins, such as albumin, transferrin, α2 macroglobulin and Factor XII, are depressed in the acute phase response.[3] Serum levels of proinflammatory cytokines, many of which stimulate the acute phase response, may also be elevated. These may include TNF-α, interferon-β, transforming growth factor-β, and interleukin (IL)-1β, IL-6, IL-8, IL-12, IL-17, and IL-23.[4] Other potential serum markers include adenosine deaminase,[5] soluble ST2,[6] and tryptophan.[7] Cellular components of blood may also indicate inflammation, as is reflected in elevations of white blood cell and platelet counts.[4] The erythrocyte sedimentation rate (ESR) is an indirect measure of inflammation, largely through an increase in plasma viscosity due to elaboration of acute phase response proteins. However, ESR is also affected by the hematocrit both in anemia and polycythemia, as well as in other diseases and physiologic states such as aging and pregnancy, reducing its accuracy and specificity in IBD.[8]

Few blood or serum markers of inflammation have been extensively validated in IBD, and fewer still are in routine use in the clinic. CRP and ESR are the most widely available and used. CRP has a relatively short half-life of $\sim$19 hours, making it a more responsive indicator of acute inflammation than most other acute phase reactants.[8] Assays vary in their sensitivity and definitions of normal cutoff values, from 0.8 mg/L for highly sensitive assays to 5 mg/L for standard sensitivity assays. However, results in the clinic span from 0.8 to 200 mg/L, providing a wide dynamic range for this test. In the typical acute phase response, hepatocytes produce CRP in response to proinflammatory cytokines, chiefly IL-6, tumor necrosis factor α and IL-1β.[8] More recently it has been recognized that CRP is also expressed in mesenteric adipocytes in patients with CD[9], accounting for the typically higher levels of CRP seen in CD than in UC. Nevertheless, in patients with acute severe UC, elevated CRP is associated with higher likelihood of colectomy[10], perhaps reflecting transmural extension of inflammation normally confined to the mucosa among those with the highest severity of UC. Genetic determinants of CRP expression are also complex[11], and as many as 25% of patients with demonstrable activity of CD on endoscopy do not express levels of CRP above the normal threshold.[12]

Stool Markers

As compared to blood or serum biomarkers, stool markers have the advantage of increased specificity for inflammatory processes localized to the bowel. While some fecal markers, such as α-1-antitrypsin and occult blood, reflect disruption of the mucosal barrier, such markers are not as accurate as others that are associated with the pathogenic inflammatory processes underlying IBD.[13] Products of leukocyte degranulation, such as lysozyme, myeloperoxidase, eosinophilic cationic protein, eosinophilic protein X, lactoferrin, matrix metalloproteinase (MMP)-9, neopterin, and polymorphonuclear elastase, are relatively stable proteins found in the stools of patients with active IBD in higher concentrations than in the stool of healthy normal individuals.[13] In addition, the damage-associated molecular pattern (DAMP) proteins S100A8/S100A9,

Table 2. Biomarkers of inflammation in IBD

Source	Indicator type	Markers
Blood	Acute phase reactants	C-reactive protein
		Erythrocyte sedimentation rate
		α-1-acid glycoprotein (orosomucoid)
		β2-microglobulin
		Sialic acid
		Serum amyloid A
		Ferritin
		Transferrin
		Haptoglobin
		Ceruloplasmin
		α-1-antitrypsin
		Fibrinogen
		Prothrombin
		Plasminogin
		Factor XII
		Serum albumin
		Complement (C1s, C2, C3, C4)
	Cytokine	Interleukin-6
		Interleukin-1β
		Interleukin-8
		Interleukin-10
		Tumor necrosis factor α
	Cellular	Platelet count
		White-blood cell count
	Other inflammatory and cell regulation	Adenosine deaminase
		MicroRNA (miRNA) species
		Calprotectin (S100A8/S100A9)
		Soluble ST2
		Serum tryptophan
Stool	Barrier/epithelial disruption	Fecal occult blood
		Fecal immunochemical test
		α-1-antitrypsin
		M2-pyruvate kinase
	Cytokine	Tumor necrosis factor α
		Interleukin-1β
		Interleukin-4
		Interleukin-10
	Inflammatory	Calprotectin (S100A8/S100A9)
		Lactoferrin (S100A12)
		Lysozyme
		Polymorphonuclear elastase
		Myeloperoxidase
		Metalloproteinase-9
		Neopterin
		Chitinase 3-Like-1
Urine	Inflammation	Neopterin
		Neutrophil gelatinase-associated lipocalin
		α-1-acid-glycoprotein
		Zn-α-2-glycoprotein
		F-2-isoprostanes
		Prostaglandin E metabolite (PGE-M)
		Leukotriene E-4
Breath	Inflammation	Volatile organic compounds ("breath print")

IBD, inflammatory bowel disease.

collectively called calprotectin, and S100A12, are also stable in stool, and are increased in active IBD.[13] Fecal M2-pyruvate kinase[14] and chitinase 3-like-1[15] levels have also been found to increase in relation to disease activity in IBD. Some limitations of fecal biomarkers include patients' disinclination to collect stool, and the lack of specificity of fecal biomarkers for IBD, as opposed to other infectious or inflammatory processes.[16] In addition, it appears that intra-individual variability of some fecal markers, including calprotectin, may be large, even when comparing different stool specimens collected over the course of a day.[17] Calprotectin and lactoferrin have been studied more extensively than other proposed fecal biomarkers, and are readily available in clinical laboratories.

Urine Markers

A small number of substances excreted in urine have been investigated as biomarkers of inflammation in IBD. These include a variety of prostaglandin and leukotriene pathway products,[18–21] neutrophil gelatinase-associated lipocalin,[22] alpha 1-acid-glycoprotein and Zn-alpha 2-glycoprotein,[23] and neopterin.[24] None is extensively validated, and none is in common use in the clinic.

Clinical Utility

Inflammation biomarkers may be useful in a variety of important clinical scenarios (see Figure 1 for applications of biomarkers of inflammation in CD). In the broadest sense, inflammation biomarkers have been used in IBD for 2 main purposes: (1) identifying patients with symptoms of IBD who should be further investigated for a possible IBD diagnosis; and (2) measuring or monitoring disease activity in response to induction or maintenance therapy. The latter purpose includes diverse settings, such as identifying patients who have successfully responded after introducing new therapy, detecting patients who are relapsing while receiving stable therapy, screening for relapse after surgical resection in Crohn's disease, and increasingly for predicting patients likely to experience clinical relapse upon withdrawal of therapy. It is important to recognize that different cutoff thresholds may optimize performance of the same biomarker assay, depending upon the clinical scenario.

Differentiating IBD from IBS

For the majority of patients with chronic abdominal pain or diarrhea, a clinically useful biomarker should balance sensitivity and negative predictive value to screen patients who would benefit from more invasive testing, such as endoscopy and histopathology or imaging, to confirm a diagnosis of IBD, while avoiding these more expensive, intrusive and riskier diagnostics for those who are very unlikely to have IBD.

A recent meta-analysis explored the utility of CRP, ESR, fecal calprotectin (FC) and fecal lactoferrin to exclude IBD in adult patients with irritable bowel syndrome (IBS). Patients with CRP $\leq$0.5 mg/dL or FC $\leq$40 μg/g were found to have a $\leq$1% probability of having IBD. ESR and fecal lactoferrin, in isolation, did not have adequate clinical utility in excluding IBD.[25] An earlier meta-analysis in adults found a pooled sensitivity of 93% and pooled specificity of 96% for FC to

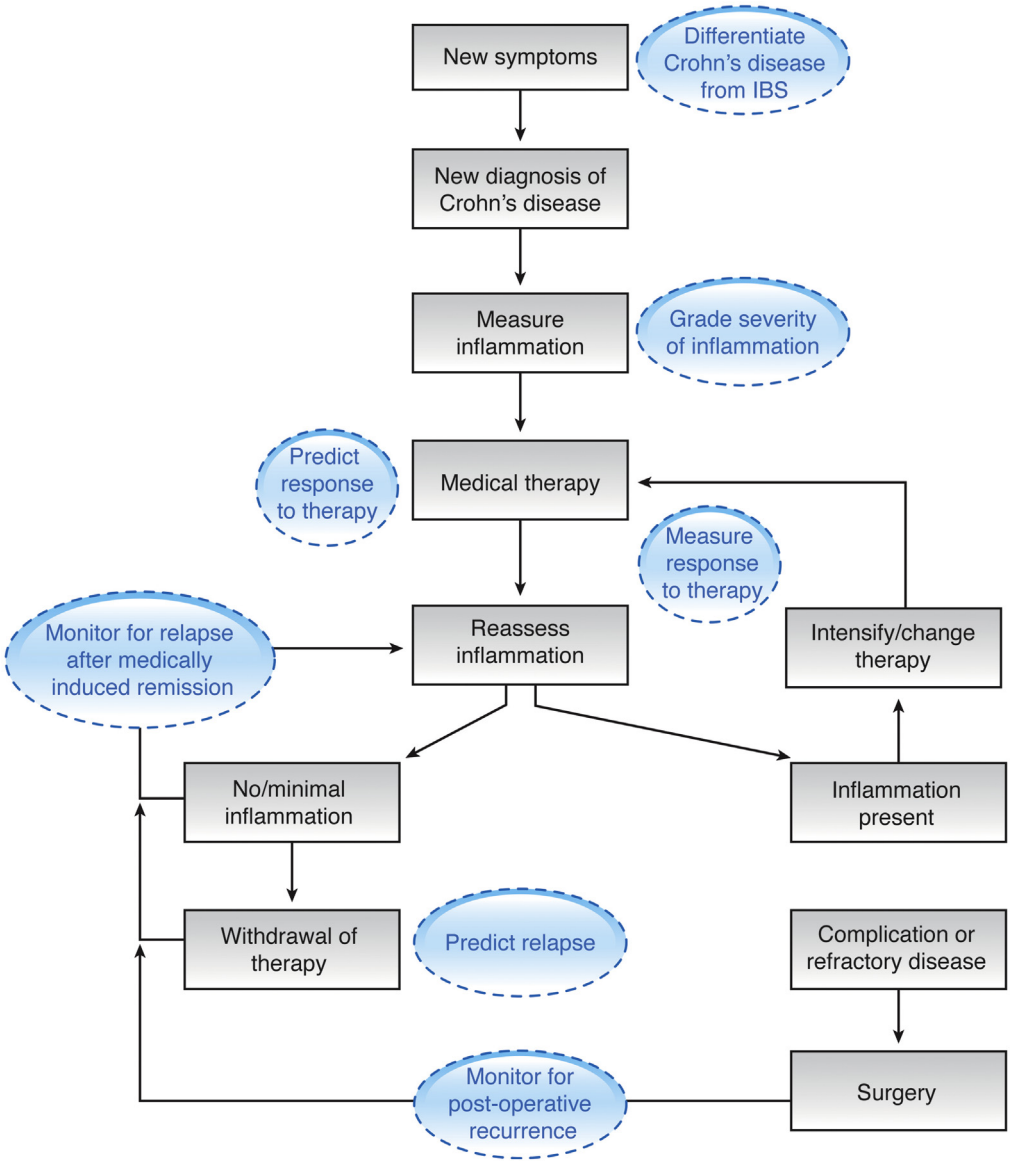

Figure 1. Applications of biomarkers of inflammation in Crohn's disease. Biomarkers of inflammation may be useful at different diagnostic and therapeutic decision points in the care of a patient with Crohn's disease. Descriptions of clinical applications are found in ovals.

diagnose IBD.[26] It should be noted that cutoff values in these studies ranged from 24 to 150 μg/g of stool. However, threshold values may be affected by differences among the commercially available assays used in the studies and in the clinic.[27] Studies point to a lower specificity of FC for IBD in children. The most recent and largest meta-analysis of FC in children found a sensitivity of 98% and specificity of 68% in diagnosing IBD, with positive and negative likelihood ratios of 3.07 and 0.03.[28] Notably, some studies indicate that disease type (CD vs UC) and disease location (colitis vs enteritis)[29] may be associated with distinct levels of FC, of potential relevance to the pediatric cohorts included in the meta-analysis.

Measuring and Monitoring Disease Activity

Once a diagnosis of IBD has been confirmed, biomarkers of inflammation may be useful in clinical situations that require objective assessment of the burden or severity of inflammation. Such scenarios include correctly categorizing disease severity, identifying those patients who have had improvement or resolution of their inflammation, and identifying patients who have had recrudescence of inflammation after medically or surgically induced remission, even in advance of the recurrence of clinical symptoms.

Categorization of Disease Activity

Nearly all studies have found biomarkers of inflammation to have higher correlation with endoscopic disease activity than with clinical disease activity indices focusing on symptoms. This discrepancy is most notable in CD, where the correlation of stool biomarkers of inflammation to endoscopic disease activity, as measured by the Crohn's Disease Endoscopic Index of Severity (CDEIS) or the Simple Endoscopic Severity Index (SES-CD), is considerably higher

than its correlation to the Crohn's Disease Activity Index (CDAI).[30,31] Patients with quiescent CD (CDAI <150) are observed to have wide variability in FC concentrations, underscoring the mismatch of symptoms and inflammation.[32] FC was highly correlated with both severity and extent of CD as measured by radiolabelled white cell scans, whereas disease activity as measured by the CDAI was not.[33] Some studies note a correlation between CRP and endoscopic severity in CD, although the association has not been as robust as with fecal markers.[29] In one study, FC levels measured by a point-of-care assay device had better correlation with the endoscopic activity measured by the CDEIS than with the leukocyte count, platelet count or CRP.[34] A threshold value of 272 μg/g was optimal for endoscopic remission (CDEIS <3), with area under the curve (AUC) 0.933, and with performance similar to ELISA-based FC assay with cutoff of 274 μg/g and AUC 0.925.[34] However, FC levels correlated best with endoscopic activity when disease was found in colon and ileum (Pearson's correlation $r = 0.88$) or colon alone ($r = 0.73$), as opposed to ileum alone ($r = 0.44$).[34] Still, fecal markers hold promise for the detection of ileal recurrence after small bowel resection, as detailed below.

Two cross-sectional studies by Schoepfer et al. compared CRP, FC and symptom-based indices to endoscopic scores of severity in UC and CD.[35,36] In ulcerative colitis, FC could accurately discriminate between patients with mild, moderate and severe endoscopic appearance.[35] Ranges for FC were identified in association with the modified Baron score of endoscopic severity for UC, with grade 0 associated with median [interquartile range] of 16 [10-30] μg/g, grade 1 with 35 [25-48] μg/g, grade 2 with 102 [44-159] μg/g, grade 3 with 235 [176-319] μg/g, and grade 4 with 611 [406-868] μg/g ($P < .001$ for discriminating the different grades).[35] In the same study, a cutoff of ≥57 μg/g of stool optimized the sensitivity and specificity at 91% and 90%, respectively to identify endoscopically active UC.[35] Similarly, FC discriminated groups of patients with CD with inactive endoscopic activity from those with mild activity (mean 104 ± 138 vs 231 ± 244 μg/g, $P < .001$), mild from moderate activity (23 ± 244 vs 395 ± 256 μg/g, $P = .008$), and moderate from high activity (395 ± 256 vs 718 ± 320 μg/g, $P < .001$). Applying a cutoff of ≥70 μg/g, FC had 87% accuracy for the detection of endoscopically active disease.[36] By contrast, clinical disease activity inidices (Lichtiger score for UC or CDAI), CRP, blood count parameters (leukocyte count in CD and UC, hemoglobin or platelet count in UC) were unable to accurately distinguish between grades of endoscopic activity, despite moderate correlations for each individual parameter.[35,36]

In addition, FC may also identify patients with UC who have ongoing histologic inflammation despite endoscopic remission. One study of 59 patients with clinically inactive UC and Mayo endoscopic subscore of 0 or 1 found that 30.5% had active inflammation on histology, associated with a significantly higher median FC (278 μg/g, IQR 136-696 μg/g) as compared to those with no histologic inflammation (68 μg/g, IQR 20-172 μg/g; $P = .002$).[37] A cut-off of 155 μg/g optimized the test characteristics, with sensitivity of 78% and specificity of 71% for histologic inflammation.[37]

It should be noted that considerable overlap in FC ranges have been observed in UC, and in CD to an even greater extent, over the levels of endoscopic disease severity. Differences in disease location in CD may account for some of the inter-individual variation in FC. However, FC and other stool biomarkers have been found to have low sensitivity in detecting isolated small bowel involvement seen on wireless capsule endoscopy.[38] In UC, some studies[39], but surprisingly not most, have found FC to correlate to extent of disease in addition to the severity of inflammation. In CD, ileocolonoscopy may fail to fully register significant inflammatory disease in the small bowel[40], which may therefore account for higher than expected FC when compared to the endoscopic appearance.

One study demonstrated that a single score for disease activity that combined evaluation by ileocolonoscopy and CT enterography (CTE) optimized the specificity of a panel of biomarkers of inflammation.[41] A combination of FC, serum MMP9 and serum IL-22, from among a large panel of known inflammation biomarkers, provided the highest correlation to inflammatory activity as measured by ileocolonoscopy-CTE score ($r = 0.699$) in a linear regression model, with the greatest contribution to the variance from FC.[41] The same study found that the Mayo endoscopic subscore was best described by a combination of FC and MMP9.[41]

Response to treatment

Cross-sectional studies categorizing groups of patients with different levels of disease activity do not prove that biomarkers such as FC will accurately track improvement in disease activity, either reduction or resolution of inflammation, for an individual patient over time with treatment. Similarly, the ability of biomarkers to detect recurrence of inflammation after resolution has been attained through medical or surgical means requires prospective study. Biomarkers capable of accurately and noninvasively determining the efficacy of treatment would be beneficial in both clinical trials and clinical practice. While studies show the promise of biomarkers in tracing individual disease activity in response to therapy, no single biomarker has gained wide acceptance.

A small study in children with IBD with active inflammation seen at colonoscopy showed that FC decreased in the first 2 months of treatment with glucocorticoids.[42] Only the minority achieved levels below the upper limit of normal (100 μg/g), even when the clinical response was considered to be good. Most experienced a rise in FC with cessation of glucocorticoid therapy.[42]

Another study of 19 adults with CD compared changes in fecal lactoferrin and calprotectin to endoscopic severity scored by SES-CD before and 4 to 6 months after initiation of new therapy for active disease.[43] A variety of treatments were administered, including metronidazole, 5-aminosalicylates, thiopurines, methotrexate and/or topical or systemic glucocorticoids. The authors defined

an endoscopic response as SES-CD $\leq$2, or a decrease by at least 2 grades in severity (ie, severe to mild or inactive, or moderate to inactive), and partial endoscopic response as 1 grade change. Median changes in FC and lactoferrin were significantly greater among responders or partial responders than among nonresponders. However, there was wide variation in the baseline values of both fecal markers and the magnitude of change.[43] These results, although in part reflecting the inconsistent therapeutic outcomes of the diverse treatments administered, underscore the challenges in defining clinically meaningful changes in biomarkers of inflammation.

A study of patients with UC in clinical remission despite elevated FC on mesalamine therapy demonstrated that dose intensification of mesalamine results in significant decreases in FC.[44] At baseline, all patients had FC >50 μg/g while on <3 g/day mesalamine. Patients were randomized to continue multimatrix mesalamine 2.4 g/day or to 4.8 g/day for 6 weeks. The primary outcome of continued clinical remission and FC <50 μg/g at 6 weeks was achieved in 26.9% of the dose-escalated patients but only 3.8% of controls ($P = .05$).[44] Significantly more patients in the dose-escalated group achieved FC <100 μg/g or <200 μg/g ($P = .04$ and $P < .005$, respectively). Clinical relapse rates did not differ over the short duration of the study, however these results indicate a dose-response relationship with regard to FC as marker of inflammation in UC under treatment with mesalamine. Patients with baseline FC $\geq$200 μg/g were also noted to have increased risk of relapse.[44]

A prospective cohort study of 53 patients with UC undergoing induction therapy with infliximab 5 mg/kg at weeks 0, 2 and 6 had FC at baseline and weekly, with colonoscopy performed at entry, weeks 6 and 10.[45] A significant decrease from baseline median FC concentration of 1260 μg/g (IQR 278.5- 3418) was seen at week 10 (72.5 μg/g, IQR 18.5 - 463; $P < .001$).[45] Overall, 58% of patients achieved both endoscopic remission and a decrease in calprotectin to <50 μg/g or at least an 80% decrease from baseline.[45] Patients who had an endoscopic remission at week 10 were more likely to have a sharp decrease in FC concentration at week 2.[45]

Response to treatment with anti-TNF antibodies is also associated with a decrease in CRP in CD. Significant reductions in baseline CRP were seen at week 26 under treatment with adalimumab in the CHARM study, and the association continued through week 56.[46] In the ACCENT I study of infliximab for CD, among patients with an elevated CRP (>8.0 mg/L) at baseline, there was a higher likelihood of durable sustained response over 54 weeks among patients who achieved a reduction in CRP. The optimal cut-off was a $\geq$60% reduction from baseline CRP at week 14, yielding an AUC of 0.75, which was equal to that achieved for a trough serum infliximab level $\geq$3.5 μg/mL, and an odds ratio for durable sustained response of 7.3 (95% confidence interval [CI], 1.4–36.7).[47] In the SONIC study, 42% of the patients who achieved clinical remission (CDAI <150) with azathioprine, infliximab, or both agents, also achieve normal CRP values and endoscopic healing (no mucosal ulceration) at week 26.[1] Response to treatment with certolizumab pegol was associated with a reduction in CRP in responders but not nonresponders.[48] In randomized controlled trials of natalizumab (anti-α4 integrin antibody) and vedolizumab (anti-$\alpha4\beta7$ integrin antibody) in CD, modest decreases in median CRP occurred with treatment.[49,50] Similarly, mongersen, an oral SMAD7 antisense oligonucleotide, demonstrated large treatment effect size for clinical remission with the two highest dose groups as compared to placebo, but failed to demonstrate a reduction in median CRP among patients with elevated CRP at baseline.[51]

Predicting Clinical Relapse

An early study followed 38 patients with CD and 41 with UC in clinical remission for 12 months after collection of FC.[52] Using multivariate regression to adjust for possible confounders, patients with a FC >150 mg/g had an increased risk of symptomatic relapse that was 14 times greater in UC but only 2 times greater in CD.[52] This work suggested that a strategy of measuring FC to predict clinical relapse would be more effective in UC than in CD.

A subsequent study followed 97 patients with UC and 65 with CD who had no symptoms of active disease at baseline for 1 year after a stool collection.[53] Calprotectin greater than 130 μg/g was considered positive, and at this cutoff, the AUC for the receiver-operator curve (AUROC) was 0.700 for relapse of UC (P =.001) and 0.649 for relapse of CD (P =.056).[53] However, a positive FC was significantly correlated with relapse in patients with colonic CD.[53]

A study measured FC at baseline in 73 children (32 CD, 41 UC) with histologically documented remission, and at the time of a second colonoscopy in follow up for a median of 36 months.[54] FC >275 μg/g was found to have optimal performance characteristics for identifying patients with histologic activity in the overall IBD population, with sensitivity of 97%, specificity 85%, positive predictive value 85%, negative predictive value 97% and likelihood ratio of 6.4 for histologic relapse.[54] However, the likelihood ratio was far greater for UC at 21.7 than for CD at 3.4,[54] indicating better utility for UC than for CD. Further analysis indicated that a cutoff of 462 μg/g had better performance in CD, with 100% sensitivity, 89% specificity, 90% positive predictive value, 100% negative predictive value and likelihood ratio of 9 for histologic relapse.[54] This finding is further corroborated in a second study where 89% of children with CD and FC levels <400 μg/g remained in remission over a median of 9 months of follow up.[55]

A fourth study in children with IBD in clinical remission for at least 3 months (41 with UC, 26 with CD, of whom 3 had isolated ileal disease, and 5 with indeterminate colitis) examined the risk of clinical relapse over a year of follow up.[56] While the positive predictive value of FC for symptomatic relapse was low (39.6% for FC >100 μg/g and 42.9% for levels >1000 mg/g), the negative predictive value for FC <100 mg/g was 75%.[56]

A prospective study of adults with IBD in clinical remission for at least 6 months looked at risk for clinical relapse within 12 months of stool collection for calprotectin

and lactoferrin.[57] Relapse occurred in 16% of the 163 patients (74 with UC and 89 with CD).[57] Relapse risk was significantly higher among those with FC >150 μg/g (30% vs 7.8%, $P<.001$), or with a qualitatively positive fecal lactoferrin (25% vs 10%, $P<.05$).[57] AUROC for prediction of relapse using FC was 0.69 for UC and 0.77 for CD.[57] For prediction of relapse in UC, the sensitivity of fecal lactoferrin was 46% and specificity was 61%, while in CD, these values were 77% and 68%, respectively.[57] However, testing was optimized when considering only colonic CD or relapse within 3 months of the test.[57]

Another study of 135 adult patients with IBD (66 with CD and 69 with UC) in clinical remission for at least 3 months examined baseline FC as a predictor of clinical relapse.[58] Patients with CD who had FC >200 μg/g had a four-fold higher likelihood of relapse over a year than those with lower concentrations, although the accuracy was less in patients with ileal disease.[58] In UC patients, those with FC >120 μg/g were 6 times more likely to have clinical relapse.[58] Confining the analysis to patients with UC and colonic CD, a cutoff of 120 μg/g had sensitivity of 80% and specificity of 60% to predict relapse.[58]

A prospective study of 53 patients with CD in clinical remission measured FC and CRP at entry.[59] Optimal cutoff values for prediction of relapse were identified by ROC for FC (340 μg/g) and CRP (9 mg/dL), with sensitivities of 80% and 70%, specificities of 90.7% and 81.4%, and AUROC 0.914 and 0.759 for FC and CRP, respectively.[59] FC >340 μg/g was associated with an 18-fold increase in risk of relapse, and was significantly more accurate than CRP in predicting relapse.[59]

One study examined the ability of FC and CRP to predict clinical relapse after clinical remission was induced by infliximab. In a study of 50 patients in clinical remission and off corticosteroids at week 14 after a standard 3-dose induction regimen, FC and CRP levels decreased dramatically among those who remitted, but week 14 values of these tests failed to predict relapse by 1 year.[60] Confining the analysis to patients with colonic CD did not improve the accuracy of either test.[60]

In contrast to these results, data from the STORI cohort study suggest the value of biomarkers of inflammation to predict relapse upon withdrawal of infliximab in the setting of CD in clinical remission on combination therapy of infliximab plus an immunomodulator.[61] Serum calprotectin and FC, as well as hsCRP, were measured prior to withdrawal of infliximab. All 3 were independently associated with subsequent relapse, with thresholds of 5675 ng/mL for serum calprotectin, 250 μg/g for FC and 5 mg/L for hsCRP.[61]

A prospective cohort of 80 patients with UC in remission for at least 3 months on mesalamine had stool collected for calprotectin and lactoferrin and were followed for a year.[62] Using an optimized cutoff of 170 μg/g for calprotectin and 140 μg/g for lactoferrin, the sensitivity and specificity of calprotectin was found to be superior, at 76% and 76%, compared to 67% and 68% for lactoferrin.[62] FC was independently associated with risk for relapse, in contrast to lactoferrin.[62]

A multicenter prospective cohort study explored monthly measurement of FC as a strategy to predict relapse in 87 patients with UC in remission on infliximab maintenance treatment.[63] By the end of the study, 34.4% had sustained deep remission (partial Mayo score <3 at all times, and endoscopic subscore of 0 at week 52), while 14.9% relapsed (endoscopic subscore ≥2 or need for change of treatment by week 52).[63] FC was observed to increase 3 months before flare to a median of 300 μg/g, and with sensitivity and specificity of 58.3% and 93.3%.[63] Two FC concentrations of ≥300 μg/g measured 1 month apart predicted relapse best, with sensitivity of 61.5% and specificity heightened to 100%.[63] These results demonstrate the potential for regular monitoring of FC to identify individuals at risk for symptomatic flare of UC.

Mao and colleagues performed a meta-analysis of FC in predicting relapse of quiescent IBD. Pooling data from 6 studies, the sensitivity and specificity to predict relapse were 78% (95% CI, 72–83) and 73% (95% CI, 68–77), with AUROC 0.83, and diagnostic odds ratio 10.31 (95% CI, 5.05–21.06).[64] Performance was similar in UC and CD. FC seemed to have improved accuracy in colonic or ileocolonic CD, although analysis was limited by low numbers of patients with ileal disease.[64]

Predicting Relapse in CD After Surgical Resection

Surgical resection of ileal or ileocolonic CD temporarily eliminates bowel inflammation, but is associated with a high risk of recurrence. Natural history studies demonstrate variable but high rates of, first, endoscopic recurrence in the neoterminal ileum, followed by recurrence of clinical symptoms. However, clinical symptoms do not accurately reflect recurrent inflammation visible at endoscopy[65], and the onset of symptoms may be delayed by 1 or more years after recurrence of inflammation. Therefore, an opportunity for early intervention may be missed if early detection of recurrent bowel inflammation relies upon symptoms. To forestall this unfavorable natural history, it has become common practice to perform colonoscopy at 6 to 12 months after resection to grade endoscopic recurrence. However, repetitive colonoscopy to monitor for recurrence is inconvenient, expensive, and bears some risks. It is highly desirable, therefore, to develop a strategy of monitoring for recurrence of CD using noninvasive markers of inflammation.

Blood markers may be insufficiently sensitive and specific for postoperative monitoring for recurrence of CD. CRP and ESR, along with clinical symptoms as measured in the CDAI, are not associated with endoscopic recurrence after surgery.[65] Fecal markers of inflammation, however, hold greater promise. Longitudinal measurement of FC and fecal lactoferrin demonstrates normalization of abnormal preoperative values within 1 to 2 months after ileal or ileocolonic resection.[66] Consistent with the observation that endoscopic recurrence after intestinal resection is often not symptomatic, many patients in clinical remission are found to have increased levels of fecal lactoferrin or calprotectin.[66,67] In

one study of 39 patients, an optimal cutoff for FC of >200 μg/mL was identified for endoscopic recurrence at 1 year after surgery, with sensitivity of 63% and specificity of 75%.[68] This approach had better sensitivity than ultrasound performed at 3 months postoperatively, which had sensitivity of only 26%, but specificity of 90%.[68] The authors suggested that colonoscopy should be performed in patients with FC >200 μg/mg when ultrasound at 3 months is negative, although the performance characteristics of this sequential testing strategy were not reported.[68]

In another study, a point-of-care device was used to measure FC, and compared to FC measured by ELISA, after surgical resection in 29 patients who underwent ileocolonoscopy to evaluate recurrence. Patients with no significant endoscopic recurrence (Rutgeerts score i0-1) had mean FC by point-of-care determination of 98 μg/g (range 30-306) as compared to 234.5 μg/g (range 100-612) for those with i2-4 (P =.012). Applying a threshold of 283 μg/g had sensitivity and specificity of 67% and 72%, respectively, and an AUC of 71.5, similar to the performance of the optimal ELISA-based FC (AUC 70.1) with a cutoff of 203 μg/g, with sensitivity and specificity of 75% and 72%.[34]

A larger prospective cohort study examined the performance characteristics of FC and highly sensitive CRP (hsCRP) in detecting endoscopic recurrence (Rutgeerts score i2,3,4).[69] All 86 patients had undergone ileocolonoscopy within 18 months (median 7 months) after ileal or ileocolonic resection for CD, and were asymptomatic. FC had a strong correlation with Rutgeerts score (r = 0.65, P<.0001), whereas hsCRP had a weaker correlation with Rutgeerts score (r = 0.34, P =.0016).[69] As defined by ROC curves, optimal cutoffs to detect postoperative recurrence were defined as FC 100 μg/g (AUC = 0.86) and hsCRP 1 mg/l for (AUC <0.70), but the overall accuracy was greater for FC than for hsCRP (77% vs 53%, respectively).[69] The sensitivity, specificity, positive and negative predictive values for FC >100 μg/g were 95%, 54%, 69%, and 93%, respectively.[69] Based upon the excellent negative predictive value at this threshold, applying a cutoff of 100 μg/g would have allowed 30% of patients to avoid colonoscopy in the setting of no endoscopic recurrence.[69]

The Post-Operative Crohn's Endoscopic Recurrence (POCER) study was a randomized controlled trial comparing early colonoscopy at 6 months with treatment escalation for endoscopic recurrence to a strategy of no colonoscopy in patients who had surgical resection of active CD, with a primary outcome of endoscopic recurrence at 18 months.[70] This study afforded an exploration of FC and serum CRP as indicators of endoscopic recurrence (Rutgeerts score ≥i2).[71] FC was significantly higher among patients with endoscopic recurrence (275 μg/mg vs 72 μg/mg, P<.001), and FC >100 μg/mg had sensitivity and specificity of 89% and 58%, respectively, and a negative predictive value of 91%, with an AUROC of 0.763.[71] By contrast, CRP and CDAI had AUROC of only 0.568 and 0.541, respectively.[71] The authors concluded that avoiding colonoscopy for patients with FC ≤100 μg/mg could have prevented unnecessary colonoscopy in 47% of patients.[71] In addition, the effect of escalated treatment based upon endoscopic recurrence at 6 months could be demonstrated, with a drop in mean FC from 324 μg/mg at 6 months to 180 μg/g at 12 months and 109 μg/mg at 18 months.[71]

In summary, the best evidence suggests that FC, rather than recurrent symptoms or elevation of CRP, may be best utilized as part of a strategy to monitor for postoperative recurrence, with values ≤100 μg/mg strongly suggesting no recurrent disease, and no need to evaluate for recurrence by ileocolonoscopy.

Future Approaches

The search continues for more specific, sensitive, and responsive markers of inflammation to assist in the management of IBD. This effort has been accelerated by the recent availability of high-throughput discovery platforms, capable of measuring thousands of analytes simultaneously. These include gene expression arrays, and metabolomic and proteomic platforms.

RNA as a Biomarker in IBD

For the most part, studies of the IBD transcriptome have focused on the pathogenesis of IBD and on differentiation between CD and UC. A small study of the transcriptome of colonocytes from patients with UC was able to differentiate actively inflamed UC mucosa from mucosa from normal control tissue or quiescent UC.[72] One recent study by Burakoff et al[73] performed gene expression arrays using mRNA from whole blood from a small number of patients with CD and UC categorized by severity of disease as noted from bowel histopathology, and from controls.[73] Using logistic regression in a combinatorial approach, data were evaluated to determine AUROC of panels of 6 individual genes expressed differentially in each category of disease and severity. Through this approach, both diseases and both levels of severity could be distinguished with AUROCs at 0.89 and higher.[73] Validation in a larger, independent data set will be required before these findings may be useful in the clinic.

Other studies have focused on microRNAs (miRNA), small, short (18–25 nucleotides), noncoding, single-stranded RNA species. The role of miRNA in IBD is incompletely understood, but their ability to interact with messenger RNA and silence expression makes them key regulators of a variety of cellular processes. In one study, colonic biopsies were taken in patients with UC and RNA was isolated and run on an miRNA gene expression array. Among the miRNA species found to be up-regulated in silico and then validated by quantitative PCR, increased expression of miR-20b and miR-98 were noted to be associated with active UC, while increased expression of miR-125b-1* and let-7e* were associated with quiescent UC.[74]

In addition to playing important roles within the cell, some miRNA species may be found in circulation, and are stable, making them potentially attractive as blood biomarkers. In a study comparing circulating miRNA species in children with CD to healthy controls and children with celiac disease, 24 miRNA species were found to be elevated.[75] Eleven of these were further investigated and confirmed in an independent set of samples.[75] In addition to good

sensitivity and specificity for a diagnosis of CD, these disease-associated miRNA species decreased after 6 months of treatment, suggesting that they may be useful as noninvasive biomarkers of inflammation.[75] A second study has found other miRNA species up- or down-regulated in active vs inactive CD or UC.[76]

Metabolomics

Metabolic profiling holds promise as a means of differentiating IBD from other diagnoses and CD from UC, and potentially to measure inflammation. A variety of methods, including NMR-spectroscopy, liquid chromatography-mass spectrometry, gas chromatography, and selective ion flow tube mass spectroscopy, have been applied to biospecimens that include colonic biopsies, urine, and stool.[77–81] More recently, volatile organic compounds have been measured in breath, with early results indicating a unique "breathprint" in children with IBD.[82]

Proteomics

Protein profiles in serum, plasma, or tissue may also be distinct in IBD. Various techniques have been applied to separate and identify protein species. These include two-dimensional gel electrophoresis, liquid chromatography, isobaric tags for relative and absolute quantification (iTRAQ), tandem mass spectrometry (MS/MS), and peptide mass fingerprinting using surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).[83,84] Pilot studies of proteomic profiling suggest these approaches may be useful in identifying serum proteins that differentiate IBD from non-IBD patients, and potentially also response to therapy.[85,86]

Conclusions

Driven by a significant need in the clinic for accurate and noninvasive means to measure intestinal inflammation objectively, biomarkers of inflammation have been the subject of intense investigation in IBD. Increasingly, blood and stool markers such as CRP and calprotectin are being used in practice as measures of intestinal inflammation, with a growing understanding of their utility and limitations. Further studies are needed to improve understanding of these markers as surrogates for the course of disease, and to identify new biomarkers with still better test characteristics, reduced cost and improved convenience.

Supplementary Material

NOTE: The first 50 references associated with this article are available below in print. The remaining references accompanying this article are available online only with the electronic version of this article. To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.07.003.

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