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Biomarkers in Inflammatory Bowel Disease: Current Practices and Recent Advances

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Abstract

Crohn's disease and ulcerative colitis represent the two main forms of the idiopathic chronic inflammatory bowel diseases (IBD). Currently available blood and stool based biomarkers provide reproducible, quantitative tools which can complement clinical assessment to aid clinicians in IBD diagnosis and management. C-reactive protein and fecal based leukocyte markers can help the clinician distinguish IBD from non-inflammatory diarrhea and assess disease activity. The ability to differentiate between forms of IBD and predict risk for disease complications is specific to serologic tests including antibodies against *Saccharomyces cerevisiae* and perinuclear antineutrophil cytoplasmic proteins. Advances in genomic, proteomic and metabolomic array based technologies are facilitating the development of new biomarkers for IBD. The discovery of novel biomarkers which can correlate with mucosal healing or predict long term disease course has the potential to significantly improve patient care. This article reviews the uses and limitations of currently available biomarkers and highlights recent advances in IBD biomarker discovery.

Keywords

Ulcerative colitis; Crohn's Disease; IBD; Biomarker; Tryptophan; C-reactive protein; ASCA; Calprotectin; indoleamine

INTRODUCTION

The two major forms of the chronic inflammatory bowel diseases (IBD) are Crohn's disease and ulcerative colitis. Together these diseases affect over a million individuals in the United States with prevalence rates close to 200 per 100,000 in some populations.(1) Healthcare utilization by patients with IBD is high and disease associated morbidity is significant for those with progressive and difficult to manage disease.(2) While the etiology remains idiopathic, evidence suggests that the ongoing inflammation in IBD results from persistent overly aggressive inflammatory responses to a subset of commensal microorganisms in a genetically susceptible host with exposure to environmental triggers.(3–5) Genetic, clinical and animal model studies have revealed important roles in disease pathophysiology for host-

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microbe interactions, autophagy and several components of both the innate and adaptive immune systems.(3)

Crohn's disease (CD) is characterized by discontinuous regions of intestinal inflammation most frequently involving the terminal ileum and colon, but can affect any part of the gastrointestinal tract from mouth to anus. Abdominal pain, weight loss and variable degrees of diarrhea are symptoms of CD. The inflammatory process of CD is transmural in nature and, as a result, potential disease complications include intestinal fibrosis, strictures and fistula formation. The inflammatory process of ulcerative colitis (UC) is limited to the mucosa and submucosa of the colon alone with disease almost invariably involving the rectum. Diarrhea, hematochezia, tenesmus and defecatory urgency are classic symptoms of active UC. Extraintestinal manifestations of IBD which may affect joints, eyes and/or the skin occur in up to 25% of patients. Disease activity is typically relapsing and remitting in both UC and CD. The disease course of CD is typically progressive. Persistent disease activity despite medical therapy or the development of disease complications frequently requires surgical intervention.

Frequent evaluative testing, often invasive, is routine for diagnosis and ongoing care of patients with IBD. The diagnosis of either CD or UC is based on clinical symptoms combined with typical findings on endoscopy, radiology and ultimately pathology. IBD care presents challenges to the most experienced clinicians in initial diagnosis, risk stratification for disease progression and therapy. In clinical trials, selection of the optimal patient population and monitoring their disease activity present additional challenges. Ongoing debate exists as to the ideal testing strategies to utilize for each of these tasks.

Biomarkers are currently used in conjunction with routine testing in clinical care of patients with IBD. Examined roles for biomarkers in IBD include support in making the IBD diagnosis, differentiation between CD and UC, determination of disease activity, risk stratification of patients for severity of disease course and in prediction of response to therapy. Most biomarkers used are not disease specific, but reflect generalized inflammation. The last decade has brought significant gains in insight to IBD genetics and pathogenesis. These insights have the potential to improve upon the utility of biomarkers currently in use. Based on the available literature, an algorithm for biomarker use in clinical care of IBD patients is depicted in figure 1. Development of an expanded repertoire of biomarkers for use in all aspects of clinical care of IBD patients and in the research of new therapies is needed. This review will summarize biomarkers currently used in clinical care, and highlight recent advances in field of IBD biomarker discovery.

HISTORICAL MEASUREMENT TOOLS OF IBD ACTIVITY

Methods of measuring disease activity in clinical trials have traditionally included patient symptoms, endoscopic appearance and basic laboratory markers. In CD, the Crohn's disease activity index (CDAI) has been in use for greater than 30 years. Both subjective and objective markers of disease are incorporated into the CDAI including diarrhea, abdominal pain, general well-being, extra-intestinal manifestations, anti-diarrheal use, abdominal mass, hematocrit, and weight.(6) Symptoms are assessed over 7 days. The Harvey-Bradshaw index was developed after the CDAI, and includes only 5 of the 8 clinical parameters above (hematocrit, weight, and anti-diarrheal use are excluded). The HBI is measured over the last 24 hours prior to assessment and is a simpler method of CD activity assessment.(7) While there have been multiple indices developed for assessment of UC severity, the Mayo score and the Ulcerative Colitis Disease Activity Index (UCDAI) are the most commonly used. These two scoring systems have four components: stool frequency, rectal bleeding, endoscopic disease assessment, and the physician's global assessment or rating of disease

activity. The Mayo score also includes a patient's functional assessment that is incorporated into the physician's global assessment.(8) Various clinical trials have modified the definitions of clinical remission or response, while retaining the basic components of the Mayo score.(8) Health-related quality of life scales such as the inflammatory bowel disease questionnaire (IBDQ) and short from of the IBDQ (SIBDQ) have also been validated as measurements of bowel and systemic symptoms, as well as emotional and social functions for CD and UC.(9)

The use of clinical parameters alone to judge IBD disease activity can present challenges. Irritable bowel syndrome (IBS) type symptoms can sometimes confound study results which are been based on clinical disease indices alone. IBS type symptoms may account for the high placebo responses sometimes seen with CDAI use in trials.(10, 11) These high placebo rates lead to difficulty in demonstrating treatment efficacy, as evidenced by a recent trial of certolizumab in CD, where the use of biomarkers as objective measures of disease activity may have enhanced the ability to reach statistical significance.(12) In UC, mucosal healing can be assessed by flexible sigmoidoscopy; however, this test is invasive and adds significant cost to care. For both CD and UC, the indices currently in use are limited in their ability to predict long-term outcomes including surgery, hospitalization, relapse, and disability.(13–15) Thus, the inadequacies of currently used clinical indices of IBD activity highlight the need for biomarkers which can assist in providing objective assessments of treatment effects.

SERUM MARKERS OF ACUTE PHASE RESPONSE

C-Reactive Protein

C-reactive protein (CRP) is a protein produced by the liver in response to a variety of acute and chronic inflammatory conditions. Cytokines associated with active IBD [interleukin (IL)-6, tumor necrosis factor alpha (TNFα) and IL-1β] stimulate increased production of CRP by hepatocytes over baseline levels which are typically less than 1mg/l.(16) During active IBD, levels can range from 5–200 mg/l depending on disease severity and the individual's capacity to produce CRP. Such elevations of CRP are not specific to IBD as levels are also increased in various viral and bacterial infections, other autoimmune disorders, malignancy and other disorders resulting in tissue necrosis.(17) Positive features for CRP as a biomarker include that it is easily and reliably measured across diagnostic laboratories and has a short plasma half-life of ~19 hours which is determined by synthesis rather than degradation.(18)

Heterogeneity exists between individuals' immune responses and elevations in CRP are more common in CD than in UC.(19) Potential explanations for this difference include the greater depth of inflammation and higher serum IL-6 found in CD verses UC.(20, 21) While most patients with active CD mount a CRP response, the level of CRP elevation varies both by individual and perhaps disease location. Florin et al found that ~10% of examined patients with active CD by clinical assessment were found to have a CRP of <10.(22) The phenotype of isolated ileal disease and low body mass index were found to be common in the low CRP producing group. However, analysis of a separate patient cohort did not confirm significance of this finding.(23) Gene polymorphisms have also been reported as an explanation for differences in CRP levels.(24)

Erythrocyte Sedimentation Rate

The rate at which red blood cells (RBC) migrate through the plasma over the period of 1 hour is termed ESR. ESR provides a crude, but rapid, assessment of the general acute phase response. When inflammation is present, pro-sedimentation factors, namely fibrinogen, cause RBCs to stick together and settle faster. Several factors influence the ESR including

age, gender, anemia, blood dyscrasias and pregnancy.(25) Compared to CRP, the ESR peaks less rapidly and resolves more slowly in response to changes in inflammation, and has a smaller degree of change making it less suited to following changes in disease activity. Nonetheless, ESR has been and remains widely used as a biomarker of IBD activity.

Other Laboratory Markers

Separate from their hemostatic function, platelets have a recognized role in inflammatory processes.(26) Their relationship to IBD pathophysiology remains an area of active investigation.(27, 28) In active IBD, the platelet count may be elevated while the mean platelet volume is low. This finding led one group to suggest platelet count as useful in distinguishing IBD from infectious diarrhea.(29) While the normal level varies considerably, in practice the platelet count is routinely available on patients and, if elevated, may alert the clinician to ongoing inflammation.

Other serum based acute phase response tests are less useful or untested as biomarkers in IBD. The white blood cell count may be elevated though it is non-specific and may be influenced by therapies such as glucocorticoids. Serum albumin may be low with acute inflammation, but is also affected by nutritional status. Orosomucoid is another hepatocyte derived acute phase protein that has been shown to correlate with IBD activity(30); however, the long half-life (5 days) has limited its utility in practice. We will focus on the clinical utility of CRP, which is the most studied acute phase reactant in IBD.

CRP in the Differentiation of IBD from non-inflammatory GI conditions

For most individuals, after abscess and infection are excluded, CRP has value as a biomarker in distinguishing active CD from functional bowel disorders.(31) Serum CRP has a lower sensitivity in UC, thus it cannot be relied upon solely to exclude the diagnosis of this disorder.(32)

CRP in Evaluating Patients with known IBD

CRP has the ability to distinguish active from quiescent IBD and trends with mucosal healing. A retrospective analysis of Mayo Clinic data showed moderate or greater clinical disease severity (OR 4.5), active disease on colonoscopy (OR 3.5) and severe inflammation on histology (OR 10.6) to associate with an elevated CRP.(33) In the same study, 51% of UC patients with active disease by colonoscopy had an elevated CRP. In a follow up investigation from the same group, quantitative assessment by CT-enterography correlated with CRP when perienteric inflammation was included, but not inflammation limited to the small bowel wall.(34) In patients with symptoms of active CD and an elevated CRP, 86% exhibited evidence of inflammation at colonoscopy.(33) This suggests that CRP is additive to clinical assessment in the ability to predict active mucosal inflammation. Across studies however, CRP is less sensitive and has a lower correlation with mucosal inflammation by endoscopy than the stool based biomarkers calprotectin and lactoferrin.(32) This is true in both UC and CD patients. In studies including CD patients, results were generally reported without distinguishing patients by disease location (ileal vs colonic vs ileocolonic); however, one study noted that abnormal small bowel radiographic findings did not associate with CRP elevation.(33)

In patients on concomitant immunosuppressive therapy for CD, elevated CRP (>5) or elevated platelet count (>298) at time of azathioprine withdrawal was predictive of future infliximab failure.(35) Though not highly sensitive, several studies have shown CRP elevations to be predictive of recurrence with CD.(36–38) A recent study identified CRP levels to associate with response to infliximab therapy in CD. Early normalization of CRP correlated with long-term response and a sustained increase of CRP levels in patients on

infliximab correlated with a loss of response to therapy.(39) In contrast, evaluations of ESR have shown only mixed results with regards to its ability to predict relapse.(37, 40)

SEROLOGIC MARKERS/ANTIBODIES

Immunologic biomarkers have been used individually and as biomarker panels in IBD care. Antibodies currently used cross-react with several bacterial and fungal antigens. The existence of these antibodies in IBD patients highlight the abnormal immune response which exists between host and commensal enteric microorganisms.

Anti-neutrophil cytoplasmic antibodies (ANCAs) and anti-Saccharomyces cerevisiae antibodies (ASCA)

ANCAs are found in a variety of immune conditions, such as Wegener's granulomatosis and rheumatoid arthritis, as well as in UC. pANCA (perinulear ANCA) is found in 20–85% of UC patients, and 2–28% of CD patients.(41) ASCA binds mannose sequences in phosphopeptidomannan located in the cell wall of *S. cervisiae* (baker's/brewer's yeast). *Candida albicans* also has epitopes that bind ASCA.(42) ASCA is most prevalent in CD patients. ANCA and ASCA were the first utilized antibodies in the setting of IBD diagnosis. (41) In some estimates, ASCA is found in 39–76% of patients with CD, up to 15% of patients with UC, and 5% of healthy controls.(43) Of note, a positive ASCA has also been seen in patients with Behcet's disease, celiac disease, autoimmune hepatitis, and primary biliary cirrhosis. ANCA positivity can be found in other forms of colitis, such as eosinophilic and collagenous colitis.(44)

Antibodies to outer membrane porin (Anti-OmpC), Flagellin (Anti-Cbir1), Pseudomonas flourescens -associated sequence I–2 (Anti-I2), and antibodies to Flagellin A4-Fla2 and Fla-X

Anti-OmpC is an antibody to an outer membrane protein isolated first from *Eschericia coli.* Adherent-invasive *E. coli* has been found in ileal CD lesions, and OmpC has been shown to be required for these organisms to thrive in the GI tract.(45, 46) Cbir1 is a flagellin related antigen that was initially identified in the enteric flora of mice, and has an ability to induce colitis in immunodeficient mice.(47) I2 is a bacterial DNA fragment that is a homolog of a bacterial transcription factor family. This sequence was identified in lamina propria mononuclear cells of active CD patients, and was later found to be associated with *P. flourescens*.(48, 49) Anti-OmpC, anti-CBir1 IgG, and anti-I2 IgA have a prevalence of approximately 50% in CD patients. (47, 48, 50) Their prevalence ranges from 5–11% in UC patients and 4–8% in healthy controls.(47, 48, 51) Flagellins A4-Fla2 and Fla-X are newly identified flagellins to which some CD patients are seropositive. In a two-year prospective cohort study of 252 patients with CD, 76% of whom had small bowel CD, 59% had antibodies to A4-Fla2 and 57% to Fla-X.(52) In a cross-sectional study, antibodies to flagellin A4-Fla2 and Fla-X were found in 29 and 26% of IBS patients, respectively.(53) These antibodies were more frequent in patients with post-infectious IBS. The prevalence of the remaining antimicrobial antibodies in IBS patients, immune-suppressed patients, and patients with gastrointestinal infections has not been adequately assessed.

Anticarbohydrate antibodies: antilaminaribioside carbohydrate IgG (ALCA), antichitobioside carbohydrate IgA (ACCA), anti-synthetic mannoside antibodies (AΣMA or AMCA)

ALCA, ACCA, and AMCA are novel antiglycan antibodies. They are similar to ASCA in that they are antibodies to sugars on the surface of microorganisms. ALCA and ACCA are associated with CD, and are found in 17–28% of CD patients, a rate lower than ASCA.(25)

They may enhance testing sensitivity because they are positive in 34–44% of CD patients who were ASCA negative.(54, 55)

AΣMA are antibodies against synthetic oligomannose epitopes.(56) These antibodies were also found to be positive in 24% of patients with CD who were negative for ASCA, and had a lower sensitivity but higher specificity when compared to ASCA.(56) Anti-C, anti-chitin carbohydrate antibody and Anti-L, anti-laminarin carbohydrate antibody, are similar in their low sensitivity but relatively high specificity in CD patients, when compared with patients with UC.(57, 58)

Serologic markers in differentiation of IBD from non-inflammatory GI conditions, and UC from CD

A meta-analysis of 60 studies (3,841 UC and 4,019 CD patients) assessed the utility of ASCA and pANCA in IBD.(59) The presence of either pANCA or ASCA was able to differentiate between IBD and non-IBD with a sensitivity of 63% and a specificity of 93%, with low heterogeneity between studies. In this meta-analysis, ASCA+/ pANCA – test had a sensitivity of 55% and a specificity of 93% for CD. Sensitivity and specificity of the pANCA+ tests for UC were 55.3% and 88.5% respectively.(59) Of note, in patients with colonic CD, ASCA was less able to distinguish between CD and UC.(59) In a pediatric study, increased pANCA levels were seen in patients with UC or those with CD who had a UC-like pancolitis picture.(60) In practice, clinicians should recognize that false positives occur in patients with non-specific GI symptoms.(25)

The addition of anti-OmpC, anti-CBir1, and anti-I2 to ASCA and ANCA constituted the commercially available serologic panel (Serology 7, Prometheus, San Diego, CA). A study of 300 pediatric patients tested the use of this panel and yielded 67% sensitivity and 76% specificity from a cohort of patients referred for IBD evaluation (IBD prevalence of 38%).(61) Using this panel of 7 antibodies, there is sensitivity of 80% and a positive predictive value of 90% for CD, but only in a population with high CD prevalence.(62) Anti-A4-Fla2 and Anti-Fla-X can be useful in distinguishing CD from UC, as they were only positive in 6% of UC patients, as compared to 50–60% of CD patients.(52) These novel antibodies are part of a recently revised panel that includes additional serologic as well as genetic and inflammatory biomarkers relevant to human IBD (IBD sgi Diagnostic, Prometheus, San Diego, CA).(63)

In a study of 1225 IBD patients, 200 controls and 113 patients with other GI inflammation a combination of ASCA, pANCA and ALCA offered the best diagnostic accuracy.(64) As mentioned above, the antiglycan antibodies improve the specificity for CD, and may be useful in ASCA negative patients.(54, 55) Antibodies have a role in predicting phenotype progression of indeterminate colitis (IC) or IBD type unclassified (10% of colitis cases).(32) A prospective study following 97 patients with IC from 3 centers for a median of 9.9 years showed that ASCA + /pANCA− predicted CD (80%) and ASCA−/pANCA+ predicted UC (63.6%), but almost 50% of patients had neither antibody positive.(65) Anti-OmpC and anti-I2, when added to ASCA and ANCA, only provided a small benefit in diagnosis of CD or UC in patients with IC.(66) Interestingly, the novel AΣMA fared better than ASCA alone in predicting CD development in patients with IBD-type unclassified (sensitivity 45 vs 27%, specificity 100% vs 71%).(56)

Serologic markers in determining prognosis and predicting disease course in CD

In CD, 50% of patients have an uncomplicated course, when followed over 10–20 years. The remaining 50% develop stricturing or penetrating complications in the first 20 years, and require surgery in the first 6 months.(67) These patients at higher risk for complications

may benefit from receiving more aggressive CD therapy upfront. Unfortunately most studies of serologic markers which aim to determine prognostication ability are performed in a cross-sectional manner; a study design that does not account for changes in the antibody profile over time.(32) In the previously mentioned meta-analysis of ASCA and ANCA, these antibodies predicted a more complex disease course inconsistently across different studies.(59) A study of 303 patients with CD by Mow et al showed that both antibody seropositivity and titer were associated with various complications. ASCA predicted small bowel disease, fibrostenosis, internal perforation and surgery. pANCA was not associated with any complications of CD. Anti-I2 was associated with increased fibrostenotic disease and small bowel surgery. Anti-Omp C was associated with stenosis, perforation, and small bowel surgery.(68) A separate study showed that antibodies to Cbir1 were associated with small bowel disease, internally penetrating disease, and fibrostenosis but not small bowel surgery.(69) Anti-A4-Fla2 and anti-FlaX were positively associated with a stricturing phenotype and small bowel disease location and negatively associated with an inflammatory CD phenotype.(52) Antiglycan antibodies have also been associated with the development of strictures and penetrating disease.(64) Prospective cohort studies following children from the time of CD diagnosis showed that 8.2%–9% of patients with one or more positive antibody (ASCA, anti-I2, anti-OmpC) had complications, compared to 2.3–2.7% with a negative serologic profile.(70, 71)

Serologic markers have been shown to be predictive of prognosis in research cohorts and cross sectional studies; however, their role in the management of the individual patient requires further study. Positive serologies occur in many CD patients who do not develop future complications. Furthermore, we do not yet have prospective studies which verify that early aggressive therapy changes long term outcomes in patients predicted to have a worse prognosis based on their serologic profile.(32)

FECAL BIOMARKERS

Fecal biomarkers are valuable in their specificity to the gastrointestinal tract. In the setting of mucosal inflammation inflammatory proteins, leukocyte products, and leukocytes themselves leak from a permeable mucosa.(25) The gold standard in this setting is the excretion of radiolabeled leukocytes in feces, which is cumbersome to use in everyday clinical practice.(72) The most frequently used fecal markers are calprotectin and lactoferrin.(32) They are inexpensive to perform and have demonstrated utility in diagnosing IBD, assessing disease activity, predicting disease relapse as well as response to therapy.(32) S100A12 is another fecal marker that has recently been studied and may be superior to the fecal markers currently used in IBD.(73)

Fecal calprotectin

Calprotectin is a 36 kilodalton protein that binds zinc and calcium and has antimicrobial effects.(74) It is resistant to bacterial degradation and stable in feces, for several days. Measurement in stool is by enzyme linked immunosorbent assays (ELISA).(74) Calprotectin makes up 50–60% of granulocyte cytosolic protein, and is released with cell death or activation, making it a sensitive marker of inflammation.(75) Other conditions with elevated fecal calprotectin include neoplasia, polyps, non-steroidal anti-inflammatory enteropathy, increasing age, celiac disease, microscopic colitis, allergic colitis, and infections.(21, 25, 76)

Fecal lactoferrin

Lactoferrin is an iron binding glycoprotein found in neutrophil granules, and possesses antimicrobial properties.(77) It is also measured by ELISA and is resistant to freeze-thaw cycles and degradation, facilitating its use as a laboratory test. Unlike calprotectin which can

be produced by monocytes and possibly epithelial cells, lactoferrin is specific to neutrophils. (78)

Fecal S100A12

S100A12 is similar to calprotectin in its calcium-binding properties.(73) This protein activates NF-κB signal transduction and increases cytokine release.(25) S1000A12 is also detectable in serum, but the fecal assay is more sensitive and specific for IBD.(79)

Other fecal biomarkers are being investigated for use in IBD. While promise exists, thus far these alternatives have shown less consistent results, lower correlation to disease activity, and overlap between patients with active and inactive disease.(78, 80) These include lysozyme, leukocyte esterase, elastase, myeloperoxidase, TNF-alpha, IL1 B, IL4, IL 10, alpha 1 anti-trypsin, and alpha-2-macroglobulin.(25, 32, 74) M2-pyruvate kinase may be the most promising of these developing fecal biomarkers.(81)

Fecal markers in differentiating IBD from non-inflammatory diarrheal disorders

Fecal biomarkers are less invasive than endoscopy and imaging, and are attractive first step when there is a clinical suspicion of IBD. In a systematic review, fecal calprotectin had a high negative predictive value in differentiating IBD from IBS in symptomatic patients without a prior diagnosis, but a lower positive predictive value.(76) A meta-analysis evaluating fecal calprotectin (30 studies and 5,983 patients) showed a sensitivity and specificity of 89% and 81% when a cutoff of 50 μg/g was used.(82) Sensitivity and specificity for IBD changed to 98% and 91% in studies with a cutoff of 100 μg/g.(82) The ideal threshold above which IBD is more likely is unknown.(32) Another meta-analysis of 6 studies evaluated the use of fecal calprotectin in patients with a clinical suspicion of IBD and showed a sensitivity and specificity of 93% and 96% respectively. However depending on fecal calprotectin alone was shown to delay diagnosis in some adults due to false negative results.(83)

Fecal lactoferrin has a sensitivity and specificity of 80% and 82% respectively, when taking a weighted mean of 19 studies including 1001 patients, where IBD patients were compared to controls of IBS and other colonic diseases.(84) Both fecal lactoferrin and calprotectin have similar diagnostic accuracy in most studies (approximately 80%), and both were superior to CRP in a study including 42 UC (60% left sided) and 43 CD patients, with 24% of CD patients having left-sided colitis, and an additional 24% having colitis and/or ileitis. (85) S100A12 has a sensitivity and specificity of 86% and 96% in differentiating chronic IBD from IBS.(73)

Fecal markers in evaluating disease activity in patients with IBD

A summary of studies showing the correlation of fecal calprotectin with endoscopic scores of disease activity in IBD yielded correlation coefficients ranging between 0.48 and 0.83. (32) Fewer studies are available for lactoferrin, but there is still a positive correlation, with coefficients between 0.19 and 0.87.(32) Fecal biomarkers are particularly useful in patients who do not exhibit an elevated CRP in the setting of active inflammation.(25, 32)

There is a higher correlation of both fecal biomarkers with colonic rather than ileal disease, as seen in a study by Sipponen et al.(86) Fecal calprotectin is also more associated with the degree of inflammation rather than disease extent in a study of UC patients.(87) The sensitivity of fecal calprotectin and lactoferrin in identifying active disease based on endoscopy is 70–100%, with specificity between 44–100%.(32) Again, the cutoff concentration of calprotectin varied between studies, ranging from 50 μg/g to 200 μg/g.(32) Lactoferrin cutoff ranged from 7.5 to 10 μg/g.(32)

Fecal markers in assessment of response to therapy, mucosal healing and post-op recurrence

Fecal calprotectin levels decreased to <50 μg/g in UC and CD patients who achieved mucosal healing in response to medical therapy.(88) Fecal lactoferrin has similarly shown utility in monitoring patients on infliximab therapy.(89) In two small studies by Sipponen and colleagues, CD patients who responded to anti-TNF therapy had decreased lactoferrin and calprotectin levels, as opposed to patients without endoscopic improvement.(90, 91) There is currently no established threshold value of fecal markers that predicts mucosal healing.(32)

After ileocolonic resection in Crohn's disease, fecal lactoferrin and calprotectin can identify postoperative recurrence.(92, 93) However since postoperative patients have higher baseline levels of the fecal biomarker, the tests' sensitivity and specificity are reduced in evaluating this population.

Fecal markers in predicting disease relapse

Multiple studies have assessed the use of fecal calprotectin in the prediction of disease relapse in patients who are in clinical remission at the time of entry into the study. A study of 79 IBD patients by Costa showed that fecal calprotectin correlates better with disease relapse in UC than with CD, using a cutoff of 150 μg/g.(94) In CD and UC, there positive prediction value of 87% and 80% respectively, however, the negative predictive value was 90% in UC and only 43% in CD.(94) Tibble et al used a calprotectin cutoff of 50 μg/g, showing a sensitivity and specificity of predicting disease relapse of 90% and 83% respectively in a 12-month period.(95) There was a large range of duration of disease remission in some studies, ranging from 3 months to 36 months, and an increase in calprotectin earlier in remission may be a better predictor of disease relapse.(32, 96, 97)

INVESTIGATION OF FUTURE BIOMARKERS

The identification and testing of new IBD biomarkers is an area of active research. Methods of biomarker discovery include candidate based approaches as well as technology platform based approaches. These technology platforms can be used to both exploit and identify differences in genomics (genotyping and gene expression), proteomics and metabolomics. Newly identified biomarkers are then validated against control (or comparator) populations and/or across states of disease activity or disease phenotype.(98) Ideally new biomarkers will be easy to obtain, inexpensive to perform, reliably and quickly quantifiable across labs, and unaffected by co-morbid factors.(25) Ultimately the discovery and validation of these biomarkers depends on effective cross talk and collaboration between expert clinicians, innovative scientists and bioinformatics specialists.

Mucosal healing has been identified as a key therapeutic endpoint and ideally new blood or stool based biomarkers will be reflective of this process in order to provide the best predictive value for IBD.(13, 99, 100) A biomarker which correlates well with mucosal healing offers the greatest opportunity to predict long-term outcomes of patients with IBD. Several potential biomarkers in IBD are currently being investigated.

Metabolome Based Biomarkers

Comparative analysis of the metabolic profile in IBD patients and animal models represents an area of active investigation which has revealed several potential targets for evaluating IBD.(101) Some studies have targeted specific metabolites while others have used total metabolomic analysis. Urine, feces and colonic tissue have been evaluated from humans while urine, serum and colon tissue has been evaluated in mouse models of colitis. A

specific study on human serum metabolics has not yet been published. While variability exists between studies, pathways which include amino acids and associated metabolic products linked to tissue inflammation or gut bacteria have thus far been elucidated. Further standardization of techniques and analyzed samples should improve accuracy of this technique which has potential to impact clinical practice.

We recently published findings on a metabolome based biomarker which exploits a component of the activated mucosal immune system in IBD that would be predicted to correlate with mucosal healing.(102) This approach will be detailed here for illustrative purposes and other potential future biomarkers are also discussed.

IDO1 mediated tryptophan catabolism pathway as a biomarker—Recent studies have identified a role for the enzyme indolemine 2,3 dioxygenase (IDO1) in intestinal inflammation and the inflammatory bowel diseases. IDO1 acts as the first and rate limiting step in tryptophan catabolism along the kynurenine pathway. This pathway has been identified as important in mediating immune tolerance via suppression of effector T-cell responses.(103) IDO1 expression is present in the gut at baseline, but is significantly elevated in the setting of intestinal inflammation.(102, 104) Proteomic and gene expression arrays studies found IDO1 to be among the most highly over-expressed genes in human IBD (105, 106), as well as in animal models of IBD.(107, 108) The functional importance of this enzyme in modifying colitis severity has now been evaluated using animal models. Inhibition of IDO1 worsens colitis severity while induction of IDO1 expression limits disease severity.(109–111)

Several lines of evidence support targeting of the IDO1 mediated tryptophan catabolism pathway as a potential biomarker of intestinal inflammation. Reduced serum tryptophan was reported in a small cohort of patients with Crohn's disease, though at the time this finding was attributed to compromised absorption.(112) Another small study found elevated serum levels of both kynurenine and kynurenic acid in CD and UC patients compared to controls. (113, 114) Metabolomic profiling of the IL10−/− model of colitis identified elevation of urinary Xanthurenic acid.(115, 116) This finding led the investigators to identify the IDO1 metabolites kynurenine and 3-hydroxykynurenine (metabolic precursors of xanthurenic acid) as elevated in the plasma of these mice with colitis. Another group confirmed these findings and showed that urinary xanthurenic acid levels correlated with colitis severity. (117) Correspondingly, it has been shown that serum levels of tryptophan are reduced by nearly 80% in mice exposed to dextran sodium sulfate colitis.(118)

Using a targeted metabolic profiling approach we recently demonstrated the potential for utilizing the IDO1 pathway as a biomarker of disease activity in human Crohn's disease. (102) IDO1 catabolizes tryptophan to kynurenine. In the setting of immune activation, the kynurenine/tryptophan (K/T) ratio is used as a surrogate marker for IDO1 activity. Using the K/T ratio rather than either alone limits potential bias related to differences in dietary intake of tryptophan.(119) This technique has been used to evaluate immune activation in several disorders including rheumatoid arthritis and lupus.(120, 121) In systematic evaluation of CD patients and a control population we found that serum tryptophan and the K/T ratio correlated with severity of CD activity as well as with the acute phase reactants ESR and CRP. The K/T ratio was useful in identifying patients with active colonic, ileo-colonic and isolated ileal disease. In a subgroup of CD patients, serial serum measurements were taken (once during active disease and once when remission was achieved). Here we found that as CD activity improved, tryptophan levels elevated while kynurenine levels and the K/T ratio lowered.

Mucosal healing was not evaluated as a component of our study. However, we did find that IDO1 was highly expressed in the colonic and small intestinal mucosa of patients with active CD. While most studies to date have focused on IDO1 expression within classical antigen presenting cells (macrophages and dendritic cells), in active IBD we identified strong IDO1 expression in the epithelium as well as likely antigen presenting cells of the lamina propria. The expression of IDO1 across several gut cell types along with the organs' large surface area likely contributes to the profound changes in serum K/T ratios we identified in active CD compared to other autoimmune disorders.(102) High expression of IDO1 protein in active IBD has been confirmed by others.(104) Furthermore the normalization of mucosal IDO1 protein expression after treatment of active CD with infliximab has been also reported.(122) Thus, measurement of serum K/T ratios has well suited potential to serve as an objective surrogate marker of gut mucosal immune activation and biomarker for CD activity.

Gut microbe influence on metabolome—Williams and colleagues used urine based metabolic profiling with high-resolution nuclear magnetic resonance spectroscopy to compare IBD patients with controls.(123) The study focused on specific metabolites influenced by gut microbes. In CD patients verses control or UC patients, hippurate and 4-cresol sulfate levels were found to be significantly lower, while formate levels were significantly higher. However, dot plot analysis revealed notable overlap between all cohorts.

L-arginine in UC—Another amino acid, L-arginine (L-Arg), has recently been investigated in IBD. L-Arg has an important role in regulation of epithelial integrity and immune function. Hong and colleagues showed that serum levels of L-arginine are increased in patients with active UC and levels correlate with histologic disease severity.(124) It was suggested that L-Arg uptake by cells in the inflamed colon is defective which could contribute to UC pathogenesis. Metabolomic array studies have also identified elevated L-Arg in colonic mucosal specimens of UC patients and serum of an animal colitis model. (125, 126) Interestingly, L-Arg supplementation was also found to limit disease severity in an animal model of colitis.(127)

Gene expression profiling

Gene expression profiling is being examined as a predictive biomarker in human IBD. Since both CD and UC are both multi-genetic disorders with complex pathophysiology, it is more likely that a panel, rather than a single biomarker, may be better able to distinguish between disorders. A propriety DNA array assay termed IBDChip tests for ~100 gene mutations relevant to IBD and is currently being investigated for use as a predictor of clinical evolution, disease complication and response to certain pharmocotherapies.(128) Arijs and colleagues have shown that mucosal gene signatures can predict response to infliximab for both UC and colonic CD patients.(129, 130) However, these assays require endoscopy with biopsy to obtain tissue. A recent study has suggested that blood-based gene expression profiling may be able to differentiate IBD from non-inflammatory diarrhea. Burakoff et al used an Affymetrix GeneChip to examine whole blood gene expression in UC, CD and control subjects.(131) Using a supervised learning method, panels of four distinct genes were able to differentiate accurately between CD, UC and controls with diarrhea. These and other promising results will need to be further evaluated in a larger cohort of patients with varying levels of disease activity to ultimately determine its clinical utility.

Using transcription profiling of circulating T cells, a group from England reported that analogous CD8+ T cell transcriptional signatures was detectable in two unrelated autoimmune disorders.(132) Furthermore, the gene signature predicted disease prognosis in

each. The expression profile identified genes involved in antigen-dependent T cell responses (both IL-7 and T-cell receptor initiated). These findings are now extended to CD and UC where the gene signatures effectively identified a patient cohort destined for frequently relapsing disease.(133) The equivalent correlation was not observed with CD4+ T cell gene expression, the T cell subset classically linked to disease pathogenesis. This gene expression signature had greater ability to predict need for escalation of therapy than standard clinical parameters (age <40, initial requirement for steroids and perianal involvement) or ASCA positivity. Confirmative studies will be needed, but this exciting development highlights the potential expression profiling in biomarker discovery.

Proteomic Array

Significant advances in proteomic array profiling technology have ignited interest in using this technique for assessment of human IBD. Current approaches include not only traditional proteomics, but also subproteomics, which analyze differences in cellular compartments, organelles and biological fluids. In the inflammatory bowel diseases, proteomic approaches have shown promise with regard to identifying active disease, differentiating between CD and UC, predicting response to anti-TNFα therapy and providing insight into disease pathogenesis.(134–137) Available reviews summarize recent studies and future directions for use of these techniques in greater detail.(138, 139)

CONCLUSIONS

Diagnosis and ongoing care of patients with CD and UC currently depends on clinical, endoscopic and histologic assessment. Radiology and standard laboratories are additive. Several established and reliable biomarkers are currently used in clinical care to aid in diagnosing IBD, differentiating between CD and UC, assessing disease activity and predicting relapse. Existing biomarkers assess stool and blood for acute phase reactants, leukocyte markers and antibody markers. Available biomarkers are limited in their ability to predict longer-range disease course. Table 1 summarizes the currently available data supporting the uses of available biomarkers in the diagnosis and management of IBD. Improvements in genomic, proteomic and metabolomic array based and mass processing technology are facilitating biomarker discovery in IBD. Rapid advances are concurrently being made in understanding of IBD etiopathogenesis. Effective bidirectional information sharing between these fields of investigation will ultimately lead to improved care of patients with IBD.

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Abbreviations

IBD	Inflammatory bowel diseases
IBS	Irritable Bowel Syndrome
CRP	C-reactive protein
ESR	erythrocyte sedimentation rate
ASCA	anti-*Saccharomyces cerevisiae* antibodies
pANCA	perinuclear anti-neutrophil cytoplasmic antibodies

CD	Crohn's disease
UC	ulcerative colitis
CDAI	Crohn's disease activity index
TNFα	tumor necrosis factor – alpha
IL	interleukin
Anti-OmpC	antibody to outer membrane porin
Anti-I2	*Pseudomonas flourescens* -associated sequence I–2
ALCA	antilaminaribioside carbohydrate IgG
ACCA	antichitobioside carbohydrate IgA
AΣMA or AMCA	anti-synthetic mannoside antibodies
GI	gastrointestinal
ELISA	enzyme linked immunosorbent assay
IDO1	indoleamine 2,3 dioxygenase-1
K/T	Kynurenine/tryptophan ratio

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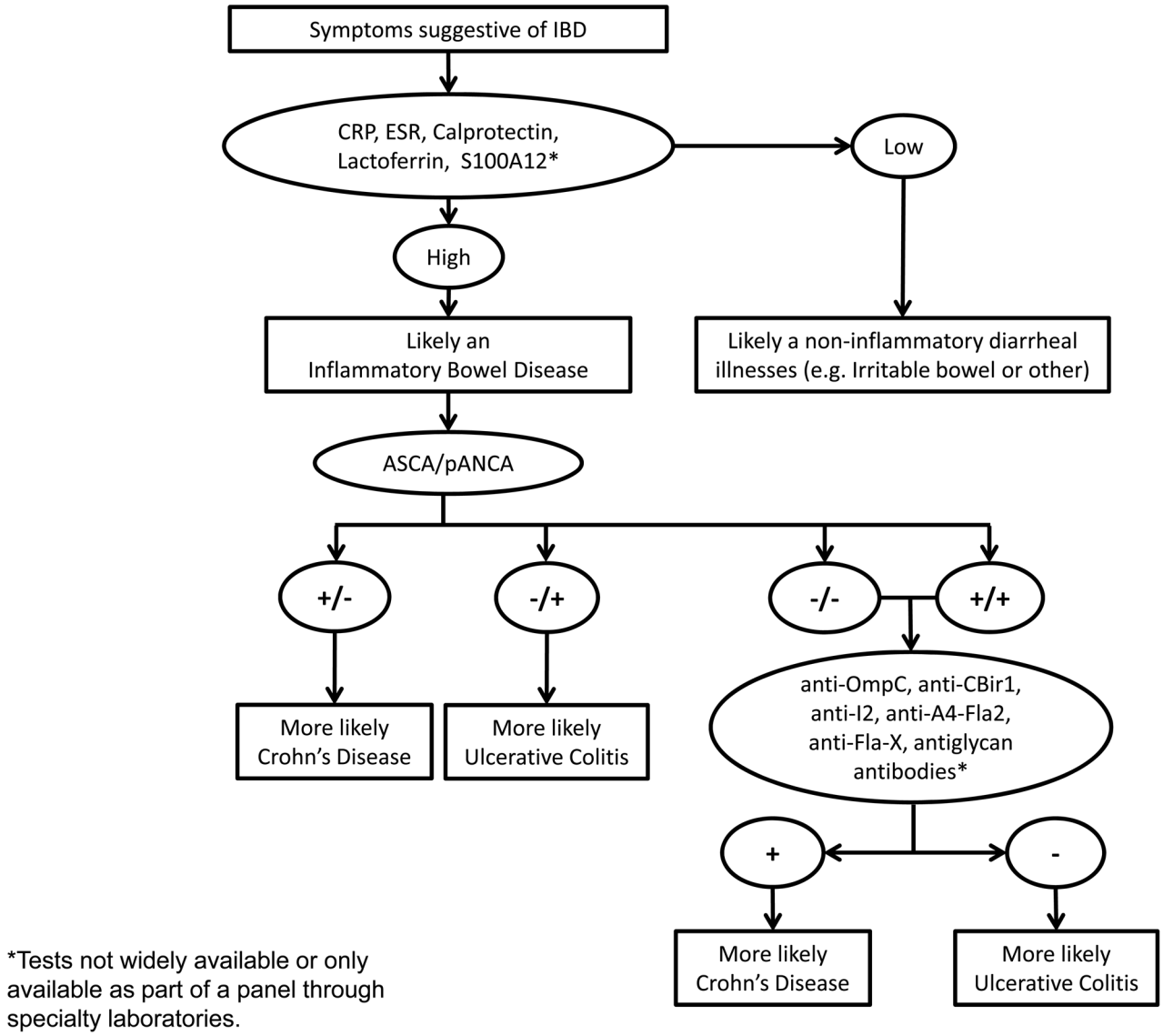

Figure 1.
Use of biomarkers as adjunct to clinical evaluation for the diagnosis of IBD

Table 1

General summary of data supporting the roles of biomarkers in IBD

Biomarker	IBD vs non IBD	CD vs UC	Disease activity	Mucosal healing	Prediction of clinical relapse	Prognosis	CD Phenotype
CRP/ESR	Useful	Not used	Useful	Possibly	Possibly	Possibly	Not used
Fecal biomarkers	Useful	Not used	Useful	Useful	Possibly	Not used	Not used
Serologic markers	Useful	Useful	Not used	Not used	Not used	Useful	Useful

* adapted from data and references 25,32