Biomarkers for Inflammatory Bowel Disease

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ABBREVIATIONS: ASCA = Anti-*Saccharomyces cervisiae* antibodies; CD = Crohn's disease; CRP = C-reactive protein; ELISA = enzyme-linked immunosorbant assay; IBD = inflammatory bowel disease; pANCA = perinuclear anti-neutrophilic cytoplasmic antibody; PPV = positive predictive value; UC = ulcerative colitis.

INDEX TERMS: Crohn's; inflammatory bowel disease (IBD); ulcerative colitis.

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Over the past ten years, the field of gastroenterology has seen the rapid development of commercially available diagnostic serological tests for a variety of intestinal diseases. Laboratory assays are routinely used to help diagnose conditions such as celiac disease, *H. pylori* infection, malabsorption, colon cancer, Zollinger Ellison syndrome, and others. More recently, biomarkers used to assist in the diagnosis of inflammatory bowel disease (IBD) have been studied. IBD refers to a heterogeneous group of disorders of unclear etiology but sharing common histopathological features. IBD patients have chronic intestinal mucosal inflammation at the microscopic level with the potential for macroscopic and extra-intestinal inflammation. IBD is divided into three entities: ulcerative colitis (UC), Crohn's disease (CD), and indeterminate colitis. UC is a chronic inflammatory disorder of the colonic mucosa which occurs from the anus and can extend proximally to involve the entire colon. In contrast, CD is a chronic inflammatory disorder that can occur anywhere from the mouth to the anus in a patchy distribution, involving the full thickness of the intestine. Currently, despite complete clinical, endoscopic, radiologic, and pathologic evaluations, 10% to 15% of adult patients with IBD cannot be differentiated; these patients fall into the category of indeterminate colitis. The clinical importance of distinguishing CD from UC is threefold: (1) defining pathogenesis, (2) guiding treatment regimens, and (3) predicting prognosis. The Crohn's and Colitis Foundation of America estimates that approximately one million Americans have IBD, evenly distributed between CD and UC, with ten percent classified as having indeterminate colitis. In the US pediatric population it is estimated that approximately 100,000 children carry the diagnosis of IBD. There has been a particular push to develop biomarkers for UC and CD in pediatric patients so that invasive procedures (colonoscopy/endoscopy) can be avoided in children.

The pathogenesis of IBD remains poorly understood. It has been hypothesized that the observed chronic inflammation may be the result of a dysfunctional immune response to gut bacteria. The exploration of the relationship between enteric bacteria and the human immune response has led to the development of several assays that detect the presence of antibodies to specific bacterial antigens. Note that none of these tests have been shown to have any direct pathophysiological significance. In clinical practice, the combination of these assays may be most useful when the results of other appropriate diagnostic evaluations are inconclusive. As of yet, none of these tests are appropriate for use in for general population screening. It should also be noted that many of the reported sensitivities and specificities of these tests are based on study populations with high disease prevalence, ranging from 42%-68%.[1] The spectrum of patients is thus very important when

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considering the value of new IBD biomarkers since factors like duration and severity could affect diagnostic sensitivity and specificity. In general, biomarkers for IBD have better specificity than sensitivity. They are typically more useful in the differentiation between UC and CD in IBD patients and less useful in detecting IBD in presenting patients. Currently available serological tests that evaluate specific microbial or leukocyte antigens include the following:

Anti-*Saccharomyces cervisiae* antibodies of the IgA or IgG class (ASCA) can be detected via an ELISA. ASCA are formed against oligomannosidic carbohydrate epitopes of the yeast *Saccharomyces cervisiase* which has been associated with CD but not UC.[2] Reported prevalence of ASCA has been described as 60%-70% in patients with CD, 10%-15% in patients with UC, and 0%-5% in control subjects.[2,3] In differentiating patients with IBD from controls, the sensitivity is 60% with a specificity of 88%-91% and a positive predictive value (PPV) of 82%.[1,3,4] ASCA results are currently reported as 'present' or 'not present'. Quantitative reporting of ASCA is not commonly used. The reporting of titers or the use of reference-ranges for ASCA have not entered routine clinical practice since they do not appear to aid in the diagnosis or prognosis or IBD.

Perinuclear anti-neutrophilic cytoplasmic antibody (pANCA) is also available to aid in the diagnosis of IBD. There are two available assays, indirect immunofluorescense, which demonstrates a specific perinuclear binding pattern when positive, and a fixed neutrophil ELISA, which provides a quantitative measure of neutrophil-specific nuclear antibodies when present. With the indirect immunofluorescense assay, the reported sensitivity and specificity in detecting patients with UC from controls is 60%-80% and 90%, respectively.[2,5] Unfortunately, up to 20% of patients with Crohn's disease are also pANCA positive; these individuals often present clinically with UC-like colitis.[2,3]

Antibodies against certain gut bacteria are also associated with IBD. Antibodies against *Escherichia coli, Pseudomonas fluorescens,* and *Bacteroides caccae* have all been evaluated as biomarkers for IBD. The anti-outer membrane protein C (anti-OmpC) antibody is made against the outer membrane porin antigens from *E. coli*. These antibodies have shown some value in IBD diagnosis and can be detected via ELISA. This antibody is found in 11%-55% patients with IBD.[1,6] Antibodies against Crohn's disease-related protein from *P. fluoresces* (I2) can be found in 50% of patients with Crohn's disease.[7] A recent study by Iltanen and others showed that anti-I2 and anti-OmpW (an antibody to a Ton-B-linked outer membrane protein of *B. caccae*) were significantly elevated in children with IBD compared to controls.[8]

Initially, CBir1 flagellin was identified as an immunodominant antigen of enteric microbial flora in the mouse model.[8,9] Flagellin is a common bacterial antigen present on most motile bacteria in the gut and is highly antigenic.[8] Using an IgG-based ELISA, a study found that 50% of patients with Crohn's have serum reactivity to CBir1 flagellin whereas patients with UC, irritable bowel syndrome, or controls had little or no reactivity.[8] Subsequent studies demonstrated that the presence of this antibody in individuals with Crohn's was associated with more clinically active disease, specifically higher prevalence of small bowel disease, internal penetrating, or fibro-stenosing complications.[9,10]

Although the presence of bacterial antibodies in patients with IBD is well documented, the pathogenic implications for these antibodies is less clear. The currently accepted hypothesis for the pathogenic mechanism for IBD is that the chronic intestinal inflammation (and the resultant or related systemic manifestations) are due to an overly aggressive immune response to resident luminal bacterial constituents. Therefore, the presence of antibodies to one or more bacterial antigens in IBD patients is perhaps not surprising.

Another biomarker whose utility in IBD is worth discussion is C-reactive protein (CRP). CRP is synthesized in the liver and is a sensitive marker of inflammation. During acute inflammation, CRP can increase as much as one thousand fold.[11] A study performed in 2002 showed that when using an ELISA for CRP, a cut-off value of 2.3 mg/L had a sensitivity of 100% and a specificity of 67% in differentiating non-IBD, functional bowel diseases from new cases of IBD.[12] CRP would thus appear to be the most sensitive marker yet found in detecting IBD but it has low specificity due to the fact that CRP is also known to be elevated in a number of other conditions, such as active infection (tuberculosis, pneumonia, and other bacterial infections), other inflammatory processes (inflammatory rheumatic arthritis, lupus, pancreatitis, myocardial infarction, and malignancy), pregnancy, and medications (such as oral contraceptives). CRP is also not promising in its ability to differentiate UC from CD. A recent review on the role of CRP in diagnosing GI disease concludes that CRP should be seen as an additional tool that can supplement clinical and physical observation but cannot replace it.[13]

In addition to serologic markers, there also exist fecal assays that may be useful to the gastroenterologist. Lactoferrin

is an iron-binding glycoprotein secreted by most mucous membranes and a major component of the secondary granules released by activated neutrophils. Neutrophils are often the first responders in the acute inflammatory response. Subsequent players in the immune response (monocytes and lymphocytes) do not contain lactoferrin. Thus, the presence of lactoferrin in feces is felt to be an early indicator of acute inflammation in the intestinal mucosa. The fecal latex agglutination test, a qualitatitive assay, has been FDA- approved as a tool in screening for colonic inflammation.[14-16] In the setting of acute diarrhea, it has been used in place of microscopic fecal leukocyte analysis to assist in the diagnosis of infectious colitis, such as bacillary dysentery or *Clostridium difficile*-induced pseudomembranous colitis.[17] Its advantages over microscopy include increased sensitivity and specificity, no refrigeration of specimen, a long time table in which the test can be performed (microscopy must be performed within 24 hours to 48 hours on refrigerated specimen), and decreased subjective bias in interpretations of results.[14] In the setting of chronic diarrhea, the fecal lactoferrin test may be of use as a non-invasive screening test for IBD. Studies have demonstrated that the rapid latex agglutination test and quantitative ELISA assay are sensitive and specific enough for detecting inflammation in chronic IBD patients. The reported sensitivity, specificity, and positive and negative predictive values of the rapid fecal latex agglutination test in IBD were 90%, 98%, 82%, and 99%, respectively. For the ELISA assay the values were 86%, 100%, 100%, and 87%, respectively.[16] It is interesting to note that there was no difference in the fecal lactoferrin content between controls and individuals with irritable bowel syndrome.[17] Presently, there are no trials comparing fecal lactoferrin levels with other inflammatory colitidies (e.g., microscopic colitis, diverticulitis, pouchitis, ischemic colitis, celiac sprue, or cancer). It is unlikely that fecal lactoferrin can discriminate IBD from other inflammatory conditions of the colon, such as microscopic colitis. Its use is currently limited to detecting inflammation in persons already diagnosed with IBD.

Another IBD-related fecal biomarker is calprotectin. Calprotectin is a calcium and zinc-binding protein derived from neutrophils and to a lesser extent, monocytes and macrophages. Measurement of calprotectin, like lactoferrin, is thus a marker of neutrophil activity in the lumen of the bowel. Calprotectin levels correlate with gut inflammation and may be more predictive of UC versus CD, however there is controversy in this claim.[18] Calprotectin appears to have very good potential in pediatric settings both in the differential diagnosis and in selecting which patients should undergo further diagnostic colonoscopy.[19,20] It has also been shown to be predictive of relapse in patients with CD and UC.[21]

Another calcium-binding protein similar to calprotectin, referred to as S100A12, has recently been reported to be a marker of gut inflammation. S100A12 levels are increased in the serum of children with IBD.[22] De Jong and others report that a sensitivity of 96% and a specificity of 92% can be achieved when 10 mg/kg fecal S100A12 was used as a cutoff. These findings give hope that non-invasive sensitive biomarkers for IBD in pediatric patients can be found.

MULTI-MARKER PANELS

With the hope of improving predictive power for diagnostic use, several investigators have examined panels using several of these biomarkers. A commercially available panel (IBD first-step®, Prometheus Laboratories, San Diego CA*)* that includes pANCA and ASCA was examined in a pediatric IBD population by Zholudev and others. They reported that if one or more of the antibodies were present, the overall sensitivity of the panel was 65% for CD and 76% for UC with specificity of 94%.[6] The prevalence of IBD in their study population was near 68% with a calculated PPV and NPV to be 96% and 59%, respectively.[1] If applied to the general population with a much lower prevalence of IBD (<5%), the PPV drops to 35% with NPV of 98%.[1] Iltanen and colleagues found that the combination of anti-OmpW, anti-I2, and ASCA identified 94% of pediatric CD patients, while a combination of anti-OmpW, anti-I2, and pANCA detected 83% of UC cases.[23] Although this was a relatively small study, the results are promising, suggesting multi-marker panels may have decent sensitivity in children. To our knowledge, a large study using most of the above mentioned markers in tandem (CRP, lactoferrin, ASCA, pANCA, anti-OmpW, anti-I2 and calprotectin, for example) has not been conducted. One would suspect that such a panel could have greatly enhanced sensitivity and specificity.

CLINICAL PRACTICE

Despite the successes in finding biomarkers of IBD, serological markers currently have a very limited role, if any, in the diagnostic work-up of adult IBD patients. For example, if the clinical suspicion is high, negative markers would not preclude the appropriate radiographic and endoscopic examinations. However, if the clinical suspicion is low, positive markers may subject the patient to unnecessary invasive testing. If clinical suspicion is high and markers are positive, the patient will still undergo radiographic and endoscopic evaluation, as the markers provide no information on extent,

location, or severity of the disease. Therefore because of their relatively low sensitivity and specificity, these tests are of little use in the diagnostic work-up of IBD and most likely subject the patient to additional blood draws and cost without providing much additional information. At present, the usefulness of these biomarkers may be limited to assisting in the differentiation between UC and CD. The importance of differentiating between the forms of IBD comes into play in the management (medical and surgical therapy) and overall prognosis of the disease. As UC is limited to the colon, there are medications that target the colon and surgical removal of the colon can "cure" the patient of the disease. At this time, despite using endoscopic, clinical, and radiographic information, ten percent of patients with IBD are unfortunately mislabeled. It is the hope therefore, that as the etiology and epidemiology of IBD continues to be studied and as new serological markers are discovered with improved sensitivity and specificity, the percentage of mislabeled patients will be diminished. Currently serological markers for IBD are not yet widely used in the adult population. In our hospital, panels of markers are available by ordering through our reference laboratory (Mayo Medical Laboratories; performed by Prometheus Labs).

FUTURE TRENDS

Not surprisingly, inflammatory cytokines are associated with IBD. Although many cytokines have been implicated in the pathogenesis of IBD, most of these cytokines are not increased in the serum of IBD patients.[24] Given the pleiotropic nature of cytokines and the fact that they play a role in so many inflammatory conditions one would assume that elevated levels would not be diagnostic for a single, particular disease. However there have been a few studies which suggest serum cytokine levels may have diagnostic value in IBD. Tumor necrosis factor-α (TNF-α) has long been associated with IBD. For example, the drug infliximab, a chimeric IgG1k monoclonal antibody targeted against TNF-α, is FDA-approved for the treatment of CD and UC. It can therefore be reasoned that inflammatory cytokines might have diagnostic use in IBD. Using the very sensitive technique of immuno-PCR, Komatsu and others found that TNF-α in the serum of IBD patients was approximately 390 times higher than in controls.[25] In another study, this group was also able to show that interleukin 18 (IL-18) was 1.7 fold higher in Crohn's disease than in normal controls and was not increased in UC, although this was a small study.[26]

Interesting data is now being published which shows that serotonin signaling is altered in IBD and even in IBS.[27] There are now numerous studies which show that these changes occur in various animal models of colitis.[28-31] These studies specifically demonstrate that there is a loss of the serotonin transporter in the intestinal mucosa of IBD patients and animals with colitis. This decrease in serotonin transporters seems to be an effect of IBD and may even contribute to the functional changes in motility, secretion, and sensation that occur in the IBD patients. Perhaps the measurement of serotonin transporters and/or receptors can be used in the future to aid in the diagnosis of IBD as well as in the development of novel treatments, improving the prognosis of IBD.

SUMMARY

Currently there is no single biomarker or panel of markers which is diagnostic of IBD generally, or CD and UC specifically. Diagnosis of IBD still requires radiographic, endoscopic, and microscopic examination. Since GI markers of inflammation are continuously being uncovered, and given that statistical power can be enhanced with multi-marker panels, we are hopeful that the medical laboratory will have the ability, in the near future, to support a non-invasive diagnosis of an inflammatory bowel disease.

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