Published in final edited form as:
Gastroenterology. 2011 May ; 140(6): 1817–1826.e2. doi:10.1053/j.gastro.2010.11.058.

The Utility of Biomarkers in the Diagnosis and Therapy of Inflammatory Bowel Disease

James D. Lewis, MD, MSCE
Center for Clinical Epidemiology and Biostatistics, Department of Medicine, Department of Biostatistics and Epidemiology, University of Pennsylvania

Abstract

Fecal and serologic biomarkers can be used in the diagnosis and management of inflammatory bowel disease (IBD). Fecal markers such as calprotectin and lactoferrin have been studied for their ability to identify patients with IBD, assess disease activity, and predict relapse. Antibodies against *Saccharomyces cerevisiae* and perinuclear antineutrophil cytoplasmic proteins have been used in diagnosis of IBD, to distinguish Crohn's disease (CD) from ulcerative colitis, and to predict the risk of complications of CD. Tests for c-reactive protein and erythrocyte sedimentation rate have been used to assess inflammatory processes and predict the course of IBD progression. Levels of drug metabolites and antibodies against therapeutic agents might be measured to determine why patients do not respond to therapy and to select alternative treatments. This review addresses the potential for biomarker assays to improve treatment strategies and challenges to their use and development.

Keywords

Crohn's disease; ulcerative colitis; biomarker; prognosis

Physicians guide management of patients with inflammatory bowel disease (IBD) using blood tests, radiology and endoscopy studies, and other tests. These diagnostic tests can be used to identify patients with IBD, determine prognosis, assess disease activity, and determine optimal therapeutic strategies (Figure 1 and Table 1).

Tests Used to Evaluate Patients with Symptoms of IBD

Numerous fecal markers can potentially be used to determine the likelihood that a patient has IBD.[1-18] The 2 most commonly utilized are calprotectin and lactoferrin. Calprotectin is 36 kDa calcium- and zinc-binding protein that represents 60% of cytosolic proteins in granulocytes.[19] It is stable in feces when stored at room temperature for up to 1 week.[20] The

Corresponding author: James D. Lewis, MD, MSCE, 720 Blockley Hall, 423 Guardian Drive, Philadelphia, PA 19104, Tel: 215-573-5137, Fax: 215-573-0813, lewisjd@mail.med.upenn.edu.

Dr. Lewis reports having previously served as an expert witness on behalf of Prometheus. He has research funding from Takeda, Centocor, and Shire. He has served as a consultant to Centocor, Novartis, Shire, Elan, Procter & Gamble, AstraZeneca, GlaxoSmithKline, Amgen, Millennium Pharmaceuticals, United Biosource Corporation, Advinus, and Allos Therapeutics.

Adapted from the following manuscripts:

Haines ML, Ajlouni Y, Irving PM, Sparrow MP, Rose R, Gearry RB, Gibson PR. Clinical usefulness of therapeutic drug monitoring of thiopurines in patients with inadequately controlled inflammatory bowel disease. Inflammatory Bowel Diseases 2010 epub ahead of print.

Afif W, Loftus EV, Jr., Faubion WA, Kane SV, Bruining DH, Hanson KA, Sandborn WJ. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. American Journal of Gastroenterology 2010;105:1133-9.

concentration of calprotectin in feces is an indirect measure of neutrophil infiltrate in the bowel mucosa. In patients with Crohn's disease (CD), the 4-day fecal excretion of ^{111}indium-labeled white blood cells (WBCs) correlates with 4-day excretion of calprotectin and even the concentration of calprotectin in a single fecal specimen .[21]

Numerous studies have addressed whether fecal calprotectin could be used to select patients with symptoms of IBD that warrant endoscopic or radiologic evaluation. Von Roon et al. summarized data from 30 studies that included 5,983 patients (1210 had IBD).[22] The estimated sensitivity and specificity values for the identification of patients with IBD, compared to those without, were 89% and 81%, respectively in studies that used a threshold fecal calprotectin concentration of 50 μg/g; these values were 98% and 91% in studies that used a threshold fecal calprotectin concentration of 100 μg/g. However, these estimates come from combinations of different studies, rather than tests of different threshold levels in a single study. In fact, it is implausible for the fecal calprotectin assay to have higher sensitivity when using a higher threshold to define a positive test. Therefore, these data cannot be used to select an optimal cut point.

van Rheenen et al. performed a similar analysis that was limited to studies that included only patients suspected to have IBD based on signs and symptoms.[23] Among the 6 studies, the sensitivity and specificity for identification of IBD in adults were 93% and 96%, respectively. In children, the test's sensitivity was 92% but the specificity was only 76%. The authors conclude that using these tests to choose what patients require further testing reduces the need for endoscopy or radiology tests in a large portion of patients but would delay the diagnosis of IBD in 6% and 8% of the adults and children with disease, respectively.

Lactoferrin is an iron-binding protein found in neutrophil granules and serum and is secreted by mucosal membranes. It is resistant to degradation and proteolysis (although less so than calprotectin), making it a useful marker of intestinal inflammation[24]. Pooling data from multiple studies and 1001 patients, Gisbert et al. estimated that the lactoferrin test identified patients with IBD with a mean sensitivity of 80% and specificity of 82%[25]. Most but not all studies reported similar performance of calprotectin and lactoferrin tests[26-28].

100A12 is a S100 protein that is similar to calprotectin. In a study of children, fecal levels of S100A12 greater than 10 mg/kg identified IBD with a sensitivity of 96% and a specificity 92%.[29] In a subsequent study of adults, S100A12 distinguished patients with IBD from those with irritable bowel syndrome with sensitivity and specificity values of 86% and 96%, respectively.[30] S100A12 can be measured in serum; although serum levels are increased in patients with IBD, this test does not distinguish IBD from irritable bowel syndrome with the same levels of sensitivity and specificity as the fecal assay.[31]

For many reasons, blood-based biomarkers might be preferred to stool-based tests. C-reactive protein (CRP) is one of many acute phase proteins that increase in the serum of patients with acute-phase IBD. Studies dating back several decades identified increased levels of CRP in nearly 100% of patients with CD and approximately 50% of those with UC[32-34]. The reason for the higher rates of increased levels of CRP in patients with CD, compared with UC, is unknown. Furthermore, many patients with established CD do not have increased levels of CRP, despite evidence of active disease, so these studies probably overestimated the sensitivity of this test in detecting CD[35]. Because of the relatively low sensitivity of the CRP test in detection of UC, use of this marker alone to identify patients with symptoms compatible with IBD that should undergo further evaluation would delay diagnosis for many.

Various serologic tests have been used in attempts to improve the diagnosis of IBD and to distinguish CD from UC, such as tests for perinuclear antineutrophil cytoplasmic antibodies (pANCAs) and anti–*Saccharomyces cerevisiae* antibodies (ASCAs)[36]. Increased titers of ASCA were reported to identify patients with CD with high levels of specificity (96%–100%) but low sensitivity (approximately 50%).[36] In contrast, increased levels of pANCA were more common in patients with UC or those with CD that had UC-like pancolitis.[36] A meta-analysis of 60 studies estimated the sensitivity and specificity of ASCA+/pANCA- for detection of CD to be 55% and 93%, respectively, and 63% and 93% for any form of IBD [37]. In pediatric patients, the test for pANCA+/ASCA- performed particularly well, identifying patients with CD with 70% sensitivity and 93% specificity [37].

Other serologic markers of CD include antibodies to *Escherichia coli* outer membrane porin (OmpC), *Pseudomonas fluorescens*-associated sequence I2, and flagellin CBir1. A recent study of more than 300 children with suspected IBD found that a test for this panel of antibodies identified patients with IBD with 67% sensitivity and 76% specificity.[38] However, studies have demonstrated comparable sensitivity and specificity using routine tests for anemia, thrombocytosis, and increased erythrocyte sedimentation rate (ESR) in the same population. [38, 39]

ASCA is believed to interact with mannose residues on mannan in the cell walls of *S cerevisiae*, although data indicate that *C albicans* also produces the ASCA-binding epitope [40, 41]. Antibodies against other sugars (particularly glycans on the surface of cells) and microorganisms have also been studied. Antibodies against laminaribioside (ALCA) and chitobioside (ACCA) have been associated with CD[42]. Tests for ALCA, ASCA, and antibodies against a covalently immobilized mannan from *S cerevisiae* (gASCA) distinguish patients with CD from healthy controls with similar operating characteristics as ASCA. Interestingly, 34%–44% of ASCA-negative patients with CD had positive results in tests for ALCA or ACCA[42, 43]. Others studies have shown that the combination of gASCA, pANCA, and ALCA is more accurate than other combinations of these serologic markers, or ACCA, antibodies to mannobioside (AMCA), and Omp, in distinguishing individuals with IBD from healthy controls[44].

In considering results from these studies, it is important to assess the sensitivity, specificity, and predictive values of diagnostic tests. The cut point for a test determines its sensitivity and specificity; higher sensitivity results in lower specificity. In comparing results between studies, it is important to assess whether comparable cut points were used to define the test operating characteristics. Similarly, because positive and negative predictive values are determined based on the prevalence of disease in the population, one must compare the study populations before drawing conclusions about predictive values.

Tests Used to Evaluate Patients Diagnosed with IBD

Differentiating between CD and UC

ASCA is associated with CD whereas increased levels of pANCA are more common among patients with UC[36]. In a meta-analysis, combinations of tests for ASCA and pANCA distinguished patients with CD from those with UC with 40%–50% sensitivity and specificity of >90%[37]. However, when the population was limited to those with colonic disease, for whom the diagnostic question is most relevant, the ASCA test was less sensitive for CD and discriminated less well between CD and UC[37].

The need for such a test is greatest in patients with IBD type unclassified (indeterminate colitis). One prospective study found that nearly half of the patients with IBD type unclassified had negative results from the ASCA and pANCA tests and that most continued

to have clinical characteristics that precluded a definitive diagnosis of CD or UC[45]. Interestingly, of the patients who had a positive result from the pANCA or ASCA test, 44% developed CD or UC over a mean follow-up period of 9.9 years. Among 26 patients that had ASCA+/pANCA- results at baseline, 8 were later diagnosed with CD and 2 with UC. Among 20 patients that had ASCA-/pANCA+ results at baseline, 4 were later diagnosed with CD and 7 with UC. Thus, among the patients with positive results from serology analyses, ASCA and pANCA were predictive of disease type, but did not have 100% accuracy[45]. Addition of the tests for I2 and anti-OmpC to the tests for ASCA and pANCA has only a marginal incremental benefit in determining which patients with IBD type unclassified have CD and which have UC[46]. Similarly, addition of tests for the anti-glycan antibodies to tests for ASCA and pANCA does not appreciably increase the ability to distinguish CD from UC in cross-sectional studies[44]. An assay that used 2 different synthetic oligomannose blocks to detect anti-mannose antibodies (antibodies to the synthetic mannose blocks known as AΣMA) demonstrated better sensitivity (45% vs. 27%) and specificity (100% vs. 71%) than the ASCA test in predicting development of CD in patients with IBD type unclassified[47].

Differentiating quiescent from active disease

Concentrations of fecal calprotectin, lactoferrin and CRP have each been correlated with histologic and endoscopic disease activity in patients with UC and CD (Table 2).[48-58] In general, calprotectin and lactoferrin correlate better with colonic than ileal disease activity, although extent of colonic disease in does not appear to be important in patients with UC[48, 50, 53]. The sensitivities of tests for calprotectin to detect any active mucosal disease range from 70%–100%, with a specificity range of 44%–100%, depending on the cut point used[49, 51, 59-61]. Sensitivities and specificities of tests for lactoferrin are similar (Table 3).

Serum biomarkers, particularly CRP, have also been used to distinguish quiescent from active disease. In general, the correlation between CRP and endoscopic activity is lower than that observed between fecal markers and activity (Table 3). Similarly, sensitivity and specificity for active mucosal inflammation is likely to be lower for CRP compared with fecal markers. For example, Solem et al. observed that the CRP test had 54% sensitivity and 75% specificity for CD in 105 patients [62]. In a study of 43 patients with UC, 19 of 37 (51%) with active disease, based on colonoscopy analysis, had increased levels of CRP whereas 0 of 6 patients without endoscopic evidence of disease activity had increased levels of CRP[62]. Perhaps most importantly from the perspective of a clinician, 37 of 43 patients (86%) with any clinical symptoms of CD and with increased levels of CRP had evidence of mucosal inflammation, based on colonoscopy analysis [62]. So, for patients with CD, the combination of increased levels of CRP and clinical symptoms is likely to be sufficient to identify active mucosal disease. Some patients have persistent, normal levels of CRP despite active disease[63]. For these patients, CRP will not be useful to differentiate quiescent from active disease. This may be a population where fecal biomarkers are particularly useful.

ESR has also been studied as a biomarker for IBD disease activity. Like CRP, some but not all studies have found it to be increased more frequently among patients with active CD than UC[63-65]. However, tests for ESR are less widely used than tests for CRP because ESR levels do not change as quickly with disease activity[63].

Using biomarkers to establish mucosal healing

In patients with UC or CD, mucosal healing in response to medical therapy correlates with a less severe future course of disease [66, 67]. Similarly, following ileal resection, the appearance of the neoterminal ileum predicts short term outcomes[68]. Thus, there is potential to use

biomarkers to assess mucosal healing following medical therapy or surgery and to predict the likelihood of relapse.

Roseth et al. demonstrated that patients with CD or UC who had remission following medical therapy had large reductions in levels of fecal calprotectin, (to below 50 μg/g)[49]. Several additional studies have shown similar results in response to therapy. Sipponen et al. performed one study of patients treated with anti-tumor necrosis factor (TNF) agents and another study of patients treated with other therapies. Among 5 patients that had mucosal healing after treatment with reagents other than anti-TNF agents, 4 (80%) also had normalized levels of fecal calprotectin and lactoferrin. Among 9 patients with no mucosal improvement after therapy, 8 (89%) had increased levels of calprotectin and 6 (67%) had increased levels of lactoferrin [61]. Eleven patients that responded to anti-TNF therapy (based on endoscopic appearance), had significant decreases in levels of fecal calprotectin and lactoferrin, whereas 3 non-responders did not have decreased levels of these markers[69]. Despite the consistency of these results, the studies were limited by small sample sizes and an inability to define an optimal cut point for predicting mucosal healing. However, within the range of cut points tested, there does not appear to be a difference between tests for calprotectin and lactoferrin in determining treatment response.

There are limited data regarding the use of biomarkers to assess CD recurrence following ileocolonic resection; and the results for fecal biomarkers demonstrated only modest sensitivity and specificity[70-72]. A possible explanation for these observations is that the initial, asymptomatic recurrence of CD results in limited mucosal injury. This small amount of injury, particularly to the ileum, is not likely to increase biomarkers to levels that can be detected in fecal samples.

Biomarkers to predict disease course

One of the major goals of treatment for CD is to prevent complications such as perforation and formation of abscesses, fistulas and strictures. Biomarkers might be used to identify patients who are at high risk for a complicated disease course. At the time of diagnosis, 5%–15% of patients already have strictures, 5%–15% have had a penetrating complication, and the remaining 70%–80% have pure inflammatory disease[73, 74]. A stricture or penetrating complication occurs in approximately 50% of patients within the first 20 years of disease; most of these patients require surgery within 6 months [74]. Similarly, estimates of the cumulative risk of surgery range from 28% to 61% at 10 years for children and adults, respectively[75, 76]. Approximately 50% of the patients with CD would be expected to have a relatively uncomplicated course during a period of 10–20 years and might be candidates for less aggressive therapy, whereas the remaining 50% would be candidates for more aggressive therapy. The challenge is to identify these populations before the complications have occurred and to find therapies that can effectively prevent these complications.

Results from tests for ASCA and pANCA have been shown to predict complicated disease courses in some, but not all, studies[37]. Other studies have evaluated the ability of other combinations of seromarkers, quantitative assessment of these markers, and combinations of markers and genetic data to predict disease course. Mow et al. studied 303 patients with CD; [77] ASCA, OmpC and I2 were each associated with various features of complicated disease (Table 4). Furthermore, the number of tests for antibodies that were positive and the concentration of the antibodies, based on sums of quartiles for each marker, were associated with complications that included stenosis, internal penetrating disease, and the need for small bowel surgery; although 40% of patients in the lowest quartiles for concentration of I2, ASCA, and OmpC levels had undergone small bowel surgery. In a follow-up study, Targan et al. associated antibodies to CBir1 with small bowel disease, an internal penetrating phenotype, and fibrostenosis[78]. Anti-CBir1 antibodies were not associated with

small bowel surgery. Similarly, ACCA, ALCA, AMCA, and gASCA have been associated with penetrating or stricturing complications and surgery.[44]

Many studies of the ability of serologic tests to predict disease course have relied on a cross-sectional design in which the disease phenotype was determined at the same time that the serologic factors were measured. However, phenotype and serologic status may change over time.[44]

To overcome this limitation, studies by Amre et al. and Dubinsky et al. [79, 80] evaluated children with CD and measured these antibodies close to the time of diagnosis or early in the course of therapy. Amre et al. associated a positive result in the ASCA test with a shorter time to fistula or abscess development. The cohort studied by Dubinsky et al. included 167 patients without internal perforations or strictures at the time of serologic analysis. Of patients with 1 or more positive result for ASCA, I2, or OmpC, 8.2% developed complications, compared with 2.7% of those with no positive result. A subsequent study by Dubinsky et al. of a larger cohort of 536 pediatric patients with CD yielded nearly identical results[81]. Similar to the findings of Mow et al.,[77] the number of positive test results and the sum of quartiles for individual results were associated with time to complications or surgery.

While these tests may be useful in carefully selected patients, the following needs to be considered. The serologic profiles associated with high risk for complications identified many patients that did not have internal perforating or stricture complications or require surgery within 5 to 10 years[81]. Additionally, the available studies did not determine how much the serologic data adds to the predictive ability of other clinical data. Finally, and as importantly, we do not know if early intervention in patients with the worst prognosis, based on positive serology test results, will change long-term outcomes.

The role of biomarkers in predicting disease relapse

Biomarkers might also be developed to identify patients that are likely to experience disease recurrence after treatment. Several studies have shown that in patients with quiescent disease, increased concentrations of fecal calprotectin predict disease relapse within 12 months, particularly in patients with UC (Table 5)[82-87]. Early studies reported that increased concentrations of fecal calprotectin identified patients that underwent relapse within 12 months with approximately 90% sensitivity and 82% specificity[82, 83]. Costa et al. reported that increased levels of fecal calprotectin had a positive-predictive value of 81% and a negative-predictive value of 90% for relapse of UC; in patients with CD, the positive predictive value was 87% and the negative-predictive value was 43% [82].

A study of calprotectin and lactoferrin levels in patients with quiescent IBD[84] found that increased levels of either biomarker identified patients who would relapse in 12 months (calprotectin sensitivity 69%, specificity 69%; lactoferrin sensitivity 62%, specificity 65%), although these values were lower than those reported in previous studies. Sipponen and Kolho reported that the relapse rate among patients with increased levels of fecal calprotectin was low, although in this study, more than 50% of the patients had been in remission for more than 1 year[86]. Increased levels of calprotectin (and likely lactoferrin) shortly after remission might be better predictors of relapse than increased levels of calprotectin 6 months or more after remission.

The abilities of CRP and ESR to identify patients that are most likely to undergo disease relapse have also been examined. In patients with CD, several studies have associated increased levels of CRP and/or ESR with relapse[88-93]; there are less data available to associate marker levels with relapse of UC. Bitton et al. examined markers of relapse of 74 patients with clinically and endoscopically quiescent UC (27 relapsed during the follow-up

period). Although the identification of basal plasmacytosis in rectal biopsy samples was associated with relapse, increased levels of CRP or ESR were not[94].

The role of biomarkers in predicting response to therapy

In addition to predicting disease relapse, biomarkers might be used to predict response to therapy. For example, it would be useful to know which patients are most likely to respond to intravenous corticosteroid therapy for UC[95-98]. Travis et al. demonstrated that the combination of stool frequency and CRP levels on the third day of therapy predicted failure to respond to intravenous steroids[96]; this finding was validated in a study of children with severe UC. However, the Pediatric Ulcerative Colitis Index (PUCAI), which is based only on symptoms, was found to more accurately identify patients that do not respond to intravenous corticosteroids than the Travis index or levels of calprotectin, CRP, M2-pyruvate kinase, or S100A12[97, 98]. In analyzing data from these types of studies, it is important to consider that the symptoms that were measured by the PUCAI may have also been used by the treating physician to make therapeutic decisions. If that is the case, it is difficult for biomarkers to outperform symptoms in predicting therapeutic decisions, such as the need for surgery.

ASCA, pANCA and other antibodies have also been tested for their association with responses to specific therapies. Taylor et al. demonstrated a lower response rate among patients with CD treated with infliximab who had positive results from a test for pANCA[99]. A subsequent study failed to confirm this association, although there was trend toward lower response rates in patients with positive results from pANCA and negative results from ASCA tests[100]. This same pattern was associated with a reduced response to infliximab therapy in patients with UC (55% of these patients responded to the drug) [101]. Most recently, in a study of children with either CD or UC, presence of a positive test for pANCA was again associated with a lower likelihood of responding to infliximab[102]. Results of tests for anti-I2, but not ASCA, pANCA, or OmpC, were associated with response to fecal diversion (94% response among patients with anti-I2 antibodies vs. 18% response among those without anti-I2 antibodies)[103].

Drug levels, metabolites and anti-drug antibodies as biomarkers

The major metabolites of thiopurines (azathioprine and 6-mercaptopurine) are 6 thioguanine nucleotides (6TGN) and 6 methylmercaptopurine (6MMP). A meta-analysis showed that patients in clinical remission were more likely to have levels of 6TGN greater than 230–260 pmol/8 × 10^8 red blood cells than patients with persistently active disease (62% vs. 36%)[104]. However, the meta-analysis included only cross-sectional studies, so drug levels were not measured before the outcomes of therapy were known. A small randomized trial reported that dose adjustment based on metabolite levels was not more effective than weight-based dose determination although the study was not completed because of slow enrollment[105]. A recent study demonstrated that data on thiopurine metabolites helped clinicians make therapeutic decisions for symptomatic patients with IBD (Table 6).[106] Less expensive alternatives to directly measure thiopurine metabolite levels, such as measuring the mean corpuscular volume, lymphocyte counts, WBC count and machine learning algorithms have been proposed[107]. However, these alternatives need to be studied prospectively to determine whether therapeutic adjustment based on these tests will improve clinical outcomes.

The effectiveness of biologic therapies, such as the anti-TNF drugs for CD and UC, depends on sufficient drug levels [108-112]. Beart et al. observed longer duration of response to therapy in those with infliximab concentrations 4 weeks post infusion of greater than 12μg/ml (median 82 days) than in those with lower drug levels (median 69 days)[108]. Among patients with CD, detectable trough serum infliximab level is associated with maintenance of

remission for the entire duration between infusions[109]. In a recent randomized trial comparing infliximab monotherapy, azathioprine monotherapy, and combination therapy, corticosteroid free remission at week 26 was more common among patients with detectable trough levels at week 30. However, it is noteworthy that 59% of the patients with undetectable infliximab levels had achieved steroid free remission, which is only modestly lower than the approximately 73% steroid free remission rate in those with detectable infliximab levels.[110] Thus, detectable trough levels are not essential to achieve clinical remission. Similar studies have demonstrated the predictive value of adalimumab drug levels and antibodies in the management of CD[111] and infliximab drug levels for moderate to severely active ulcerative colitis[112].

Factors other than the administered dose also contribute to anti-TNF drug levels. Biologic therapies can induce production of anti-drug antibodies, which are associated with shorter duration of response and a higher incidence of adverse events[108].

There is uncertainty regarding in what clinical setting measurement of infliximab drug levels and anti-infliximab antibodies is useful, but they are most commonly measured when patients lose response to therapy. Afif and Sandborn found that only 17% of patients with antibodies to infliximab responded to an increased dose of this drug[113]. However, switching patients to another anti-TNF agent resulted in a clinical response in 92% of these patients. These data led Afif et al. to propose an algorithm (Table 6) in which detection of anti-drug antibodies in serum samples indicates that patients should be given an alternative drug in the same class. Furthermore, undetectable trough levels or low levels of the drugs by 4-weeks after administration indicates that the dose or frequency should be increased (or that an alternate anti-TNF agent should be given); patients with therapeutic drug levels in serum should be evaluated for active disease and switched to a therapeutic with a different mechanism of action.

Conclusions

Biomarkers could have a role at nearly every point in the disease management. When patients present with symptoms suggestive of IBD, combinations of fecal and serologic markers might be used to identify patients that should undergo invasive testing and to help distinguish CD from UC. Tests for serologic biomarkers have been developed to identify patients that are most likely to have a severe course of disease progression, but require further evaluation in prospective studies that also assess clinical predictors (e.g., disease location, nutritional status, routine blood tests. Failure to achieve mucosal healing with therapy has been associated with worse disease course. Biomarkers such as calprotectin and lactoferrin can be used to assess mucosal healing, without the need for invasive testing or radiation. Tests for levels of drug metabolites or anti-drug antibodies can be used to determine why certain patients don't respond to specific therapies and identify alternative treatment strategies.

Acknowledgments

This work was supported in part by NIH grant K24-DK078228.

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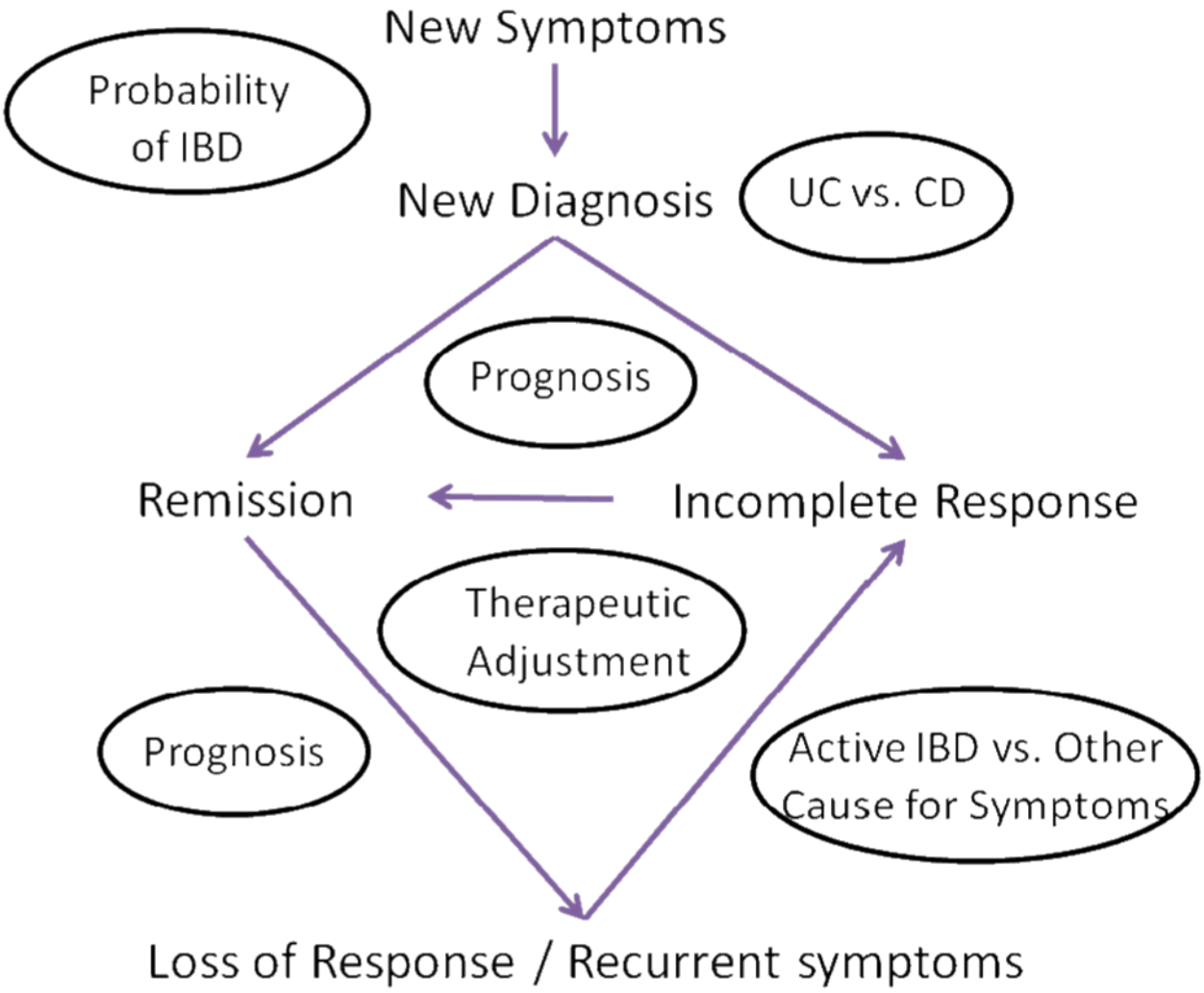

Figure 1.
The potential role of biomarker assays in the care of patients with suspected or established IBD. Biomarkers might be used in all phases of the care. For patients with suspected IBD, biomarkers can be used to select which patients are unlikely to have IBD and could forgo further testing. Once patients are diagnosed, biomarkers can be used to determine which patients have CD or UC and to predict disease course. Biomarkers might be used to determine which patients are most likely to respond to therapies and determine prognosis, to identify those that require more aggressive therapies. In patients with recurrent symptoms, biomarkers can differentiate patients with active inflammation from those likely to have symptoms from other causes.

Table 1

Applications for selected biomarkers*

Test	IBD vs. Other Disease	UC vs. CD	Risk of Complications	Active Disease vs. Remission	Assess Mucosal Healing	Predict Relapse	Predict Response to Therapy	Therapeutic Adjustment
Calprotectin	+			+	+	+	+	
Lactoferrin	+			+	+	+	+	
S100A12	+			+	+	+	+	
CRP	+			+	+	+	+	
ESR	+			+		+		
Serologies	+	+	+				+	
6MP metabolite levels						+	+	+
Anti-TNF drug levels						+	+	+
Antibodies against biologic therapies						+	+	+

*The table includes both confirmed and theoretical roles for these biomarkers.

Gastroenterology. Author manuscript; available in PMC 2013 August 21.

Table 2

Correlation of biomarkers with disease activity, determined by endoscopy.

Author	Patient population	Assessment of endoscopic disease activity	Lactoferrin (correlation coefficient)	Calprotectin (correlation coefficient)	CRP (correlation coefficient)
Sipponen[50] (2008)	CD	CDEIS	0.77	0.73	0.55
D'Inca[51] (2007)	CD	SES-CD	0.19	0.48	
D'Inca[51] (2007)	UC	Mayo score	0.35	0.51	
Hanai 2004[52]	UC	Matt's Index		0.81	
Siponnen[53] (2008)	CD	SES-CD	0.63	0.64	0.52
Fagerberg[54] (2007)	IBD	Saverymuttu[114]		0.52	
Roseth[48] (1997)	UC	Mayo score		0.57	
Jones[55] (2008)	CD	SES-CD	0.76	0.72	0.46
Sipponen[56] (2008)	CD	CDEIS	0.87	0.83	0.61
Schoepfer[58] (2009)	UC	Rachmilewitz Index		0.83	0.50
Schoepfer[57] (2010)	CD	CDEIS		0.75	0.53

CDEIS – Crohn's Disease Endoscopic Index of Severity; SES-CD - Simple Endoscopic Score for Crohn's Disease

Table 3

Sensitivity and specificity of biomarkers to identify active disease, based on endoscopy.

Author	Patient population	Assessment of endoscopic disease activity	Lactoferrin (Sensitivity / Specificit)	Calprotectin (Sensitivity / Specificity)	CRP (Sensitivity / Specificit)
Roseth[#49] (2004)	CD and UC	Farup method[115]		100% / 100%	
Solem[62] (2005)	CD	Study specific index[##]			54% / 75%
Siponnen [*] (2008)[59]	CD	CDEIS	77% / 100%	87% / 100%	
Siponnen[60†] (2008)	CD	CDEIS	66%-71% / 83%-92%	70%-91% / 44%-92%	48% / 91%
Siponnen[61‡] (2010)	CD	SES-CD	80% / 67%	80% / 89%	
D'Inca [**][51] (2007)	CD	SES-CD	77% / 80%	81% / 80%	
D'Inca [**][51] (2007)	UC	Mayo score	75% / 60%	78% / 70%	
Schoepfer [††][58] (2009)	UC	Rachmilewitz Index		86% - 93% / 71% - 88%	
Schoepfer[#57] (2010)	CD	CDEIS		89% / 58%	68%/58%

CDEIS – Crohn's Disease Endoscopic Index of Severity

SES-CD - Simple Endoscopic Score for Crohn's Disease

[#]Threshold concentrations - calprotectin 50 μg/g

[*]Threshold concentrations - calprotectin 200 μg/g; lactoferrin 10 μg/g

[†]Threshold concentrations - calprotectin 50-200 μg/g; lactoferrin 7.25 – 10 μg/g

[‡]Threshold concentrations - calprotectin 100 μg/g; lactoferrin 7.25 μg/g

[##]Findings of erosions or ulcerations; spontaneous bleeding; exudate; inflammatoryappearing nodularity, masses, or fistulas; friability; granularity; cobblestoning; extensive erythema; and concomitant erythema with edema.

[**]Threshold concentrations - calprotectin 80 μg/g; lactoferrin not specified in μg/g

[††]Threshold concentrations - calprotectin 50-100 μg/g

Table 4

Associations of serologies and NOD2 mutations with disease phenotype

	Small bowel disease	Fibrostenosis	Internal Perforation	Small bowel surgery	Perianal disease
ASCA	+	+	+	+	Not associated
pANCA	-	-	Not associated	-	Not associated
I2	+	+	Not associated	+	Not associated
OmpC	Not associated	+	+	+	Not associated

Adapted from Mow WS, Vasiliauskas EA, Lin Y-C, Fleshner PR, Papadakis KA, Taylor KD, Landers CJ, Abreu-Martin MT, Rotter JI, Yang H, Targan SR. Association of antibody responses to microbial antigens and complications of small bowel Crohn's disease. Gastroenterology 2004;126:414-24.

Table 5

Studies associating increased concentrations of fecal calprotectin with relapse in patients with exacerbations in ulcerative colitis.

Author	Patient population	Duration of remission at entry	Calprotectin Concentration to Define Elevated Level	Relapse Rate with Low Calprotectin	Relapse Rate with High Calprotectin
Gisbert[84]	UC	>6 mos.	>150μg/g	9%	31%
Tibble[83]	UC	1-4 mos.	>50μg/g	10% *	85% *
Tibble[83]	CD	1-4 mos.	>50μg/g	15%	85%
Costa[82]	UC	1-12 mos.	>150μg/g	10%	81%
Costa[82]	CD	1-12 mos.	>150μg/g	57%	87%
D'Inca[85]	UC	3-36 mos.	>130μg/g	30%	79%
Sipponen[86]	UC+CD	> 3mos (51% >12 months)	>100μg/g	25%	39%
Walkiewicz[87#]	CD	Not stated	>400μg/g	11%	56%

* estimated from Kaplan Meier curves

Table 6

Proposed algorithms in response to measured drug levels in the setting of symptoms of active disease

6TGN concentration	6MMP concentration	Interpretation	Strategy
In therapeutic window *	Normal or high ^	Refractory to thiopurines	Change therapy – can discontinue thiopurine or continue at same dose in conjunction with the new therapy
Low	Low or normal	Too low of dose or noncompliant	Increase dose or educate regarding compliance
High	Normal or high	Refractory to thiopurines	Change therapy and discontinue thiopurine or continue at same dose or lower dose
Low	High	Preferential shunting to 6MMP †	Change therapy or reduce dose and add allopurinol

Anti-Infliximab antibody	Infliximab concentration	Additional testing	Strategy
Positive			Change to another anti-TNF therapy. If persistent disease activity after changing agents, change to agent with different mechanism
	In therapeutic window	Active disease on endoscopy / radiology	Change to agent with different mechanism
	In therapeutic window	Inactive disease on endoscopy / radiology	Investigate for alternative etiology of symptoms
	Sub-therapeutic #		Increase dose or frequency. If persistent disease activity, switch to a different anti-TNF agent.
	Sub-therapeutic #		Alternative strategy - Switch to a different anti-TNF agent. If persistent disease activity, change to agent with different mechanism.

* Therapeutic window for 6TGN defined as 235–450 pmol/8 _ 108 red blood cells

^ Therapeutic level of 6MMP is less than 5700 pmol/8 _ 108 RBC

† A ratio of 6MMP:6TGN greater than 11

Sub-therapeutic infliximab concentration defined as <12 mcg/ml at 4 weeks or undetectable trough level