Inflammatory Bowel Disease: Serologic Markers and Pharmacogenomic and Metabolic Assessment of Thiopurine Therapy

Number: 0249

Policy

*Please see amendment for Pennsylvania Medicaid at the end of this CPB.

I. Aetna considers TPMT gene mutation or TPMT phenotypic assays (e.g., Prometheus TPMT Genetics, Prometheus TPMT Enzyme) medically necessary prior to initiation of 6-mercaptopurine or azathioprine therapy. Only 1 genotypic or phenotypic assay of TPMT activity is necessary per member per lifetime. TPMT gene mutation assays and TPMT phenotypic assays are considered experimental and investigational for all other indications because their effectiveness for indications other than the one listed above has not been established.

II. Aetna considers testing for anti-chitobioside carbohydrate antibodies (ACCA), anti-laminaribioside carbohydrate antibodies (ALCA), anti-mannobioside carbohydrate antibodies (AMCA), anti-chitin IgA (Anti-C), and anti-laminarin IgA (Anti-L), anti-neutrophil cytoplasmic antibodies (ANCA), anti-Saccharomyces cerevisiae antibodies (ASCA), anti-outer membrane porin C (OmpC) antibodies, anti-CBir1 flagellin (anti-CBir1) antibodies, and I2 antibodies experimental and investigational to diagnose inflammatory bowel disease, to distinguish ulcerative colitis from Crohn's disease, and for all other indications because their effectiveness has not been established.

III. Aetna considers anti-smooth muscle antibodies (ASMA) experimental and investigational to diagnose inflammatory bowel disease or to distinguish ulcerative colitis from Crohn's disease because its effectiveness for these indications has not been
established. (Note: ASMA may be medically necessary to diagnose autoimmune hepatitis).

IV. Aetna considers measurements of 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine nucleotide (6-MMPN) (e.g., Prometheus Thiopurine Metabolites) medically necessary to monitor compliance in those not responding to 6-mercaptopurine and azathioprine therapy and to assess suspected toxicity.

V. Aetna considers fecal measurement of calprotectin experimental and investigational for the management of inflammatory bowel diseases (e.g., Crohn's disease, ulcerative colitis) and other indications because its clinical value has not been established.

VI. Aetna considers NOD2/CARD15 genotyping experimental and investigational because its clinical value has not been established.

VII. Aetna considers CD14 gene C-260T polymorphism testing, interleukin-10-1082A/G polymorphism testing, Prometheus Crohn's Prognostic, Prometheus IBD sgi diagnostic panel and the Prometheus IBS diagnostic panel (including BDNF [brain-derived neurotrophic factor], GRO-a [growth-regulated oncogene-alpha], IL-1β [interleukin-1 beta], IL-6, IL-8, macrophage inflammatory protein-1β (MIP-1β), NGAL [neutrophil gelatinase-associated lipocalin], TIMP-1 [tissue inhibitor of metalloproteinase-1], tTG [anti-human tissue transglutaminase IgA], and TWEAK [TNF-related weak inducer of apoptosis]) experimental and investigational for the diagnosis and management of inflammatory bowel diseases because their clinical values have not been established.

VIII. Aetna considers pre-operative serologic screening to identify individuals at increased risk of recurrence of Crohn’s disease experimental and investigational because the effectiveness of this approach has not been established.

IX. Aetna considers the following tests experimental and investigational because their clinical values have not been established.

- Crohn's disease peptide antibody testing
- ECM1 and Stat-3 testing for ulcerative colitis
- eXaIBD test (a quantitative PCR test for 6 genes [BLCAP, UBE2G1, GPX1, RAP1A, CALM3 and NONO]),
- Measurement of serum mannose-binding lectin
- Myeloperoxidase antibody testing for inflammatory bowel disease,
Proteinase-3 antibody testing,

Raman spectroscopy for inflammatory bowel disease

Serum amyloid A as a biomarker of disease activity in Crohn's disease

Whole blood gene expression analysis in the evaluation of Crohn's disease and ulcerative colitis

See also CPB 0341 - Infliximab (for measurements of serum levels of infliximab and antibodies to infliximab), and CPB 0715 - Pharmacogenetic and Pharmacodynamic Testing.

Background

Serologic Markers of Inflammatory Bowel Disease:

A serology panel including anti-neutrophil cytoplasmic antibodies (ANCA), anti-Saccharomyces cerevisiae IgG and IgA antibodies (ASCA), and anti-OmpC antibodies (outer membrane porin from E. coli) are marketed by Prometheus Laboratories (San Diego, CA) as the IBD First Step. This panel has not been shown to have levels of specificity sufficient to distinguish ulcerative colitis (UC) from Crohn's disease (CD) in indeterminate cases.

Research into the pathogenesis of inflammatory bowel disease in the areas of mucosal immunology, genetics, the role of bacterial products, and mediators of tissue damage has identified new sets of “subclinical” serological markers known as anti-neutrophil cytoplasmic antibodies (ANCA). ANCA have also been found to be associated with Wegener's granulomatosis and other forms of systemic vasculitides, and more recently with sclerosing cholangitis and other autoimmune liver diseases.

“Atypical” ANCA yielding a perinuclear staining pattern (pANCA) with alcohol-fixed neutrophils is primarily found in patients with UC; pANCA has been found to be detectable in 50 to 80 % of patients with UC, and 10 to 40 % of patients with CD. Anti-Saccharomyces cerevisiae antibody (ASCA) is primarily detected in patients with CD; ASCA has been found to be detectable in 46 to 70 % of patients with CD and 6 to 12 % of patients with UC.

These tests, however, have insufficient sensitivity to diagnose UC or CD. In a paper for the North American Society for Pediatric Gastroenterology and Nutrition, Griffiths concluded that “the relatively low sensitivities of serology for [Crohn's disease] and [ulcerative colitis] as documented in all studies argue against there being any greater value of ASCA/ANCA as routine or first-line screening tests for [inflammatory bowel disease] in comparison to clinical acumen and the equally sensitive (albeit less specific) measurement of acute phase reactants.
Moreover, the need for performance of definitive radiologic and endoscopic studies to guide therapy by defining the extent and nature of inflammatory bowel diseases (IBD) will not be averted by positive serologic tests.” Gupta et al (2004) examined the concordance of serologic testing for inflammatory bowel disease with clinical diagnosis established by traditional testing in children. The investigators found that the sensitivity of serologic testing is insufficient to replace traditional studies when evaluating children for inflammatory bowel disease. The investigators evaluated the results of ANCA and ASCA testing in 107 children who had serologic testing for inflammatory bowel disease (IBD) at their center, and compared these results with their clinical diagnosis. The investigators calculated that the sensitivity, specificity, positive and negative predictive values of serologic testing for ulcerative colitis were 69.2, 95.1, 90.0 and 87.1 %, respectively, and for CD were 54.1, 96.8, 90.9 and 80.8 % respectively. The investigators concluded that “[t]he low sensitivity, especially for Crohn’s disease, precludes the possibility that the IBD Diagnostic System can replace traditional studies when evaluating for inflammatory bowel disease.”

Some investigators have proposed using these serologic tests to differentiate CD from UC (e.g., Kornbluth and Sachar, 2004). Differentiation of CD from UC is clinically problematic only when inflammation is confined to the colon. A number of studies have reported that IgA and IgG ASCA titers are significantly greater and highly specific for CD, and that and that pANCA positivity is highly specific for UC. However, there is much less published information concerning the subgroup of IBD patients with colitis only, where differentiation of UC from CD is clinically problematic. One investigator reported ASCA positivity in only 47 % of 17 patients with Crohn's colitis. Another investigator found only 32 % of 37 patients with Crohn's colitis were ASCA positive and pANCA negative. Conversely, studies have found that the majority of Crohn’s patients positive for pANCA have a UC-like presentations (Bentley et al, 2001; Ruemmele, 1998; Vasiliauskas et al, 1996). Griffiths explained, “hence, the usefulness of serology is less (where it is needed most), given the higher prevalence of pANCA positivity and the lower prevalence of ASCA positivity in CD confined to the colon”.

Similarly, whether or not ASCA/ANCA measurement may be helpful in classifying otherwise 'indeterminate' colitis can not as yet be ascertained. Only a few patients have been studied, and follow-up is too limited. In the only prospective study of serologic testing in indeterminate colitis, Joosens et al (2002) examined the results of serologic testing for ASCA or ANCA and final diagnosis of CD or UC after 6-year follow-up of 97 patients with indeterminate colitis. The largest group (47) of subjects were negative for both ANCA and ASCA; 3 of these had a final diagnosis of CD, 4 had a final diagnosis of UC, and 40 had a final diagnosis of indeterminate colitis. Of 26 subjects who were ASCA-positive and ANCA-negative, 8 had a final diagnosis of CD after 6 years follow-up, 2 had a final diagnosis of UC,
and 16 remained with a diagnosis of indeterminate colitis. Of 20 subjects who were ASCA-negative and ASCA-positive, 4 had a final diagnosis of CD, 7 had a final diagnosis of UC, and 9 had a final diagnosis of indeterminate colitis. Only 4 of the 97 subjects were positive for both ANCA and ASCA; 2 of these had a final diagnosis of CD, 1 had a final diagnosis of UC, and 1 remained with a final diagnosis of indeterminate colitis. Thus, about one-third (31%) of subjects who were ASCA-positive and ANCA-negative progressed to CD during the 6-year follow-up period, and about one-third (35%) of subjects who were ASCA-negative and ANCA-positive progressed to UC during the 6-year follow-up period. Joosens et al (2002) calculated that, thus far, the sensitivity of ASCA+/ANCA- for CD was 66.7% and the specificity is 77.8%, and the sensitivity of ASCA-/ASCA+ for UC is 77.8% and the specificity is 66.7%. Noting that these calculations exclude subjects who remained with the diagnosis of indeterminate colitis, a technology assessment of serologic testing for IBD by the Institute for Clinical Systems Improvement noted that only a “small number” (21) of subjects were included in this analysis of sensitivity and specificity. Based on their analysis of this prospective study and other published studies of serologic testing in indeterminate colitis, the ICSI concluded that the clinical utility of serologic testing in indeterminate colitis has not been established. It has also been noted that this study does not provide direct evidence of improvement in clinical outcomes by basing the management of persons with indeterminate colitis on serologic testing.

ANCA and ASCA testing has not been proven to be useful in selecting therapeutic interventions. “Although this would be desirable, there is no evidence as yet that serological test results can be used to predict the likelihood of therapeutic response to specific interventions,” Griffiths explained. In a prospective clinical study of CD patients, Esters et al (2002) found no significant relationship between these serologic markers and response to anti-tumor necrosis factor (TNF) therapy.

In addition, studies have not demonstrated correlation of ANCA or ASCA with disease activity, duration of illness, extent of disease, extra-intestinal complications, or surgical or medical treatment in patients with IBD. The Institute for Clinical Systems Improvement (ICSI) (2002) technology assessment of serologic testing for IBD concluded “[t]he clinical utility of serological testing is not yet established for the diagnosis of inflammatory bowel disease in patients presenting with symptoms suggestive of IBD” and “[t]he clinical utility of serological testing is not yet established for differentiating between UC and CD in patients with inflammatory bowel disease”.

Additional assays have been developed to use in conjunction with ANCA and ASCA in an effort to improve the diagnostic capabilities of serologic testing. OmpC IgA is an autoantibody to outer membrane porin to E. coli included in the Prometheus serology panel to enhance
detection of CD (Landers et al, 2002). I2 is an IgA antibody that has been detected in patients with CD. The I2 serologic response recognizes a novel homologue of the bacterial transcription-factor families from a Pseudomonas fluorescens associated sequence (Sandborn, 2004). However, there are no studies of the clinical utility of anti-OmpC and I2 IgA antibodies in distinguishing CD from UC in persons with IBD whose diagnosis cannot be established by standard methods.

Landers et al (2002) reported on serologic test results of a referral center population of 151 patients with CD. This study found that immune responses to specific antigens (ASCA, pANCA, OmpC, and I2) are not uniform among CD patients. ASCA was detected in 56 % of patients, OmpC in 55 % of patients, I2 in 50 % of patients, and ANCA in 23 % of patients. The investigators reported that 85 % of patients responded to at least 1 antigen and that only 4 % responded to all 4. This study did not demonstrate, however, how these serologic test results relate to clinical behavior and response to therapy. The authors stated that “[t]he relationship of these different patterns of immune responses to clinical behavior is not yet clear.” The authors concluded “[d]efining how these antibody reactivities relate to clinical behavior and response to therapeutic modalities will require larger numbers of phenotypically well-characterized patients.”

Mow et al (2004) reported on the results of an hypothesis generating study, the aim of which was to determine whether Crohn's patients with predominant serum antibody reactivity toward bacterial antigens OmpC and/or I2 were more likely to achieve remission with antibiotics. Study subjects were patients with moderately active right-sided colonic and/or small bowel CD who were participating in an 8-week randomized clinical trial comparing the steroid budesonide with or without the antibiotics metronidazole and ciprofloxacin. Subject's serum was analyzed for ASCA, pANCA, anti-OmpC, and anti-I2 antibodies, and subjects were put into 1 of 4 “profile groups” (ASCA, pANCA, anti-OmpC/I2, and no or little antibody) depending upon the subjects' levels of antibody response. Twenty-five of 121 subjects were excluded from the analysis because their level of antibody response did not fit the 4 predominant profile groups. Only 2 subjects had an ANCA predominant profile, and these subjects were excluded from the analysis. In the steroid plus antibiotic group, 5 of 11 subjects (45.5 %) with predominant OmpC and I2 antibodies achieved remission, 5 of 16 subjects with predominantly ASCA antibody (31.3 %) achieved remission (similar to the overall remission rate), and 5 of 21 subjects (23.8 %) with little or no antibodies achieved remission. In the steroid only group, 7 of 16 subjects (43.8 %) with little or no antibodies achieved remission, 8 of 20 (40 %) with predominantly ASCA antibody achieved remission, and 3 of 10 (30 %) subjects with predominant OmpC and I2 antibodies achieved remission. Although there was a trend toward greater responsiveness to therapy that includes antibiotics in subjects with
predominant OmpC and I2 antibodies, and a trend toward less responsiveness in subjects with little or no antibodies, these trends did not achieve statistical significance. The authors concluded that this hypothesis-generating study provides preliminary evidence to suggest that serologic information about CD patients may be helpful in defining patients who would best respond to therapy. The authors noted, however, that, "[a]lthough these trends are provocative, they lack statistical significance.” The authors concluded that 'p]rospective randomized placebo controlled trials that do not limit patient selection by disease location and do not have concomitant therapy are warranted to test this hypothesis.'

Mow et al (2004) evaluated the sera of 303 patients with CD to determine whether expression of certain antibodies is associated with phenotypic manifestations. The investigators found that patients expressing I2 were significantly more likely to have fibrostenosing CD (64.4 % versus 40.7 %), and to require small bowel surgery (62.2 % versus 37.4 %). Patients with anti-OmpC were more likely to have internal perforating disease (50 % versus 30.7 %) and to require small bowel surgery (61.4 % versus 44.2 %). The investigators stated that these findings suggest an association between these immune responses and CD complications. The investigators concluded that "[i]n the future, knowledge of serological response may help the clinician determine the risk for more severe disease characteristics and predict disease behaviors. As a result, it may be possible to tailor therapy more effectively on the basis of specific serological responses. However, these findings must be confirmed by prospective studies that evaluate the presence of these antibody responses and the development of complicated small bowel disease phenotypes." An editorial accompanying this study explained that this study is limited by its retrospective nature (Vermeire and Rutgeerts, 2004): "Therefore, it is important that these findings first be confirmed in independent series and more importantly, that prospective studies with these markers be conducted to assess the risk of microbial responses on the development of strictures and perforations and subsequent need for surgery."

The flagellin-like antigen, CBir1, is a associated with the presence of IBD. In particular, serum response to anti-CBir1 identifies the presence of CD and is associated with a subset of patients with this form of IBD. CBir1 is found in the C3H/HeJ Bir mouse model. It is believed that the Cdcs1 locus of the C3H/HeJ Bir mouse confers severe colitis associated with a decrease in innate immune function and an increase in adaptive T-cell responses to commensal bacterial products. No evidence-based clinical guidelines from leading medical professional organizations recommend the use of this marker.

Targan et al (2005) assessed serum response to CBir1 flagellin in CD patients and compared this response to responses defined previously to ASCA, I2, OmpC, and pANCA, and determined anti-CBir1-associated phenotypes. A total of 484 sera from the Cedars Sinai Medical Center
repository, previously typed for anti-Saccharomyces cerevisiae antibody, anti-I2, anti-OmpC, and pANCA were tested for anti-CBir1 by enzyme-linked immunosorbent assay, and results were evaluated for clinical phenotype associations. The presence and level of immunoglobulin G anti-CBir1 were associated with CD independently. Anti-CBir1 was present in all antibody subgroups and expression increased in parallel with increases in the number of antibody responses. pANCA+ CD patients were more reactive to CBir1 than were pANCA+ UC patients. Anti-CBir1 expression is associated independently with small-bowel, internal-penetrating, and fibrostenosing disease features. Levels of anti-CBir1 are increased in 50 to 55 % of patients with CD. The authors concluded that serum responses to CBir1 independently identify a unique subset of patients with complicated CD.

Dubinsky et al (2006) examined the association of immune responses to microbial antigens with disease behavior and prospectively determined the influence of immune reactivity on disease progression in pediatric CD patients. Sera were collected from 196 pediatric CD cases and tested for immune responses: anti-I2, anti-OmpC, anti-CBir1, and ASCA using enzyme-linked immunosorbent assay (ELISA). Associations between immune responses and clinical phenotype were evaluated. A total of 58 patients (28 %) developed internal penetrating and/or stricturing (IP/S) disease after a median follow-up of 18 months. Both anti-OmpC (p < 0.0006) and anti-I2 (p < 0.003) were associated with IP/S disease. The frequency of IP/S disease increased with increasing number of immune responses (p trend = 0.002). The odds of developing IP/S disease were highest in patients positive for all 4 immune responses (odds ratio [OR] (95 % confidence interval [CI]): 11 (1.5 to 80.4); p = 0.03). Pediatric CD patients positive for greater than or equal to 1 immune response progressed to IP/S disease sooner after diagnosis as compared to those negative for all immune responses (p < 0.03). The authors concluded that the presence and magnitude of immune responses to microbial antigens are significantly associated with more aggressive disease phenotypes among children with CD. This was the first study to prospectively demonstrate that the time to develop a disease complication in children is significantly faster in the presence of immune reactivity, thus predicting disease progression to more aggressive disease phenotypes among pediatric CD patients.

In a review on serological markers in IBD, Bossuyt (2006) stated that several antibodies have been associated with IBD, the 2 most comprehensively studied being autoantibodies to neutrophils (atypical pANCA) and ASCA. New microbial target antigens such as OmpC, I2, and the flagellin CBir1 have been described in patients with CD. There is evidence that the number and magnitude of immune responses to different microbial antigens are associated with the severity of the disease course. However, this should be confirmed by additional studies.
Benor et al (2010) compared the predictive values of the Prometheus Inflammatory Bowel Disease (IBD) Serology 7 (IBD7) panel (Prometheus Laboratories, San Diego, CA) with the predictive values of routine blood tests in a population of children referred for initial evaluation of suspected IBD. Medical records of pediatric patients referred for evaluation of IBD for whom IBD7 testing was performed at Prometheus Laboratories between January 2006 and November 2008 were reviewed. Patients underwent diagnosis by pediatric gastroenterologists on the basis of clinical, radiologic, endoscopic, and pathologic evaluations. A total of 394 records were identified. These investigators excluded 90 records on the basis of age of greater than 21 years, previous diagnosis of IBD, or unclear diagnosis. The prevalence of IBD in this cohort was 38%. The sensitivity, specificity, positive predictive value, negative predictive value, and kappa value for the serological panel were 67%, 76%, 63%, 79%, and 42%, respectively, compared with values for a combination of 3 abnormal routine laboratory test results of 72%, 94%, 85%, 79%, and 47%. The anti-flagellin antibody assay, the newest assay added to the panel, had sensitivity of 50% and specificity of 53%. Repeat serological testing failed to produce consistent results for 4 of 10 patients. The authors concluded that despite its recent inclusion of the anti-flagellin assay, the IBD7 panel has lower predictive values than routine laboratory tests in pediatric screening for IBD.

In summary, the testing for ANCA, ASCA, OmpC antibodies, I2 antibodies, and anti-CBir1 antibodies appears to be a promising approach to diagnose IBD, and to distinguish UC from CD.

Anti-glycan antibodies are also serological markers that are reportedly being utilized for testing in IBD. These markers are directed against microbial carbohydrate antigens and it’s purported that these markers are useful in the differentiation of CD versus UC. Anti-glycan antibodies include: anti-chitin (Anti-C), anti-chitobioside (ACCA), anti-laminaribioside (ALCA), anti-laminarin (Anti-L), anti-mannobioside (AMCA), and anti-smooth muscle antibody (ASMA), which detects autoantibodies directed against smooth muscle.

The anti-smooth muscle antibody (ASMA) test detects autoantibodies directed against smooth muscle. While this test has been employed in the management of patients with autoimmune hepatitis and cirrhosis, its use in the diagnosis of IBD has not been established.

The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn’s and Colitis Foundation of America’s consensus conference report on differentiating UC from CD in children and young adults (Bousvaros et al, 2007) stated that the clinical value of serology in patients with indeterminate colitis (IC) remains a topic of research, and further investigation should ascertain, among other areas, the role of surrogate laboratory markers (e.g., genetics, microbiology, and serology) in distinguishing these entities. A
proposed algorithm to aid clinicians in differentiating UC from CD does not include serological testing.

Mokrowiecka et al (2009) stated that the role of pANCA and ASCA assessment in IBD diagnosis and differentiating is still imprecise and controversial. These researchers determined the accuracy of pANCA and ASCA in patients with IBD subgroups. The study was performed in 125 patients: 71 patients with UC, 31 with CD and 23 with IC. Control group consists of 45 patients with functional intestinal disorders; pANCA and ASCA (IgA and IgG) were measured with ELISA, using commercial antibody panel. In UC patients the prevalence of pANCA was 68 %, which was significantly higher than in CD -- 29 %. ASCA were found significantly more often in CD -- 80.6 % than in UC patients -- 26.8 %. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of pANCA for UC diagnosis was 68 %, 84 %, 75 % and 78 %; and ASCA for CD: 81 %, 78 %, 45,5 % and 95 %, respectively. The combined use of these 2 markers gave changes in diagnostic accuracy: pANCA+/ASCA- in UC: 42 %, 100 %, 100 % and 43 %, and for pANCA-/ASCA+ in CD: 52 %, 98.6 % 94 % and 82 %, respectively. The authors concluded that the specificity of these combined markers tends to be higher than sensitivity, what made them more useful in the differentiation of the IBD subtypes rather than population screening. The characteristic IC serotype pANCA(-)ASCA(-) leads to further controversies about origin of this IBD subtype.

The American College of Gastroenterology practice guidelines for management of CD in adults (Lichtenstein et al, 2009) stated that serological studies evaluating ASCA, ANCA, anti-CBir1, anti-OmpC are evolving to provide adjunctive support for the diagnosis of CD; however, they are not sensitive or specific enough to be recommended for use as a screening tools.

Lembo and colleagues (2009) stated that currently, no single serum biomarker can reliably differentiate irritable bowel syndrome (IBS) from other functional gastrointestinal disorders or organic diseases of the gastrointestinal tract. These researchers attempted to develop and validate a diagnostic test using serum biomarkers to detect IBS. A total of 10 serum biomarkers (ANCA, ASCA-IgA, BDNF [brain-derived neurotrophic factor], CBir1, GRO-a [growth- regulated oncogene-alpha], IL-1β [interleukin -1 beta], NGAL [neutrophil gelatinase-associated lipocalin], TIMP-1 [tissue inhibitor of metalloproteinase-1], tTG [anti-human tissue transglutaminase IgA], and TWEAK [TNF-related weak inducer of apoptosis]) were selected from a potential panel of 140 for their ability to differentiate IBS from non-IBS disease in blood samples from patients with IBS, other gastrointestinal disorders and healthy volunteers. A predictive modelling tool was developed to assess patterns and relationships among the 10 serum biomarkers that best differentiated IBS patients from healthy controls and patients with non-IBS gastrointestinal disease. This model was tested in a different cohort of patients and healthy controls (n = 516) to determine the predictive accuracy of differentiating IBS from
non-IBS. The sensitivity and specificity of the 10-biomarker algorithm for differentiating IBS from non-IBS was 50 % and 88 %, respectively. The positive predictive value was 81 %, and the negative predictive value was 64 % at 50 % IBS prevalence in the validation cohort. Overall accuracy was 70 %. The value of this panel of biomarkers in the management of patients with IBS needs to be validated.

In a review on the importance of early diagnosis in patients with IBS, Halpert (2010) stated that the diagnosis of IBS is challenging because symptoms can vary between patients and overlap with those of other disorders. This investigator examined the current diagnostic approach in IBS and discussed new tools that may improve diagnostic confidence earlier in the process. The author noted that accumulating evidence suggests that fecal and/or serum biomarkers may be helpful in differentiating IBS from non-IBS disorders. These tools may help minimize unnecessary testing and diagnostic delays. As biomarkers are further studied and developed, they are likely to become an integral part of the diagnosis of IBS and reduce the potential for incorrect diagnosis and treatment delays.

Dotan (2010) stated that IBD are chronic intestinal disorders where, in genetically susceptible hosts, an intestinal microorganism triggers an over-reactive immune response. Antibodies against luminal antigens are specifically associated with CD. In addition to the previously described ANCA, ASCA, OmpC, I2 and CBir1 Flagellin, new anti-glycan antibodies were recently added to the armamentarium of serologic markers in IBD. The anti-glycan antibodies are directed against laminaribioside, chitobioside, mannobioside and mannan residues and are designated anti-laminaribioside carbohydrate antibodies (ALCA), anti-chitobioside carbohydrate antibodies (ACCA), anti-mannobioside carbohydrate antibodies (AMCA) and gASCA, respectively. Anti-laminarin IgA (Anti-L), and anti-chitin IgA (Anti-C) are new members of this family. Laminarin and chitobioside are capable of stimulating the innate immune system, thus the finding of antibodies against these glycans suggests a connection between the adaptive and innate arms of the immune response in CD patients. The contribution of serologic markers, specifically the anti-glycan antibodies, to IBD diagnosis may be in differentiating IBD from other gastrointestinal diseases, and between CD and UC, in better classifying undetermined colitis and for decision-making prior to proctocolectomy in UC patients. The anti-glycan antibodies are specifically important in ASCA-negative CD patients. Correlation between serologic markers and genetic variations may contribute to re-classifying IBD into new and more homogeneous subclasses. Their significance in diagnosing populations at risk, such as unaffected relatives of IBD patients and CD patients prior to diagnosis, is under current investigation.

Silberer et al (2005) compared 5 different leukocyte proteins in feces of patients with chronic IBD, irritable bowel syndrome (IBS) and healthy persons who underwent prophylactic
colonoscopy. The leukocyte proteins calprotectin, lactoferrin, lysozyme, myeloperoxidase (MPO), and PMN-elastase were determined with immunoassays in fecal samples of 3 consecutive feces (e.g., 3 days) in 40 healthy persons, 39 patients with chronic IBD (of these 21 with Crohn's disease and 18 with ulcerative colitis), and 40 patients with IBS. Receiver operating characteristics (ROC) curves calculated for healthy persons and patients with IBD yielded the following areas under the curves (AUCs): PMN-elastase 0.916, calprotectin 0.872, MPO 0.750, lysozyme 0.726, and lactoferrin 0.693. The AUCs of PMN-elastase and calprotectin were not significantly different (p = 0.327), whereas PMN-elastase or calprotectin versus the other proteins were significantly different (p < 0.001). PMN-elastase and calprotectin correlated with the endoscopically classified severity of inflammation. All fecal leukocyte markers in IBS were found in the range of the healthy persons. Data on storage stability of leukocyte proteins in fecal supernatants are given. The authors concluded that fecal PMN-elastase and calprotectin support the differentiation of chronic IBD from IBS and correlate with the severity of inflammation.

Lettesjo et al (2006) examined if inflammatory markers could be detected in feces from patients with IBS and collagenous colitis (CC), and elucidated if such analyses could be used as non-invasive tools to distinguish between these disorders. Stool samples were obtained from 18 patients with CC, 46 patients with IBS and 20 healthy controls (HC). Eosinophil protein X (EPX), MPO, tryptase, interleukin-1 beta (IL-1beta) and tumor necrosis factor alpha (TNFalpha) were measured in supernatants from processed feces using immunoassays. EPX levels were enhanced in feces from CC patients (median 3.8 microg/g (0.47 to 16.2)) compared to patients with IBS (0.44 microg/g (0.25 to 1.8)), p < 0.001, and HC (0.46 microg/g (0.21 to 1.3)), p < 0.001. In addition, MPO was increased in CC patients (11.7 microg/g (2.0-124)) compared to IBS patients (1.7 microg/g (0.81 to 5.2)), p < 0.01, and HC (2.5 microg/g (1.1 to 6.3)), p < 0.05. Tryptase was found in 9/18 patients with CC, 6/46 with IBS and 1/19 HC. IL-1beta was only enhanced in 2/11 CC patients and TNFalpha was not detected in any sample. The authors concluded that increased levels of EPX, MPO and tryptase were observed in stools from CC patients, whereas the levels in IBS patients did not differ from healthy controls. These findings suggested that fecal markers could be used as part of the clinical work-up to determine which patients should be biopsied and evaluated for CC.

A recent review by Navaneethan et al (2009) on laboratory tests for ileal pouch-anal anastomosis noted the need for further studies validating the role of anti-CBIR1 as a laboratory marker to predict pouchitis, and concluding that serologic tests are primarily for research purposes.

Booth et al (2011) noted that the evidence for testing TPMT enzymatic activity or genotype before starting therapy with thiopurine-based drugs is unclear. These investigators examined
the sensitivity and specificity of TPMT genotyping for TPMT enzymatic activity, reducing harm from thiopurine by pre-testing, and the association of thiopurine toxicity with TPMT status in adults and children with chronic inflammatory diseases. MEDLINE, EMBASE, the Cochrane Library, and Ovid HealthSTAR (from inception to December 2010) and BIOSIS and Genetics Abstracts (to May 2009) were used for this review. Two reviewers screened records and identified relevant studies in English. Data on patient characteristics, outcomes, and risk for bias were extracted by one reviewer and independently identified by another. A total of 54 observational studies and 1 randomized, controlled trial were included. Insufficient evidence addressed the effectiveness of pre-testing. Genotyping sensitivity to identify patients with low and intermediate TPMT enzymatic activity ranged from 70.33 % to 86.15 % (lower-bound 95 % CI: 54.52 % to 70.88 %; upper-bound CI: 78.50 % to 96.33 %). Sparse data precluded estimation of genotype sensitivity to identify patients with low to absent enzymatic activity. Genotyping specificity approached 100 %. Compared with non-carriers, heterozygous and homozygous genotypes were both associated with leukopenia (OR, 4.29 [CI: 2.67 to 6.89] and 20.84 [CI: 3.42 to 126.89], respectively). Compared with intermediate or normal activity, low TPMT enzymatic activity was significantly associated with myelotoxicity and leukopenia. The authors concluded that insufficient evidence addresses the effectiveness of TPMT pre-testing in patients with chronic inflammatory diseases -- insufficient evidence demonstrating that this strategy is effective in reducing harm or is superior to the established clinical standard of hematological monitoring.

The new PROMETHEUS IBD sgi Diagnostic is the 4th-generation irritable bowel disease (IBD) diagnostic test and the first and only test to combine serologic, genetic, and inflammation markers in the proprietary Smart Diagnostic Algorithm for added diagnostic clarity. This test aids healthcare providers in differentiating IBD versus non-IBD as well as CD versus UC in 1 comprehensive blood test. This assay includes 9 serological markers including the proprietary Anti-Fla-X, Anti-A4-Fla2, anti-CBir1, anti-OmpC, and DNAse-sensitive pANCA that helps identify patients with IBD and utilizes Smart Diagnostic Algorithm Technology to improve the predictive accuracy. Genetic susceptibility influences immune responses and this assay includes evaluation of ATG16L1, STAT3, NXX2-3, and ECM1. Inflammatory markers include VEGF, ICAM, VCAM, C-reactive protein (CRP), SAA. While most other labs only offer assay values, PROMETHEUS IBD sgi Diagnostic provides added clarity in diagnosing IBD, UC, and CD.

One of the references cited in the Prometheus website was a study on genetics of the ulcerative colitis by Thompson and Lees (2010). The authors concluded that "[t]hese advances will not in short term provide additional diagnostic or prognostic value. There is also no reason necessarily why polymorphisms that contribute to disease susceptibility should predict disease course, and almost certainly have no bearing on treatment response and intolerance."
Prospective studies are required to address the genetic contributions to these clinically important areas, and will become a major focus in the next few years”.

Adler et al (2011) noted that CD is often purely inflammatory at presentation, but most patients develop strictures and fistulae over time (complicated disease). Many studies have suggested that nucleotide-binding oligomerization domain 2 (NOD2) mutations are associated with a varying but increased risk of complicated disease. An accurate and sufficiently powerful predictor of complicated disease could justify the early use of biological therapy in high-risk individuals. These researchers performed a systematic review and meta-analysis to obtain accurate estimates of the predictive power of the identified mutations (such as p.R702W, P.G908R, and p.Leu1007fsX1008) in NOD2 for the risk of complicated disease. An electronic search of MEDLINE, Embase, and Web of Science identified 917 relevant papers. Inclusion required specification of genetic mutations at the individual level and disease phenotypes by Vienna classification (inflammatory (B1), stricturing (B2), and fistulizing (B3)). A total of 49 studies met these criteria, which included 8,893 subjects, 2,897 of whom had NOD2 mutations. Studies were weighted by median disease duration. Studies not providing duration data were weighted at the level of the study with the shortest disease duration (3.9 years). The relative risk (RR) of the presence of any NOD2 mutant allele for complicated disease (B2 or B3) was 1.17 (95 % confidence interval (95 % CI): 1.10 to 1.24; p < 0.001). P.G908R was associated with an RR of complicated disease of 1.33 (95 % CI: 1.11 to 1.60; p = 0.002). NOD2 did not predict peri-anal disease (p = 0.4). The RR of surgery was 1.58 (95 % CI: 1.38 to 1.80; p < 0.001). There was substantial heterogeneity across all studies (I(2) = 66.7 %). On the basis of logistic regression of these data, the sensitivity of any mutation in predicting complicated disease was 36 % and specificity was 73 %, with the area under the receiver operating characteristic curve 0.56. The authors concluded that the presence of a single NOD2 mutation predicted an 8 % increase in the risk for complicated disease (B2 or B3), and a 41 % increase with 2 mutations. Surgery risk is increased by 58 % with any NOD2 mutation, whereas peri-anal disease was unchanged. The predictive power associated with a single NOD2 mutation is weak. The RR of any NOD2 mutations for complicated disease was only 17 % across 36 studies. However, the presence of two NOD2 mutations had 98 % specificity for complicated disease. These data provide insufficient evidence to support top-down therapy based solely on single NOD2 mutations, but suggest that targeted early-intensive therapy for high-risk patients with two NOD2 mutations might be beneficial, if prospective trials can demonstrate changes in the natural history in this subset of patients.

Bi et al (2011) stated that UC and CD are 2 distinct forms of IBD that can overlap radiologically, endoscopically, and pathologically. This difficulty complicates surgical options. The development of new technologies providing accurate diagnosis of IBD is needed. Raman
spectroscopy is a non-invasive method that uses the intrinsic properties of tissue and that tissue’s vibrational energy in reaction to light. These investigators hypothesized that Raman spectroscopy can detect the structural and compositional changes that occur in the tissue during the development of IBD, and thus may offer increased diagnostic certainty in the differentiation between CD and UC. Fresh frozen colon tissue biopsies from patients with UC (n = 12) and with CD (n = 9) were measured in-vitro using a custom-designed Raman fiber-optic probe. For spectra collection, the probe was placed in gentle contact with the mucosa surface for 3 seconds, with excitation power at 150 mW. Five spectra were acquired from each biopsy to increase the signal-to-noise ratio and to ensure repeatability of data collection. Mean spectra were analyzed for peak difference and molecular origin. Significant difference was observed between the spectra from each disease in the spectral regions assigned to nucleic acid, phenylalanine, and lipids. Tissue samples from patients with UC demonstrated higher content of lipid and lower amount of phenylalanine and nucleic acid. These characteristic Raman features could serve as spectral markers that can potentially be applied to distinguish UC and CD. The authors concluded that this study presented the only application of Raman spectroscopy in the diagnosis of IBD. The feasibility of this technique in differentially detecting molecular alterations in UC and CD has been demonstrated, indicating the potential to improve diagnostic accuracy of IBD.

Wang et al (2012) noted that the gene encoding CD14 has been proposed as an IBD-susceptibility gene with its polymorphism C-260T being widely evaluated, yet with conflicting results. These researchers investigated the association between this polymorphism and IBD by conducting a meta-analysis. A total of 17 articles met the inclusion criteria, which included a total of 18 case-control studies, including 1,900 UC cases, 2,535 CD cases, and 4,004 controls. Data were analyzed using STATA software. Overall, association between C-260T polymorphism and increased UC risk was significant in allelic comparison (OR = 1.21, 95 % CI: 1.02 to 1.43; p = 0.027), homozygote model (OR = 1.44, 95 % CI: 1.03 to 2.01; p = 0.033), as well as dominant model (OR = 1.36, 95 % CI: 1.06 to 1.75; p = 0.016). However, there was negative association between this polymorphism and CD risk across all genetic models. Subgroup analyses by ethnicity suggested the risk-conferring profiles of -260T allele and -260 TT genotype with UC in Asians, but not in Caucasians. There was a low probability of publication bias. The authors concluded that expanding previous results of individual studies, these findings demonstrated that CD14 gene C-260T polymorphism might be a promising candidate marker in susceptibility to UC, especially in Asians.

Zhu et al (2013) stated that a large number of studies have shown that the interleukin-10 (IL-10)-1082A/G polymorphism is implicated in susceptibility to IBD. However, the results are inconsistent. These investigators performed a meta-analysis to estimate the association
between -1082A/G polymorphism in the IL-10 gene and IBD susceptibility. A total number of 18 case-control studies including 17,585 subjects were identified. No association was found between -1082A/G polymorphism and UC susceptibility. However, increased risk of CD was associated with -1082A/G polymorphism in the dominant genetic model (GG+GA versus AA: OR = 1.22, 95 % CI: 1.02 to 1.46, p = 0.028) and the heterozygote comparison (GA versus AA: OR = 1.28, 95 % CI: 1.05 to 1.55, p = 0.015). The authors concluded that the results of this meta-analysis provided evidence for the association between IL-10-1082A/G polymorphism and susceptibility of CD. However, they noted that due to several limitations in the present study, well-designed epidemiological studies with large sample size among different ethnicities should be performed in the future.

Kovacs and associates (2013) stated that mannose-binding lectin (MBL) is a pattern-recognition molecule of the innate immune system and may be involved in the pathogenesis of IBD. These researchers evaluated the prevalence of MBL deficiency in a cohort of patients with pediatric-onset IBD and examined if it is associated with the clinical manifestations, serum antibody formation, or genetic factors. This prospective study included 159 pediatric patients (mean age of 14.0 years) with IBD (107 patients with CD and 52 patients with UC). Furthermore, 95 controls were investigated. Serum samples were determined for MBL by ELISA and for serologic markers (ASCA and pANCA) by indirect immunofluorescent assay. NOD2/CARD15 variants were tested by PCR/restriction fragment length polymorphism. The MBL serum concentration was significantly lower in IBD patients (both with CD and UC) compared to controls (IBD, p = 0.007; CD, p = 0.04; UC, p = 0.004). Prevalence of low MBL level (less than 500 ng/ml) was significantly higher in both CD and UC groups compared to controls (p = 0.002 and p = 0.006). Furthermore, low MBL level was associated with isolated ileal involvement (p = 0.01) and MBL deficiency (less than 100 ng/ml) with male gender (p = 0.004) in patients with CD. These investigators failed to confirm any correlation between MBL deficiency and serum autoantibodies or NOD2/CARD15 variants. The authors concluded that these findings suggested that low MBL associated with pediatric-onset IBD and ileal CD may be considered an additional marker of the IBD pathogenesis.

Moreover, an UpToDate review on “Clinical manifestations, diagnosis and prognosis of Crohn's disease in adults” (Peppercorn, 2013) does not mention measurement of mannose-binding lectin as a management tool.

Mitsuyama and colleagues (2014) stated that various non-invasive tests have been studied to screen for patients with CD, and were found to have limited accuracy and sensitivity, particularly in Asian populations. These investigators explored the possible diagnostic utility of antibodies to the CD peptide (ACP) in patients with CD. In a multi-center study using ELISA, serum ACP levels were determined in 196 patients with CD, 210 with UC, 98 with other
intestinal diseases, 132 with other inflammatory diseases, and 183 healthy controls; and then examined for correlation to clinical variables. The diagnostic utility of ACP was evaluated by ROC analysis and compared with ASCA. Levels of ACP were significantly elevated in the CD patients, but not in the other groups that included UC, other intestinal diseases, other inflammatory diseases and the healthy controls. Among these other groups, ACP levels were not significantly different. In the CD patients, ACP had a higher sensitivity and specificity (63.3 % and 91.0 %, respectively) than ASCA (47.4 % and 90.4 %). Levels of ACP were negatively associated with disease duration, but not with Crohn’s Disease Activity Index (CDAI), disease location, or medical treatment. The authors concluded that ACP, a newly proposed serologic marker, was significantly associated with CD and was highly diagnostic. Moreover, they stated that further investigation is needed across multiple populations of patients and ethnic groups, and more importantly, in prospective studies to ascertain the clinical value of ACP.

Furthermore, an UpToDate review on “Clinical manifestations, diagnosis and prognosis of Crohn’s disease in adults” (Peppercorn, 2013) does not mention measurement of antibodies to the CD peptide as a management tool.

Adedokun et al (2014) analyzed data collected during the Active Ulcerative Colitis Trials (ACT-1 and ACT-2) to assess relationships between serum concentrations of infliximab and outcomes of adults with moderate-to-severe UC. These investigators compared serum concentrations of infliximab with outcomes of 728 patients with moderately-to-severely active UC who participated in ACT-1 or ACT-2; efficacy data were collected at weeks 8, 30, and 54 (for ACT-1 only). Relationships between serum concentration of infliximab and efficacy outcomes were assessed using trend, logistic regression, and receiver operating characteristic curve analyses. They also evaluated factors that affected the relationship between exposure and response. Median serum concentrations of infliximab at weeks 8, 30, and/or 54 were significantly higher in patients with clinical response, mucosal healing, and/or clinical remission than in patients who did not meet these response criteria. There were statistically significant relationships between quartile of infliximab serum concentration and efficacy at these time points (p < 0.01). Infliximab therapy was effective for a smaller proportion of patients in the lowest quartile, and these patients had lower serum levels of albumin and a higher incidence of antibodies to infliximab than patients in other quartiles. Although the relationship between exposure to infliximab and response varied among patients, approximate serum concentrations of 41 μg/ml infliximab at week 8 of induction therapy and 3.7 μg/ml at steady-state during maintenance therapy produced optimal outcomes in patients. The authors concluded that serum concentrations of infliximab are associated with efficacy in patients with moderate-to-severe UC; however, complex factors determine the relationship between exposure to this drug and response. They stated that a prospective evaluation of the value of
measuring serum concentrations of infliximab should be performed before these data can be included in patient management strategies.

An UpToDate review on “Clinical presentation and diagnosis of inflammatory bowel disease in infants, children, and adolescents” (Higuchi and Bousvaros, 2016) states that “The anti-OmpC antibody has been identified as a potential serologic marker of IBD. The OmpC is an outer membrane porin, E. coli protein that is immunoreactive to P-ANCA monoclonal antibodies. In a study of 198 children, anti-OmpC was detected in 25 % of patients with CD (n = 81), 11 % of patients with UC (n = 54), and 5 % of controls (n = 63). Because anti-OmpC was positive in 9 children with IBD who were not detected by ASCA (IgA and IgG) or P-ANCA, the addition of anti-OmpC to these antibody assays increased the sensitivity from 63 to 70 %, but decreased the specificity from 97 to 94 %”.

Pre-Operative Serologic Screening to Identify Patients at Increased Risk of Recurrence of Crohn’s Disease:

Hamilton and colleagues (2016) stated that disease recurs frequently after CD resection; and the role of serological anti-microbial antibodies in predicting recurrence or as a marker of recurrence has not been well defined. In this study, a total of 169 patients (523 samples) were prospectively examined, with testing peri-operatively, and 6, 12 and 18 months post-operatively. Colonoscopy was performed at 18 months post-operatively. Serologic antibody presence (pANCA, ASCA IgA/IgG, anti-OmpC, anti-CBir1, anti-A4-Fla2, anti-Fla-X) and titer were tested. Quartile sum score (range of 6 to 24), logistic regression analysis, and correlation with phenotype, smoking status and endoscopic outcome were assessed. Patients with greater than or equal to 2 previous resections were more likely to be anti-OmpC positive (94 % versus 55 %, greater than or equal to 2 versus less than 2, p=0.001). Recurrence at 18 months was associated with anti-Fla-X positivity at baseline (49 % versus 29 %; positive versus negative, p=0.033) and 12 months (52 % versus 31 %, p=0.04). Patients positive (n=28) for all 4 antibacterial antibodies (anti-CBir1, anti-OmpC, anti-A4-Fla2 and anti-Fla-X) at baseline were more likely to experience recurrence at 18 months than patients negative (n=32) for all 4 antibodies (82 % versus 18 %, p=0.034; OR 6.4, 95 % CI: 1.16 to 34.9). The baseline quartile sum score for all 6 anti-microbial antibodies was higher in patients with severe recurrence (Rutgeert’s i3 to i4) at 18 months, adjusted for clinical risk factors (OR 1.16, 95 % CI: 1.01 to 1.34, p=0.039); smoking affected antibody status. The authors concluded that anti-Fla-X and presence of all anti-bacterial antibodies identified patients at higher risk of early post-operative CD recurrence. They stated that serologic screening pre-operatively may help identify patients at increased risk of recurrence. These preliminary findings need to be validated by well-designed studies.
Serum Amyloid A as a Biomarker of Disease Activity in Crohn's Disease:

Yarur and co-workers (2017) noted that serum amyloid A (SAA) is an acute-phase protein, but its role as a biomarker of disease activity in CD is unclear. These researchers evaluated the correlation between SAA, inflammatory cytokines, and mucosal inflammation in patients with CD and examined if this marker might be useful in patients who do not have elevated CRP levels despite having active disease. This was a cross-sectional study that included patients with CD who underwent colonoscopies for assessment of disease activity. Predictive variables were recorded at the time of the procedure and included demographics, phenotype of disease, medications, and collection of serum for cytokine analysis (SAA, CRP, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and interleukins 8, 1β, and 6). The primary outcome was the presence of mucosal healing (MH) (absence of macroscopic and microscopic inflammation). A total of 94 patients were included; 68 (72.3 %) had not achieved MH; SAA, CRP, intercellular adhesion molecule, and interleukin-6 levels were significantly lower in those patients with MH; SAA was the only test that performed well in the sensitivity/specificity analysis (ROC: 0.81, p = 0.046). A high SAA was able to identify 70 % of the patients with a normal CRP but active inflammation. The authors concluded that high circulating SAA levels can correlate with lack of MH and may be used as a surrogate marker for disease activity, even in those patients in whom CRP levels do not correlate with disease activity. These preliminary findings need to be validated by well-designed studies.

Furthermore, UpToDate review son “Clinical manifestations, diagnosis and prognosis of Crohn disease in adults” (Peppercorn and Kane, 2016) and “Clinical presentation and diagnosis of inflammatory bowel disease in children” (Higuchi and Bousvaros, 2016) do not mention serum amyloid A as a marker.

Whole Blood Gene Expression Analysis:

Barnes and associates (2015) noted that despite significant improvements in the understanding CD and UC in recent years, questions remain regarding the best approaches to assessment and management of these chronic diseases during periods of both relapse and remission. Various serologic biomarkers have been used in the evaluation of patients with both suspected and documented IBD, and while each has potential utility in the assessment of patients with IBD, potential limitation remain with each method of assessment. Given these potential shortcomings, there has been increased interest in other means of evaluation of patients with IBD, including an expanding interest in the role of gene expression profiling. Among patients with IBD, gene expression profiles obtained from whole blood have been used to differentiate active from inactive CD, as well as to differentiate between CD, UC, and non-inflammatory diarrheal conditions. There are many opportunities for a non-invasive,
blood based test to aid in the assessment of patients with IBD, particularly when considering more invasive means of evaluation including endoscopy with biopsy. Furthermore, as the emphasis on personalized medicine continues to increase, the potential ability of gene expression analysis to predict patient response to individual therapies offers great promise. The authors concluded that while whole blood gene expression analysis may not completely replace more traditional means of evaluating patients with suspected or known IBD, it offers significant potential to expand the knowledge of the underlying genes involved in the development of these diseases. The main drawbacks of this study were: (i) the majority of the studies evaluating the use of whole blood gene expression analysis in the evaluation of patients with IBD have examined small populations. These small study populations may result in evaluations of heterogeneous patient groups, including patients with varying degrees of disease activity. This introduced heterogeneity into the ultimate population of cells used for the sample analysis, and thus larger studies are needed for further exploration, and (ii) when target genes have been identified in IBD and other inflammatory conditions, difficulty in the evaluation of which genes represent underlying etiologies and which represent consequences of the disease remained.

*Immunological Biomarkers (e.g., Interleukin-6 [IL-6], IL-8, and Macrophage Inflammatory Protein-1β [MIP-1β])*

Pike and colleagues (2015) noted that there is a recognized need for biological markers to facilitate diagnoses of IBS and to distinguish it from other functional and organic disorders. As post-infectious IBS (PI-IBS) is believed to account for as many as 1/3 of all IBS cases, these researchers sought to identify differences in specific cytokines and serologic responses across patients with idiopathic IBS and PI-IBS and healthy controls. A total of 120 US military personnel were identified from the Defense Medical Surveillance System-based International Classification of Diseases, 9th Revision, Clinical Modification (ICD9-CM) codes recorded during medical encounters and were grouped based on infectious gastro-enteritis (IGE) episode (shigella, campylobacter, salmonella, or an unspecified pathogen) followed by IBS, IBS without antecedent IGE, or IGE without subsequent IBS within 2 years of the IGE exposure. Sera from subjects were assayed for cytokine levels and antibodies against a panel of microbiome antigens. In total, 10 of 118 markers considered were shown to differ between IBS patients and healthy controls, including cytokines interleukin-6 (IL-6), IL-8, IL-1β, and macrophage inflammatory protein-1β (MIP-1β), as well as antibody responses to microbial antigens. Antimicrobial antibody response profiles also differed between PI-IBS cases compared with IBS cases without an antecedent episode of acute IGE. Comparisons also suggested that immunoglobulin A (IgA) and IgG profiles may point to pathogen-specific origins among PI-IBS cases. The authors concluded that taken together, these results provided further evidence as
to the molecular distinctness of classes of IBS cases and that serum biomarkers may prove useful in elucidating their pathobiological pathways.

**Fecal Markers of Inflammatory Bowel Disease**

The fundamental pathological process behind IBD is intestinal inflammation. As the precise cause of IBD is not yet completely understood, current treatment strategies are aimed at reducing or eliminating the inflammation. Endoscopic examination and histological analysis of biopsy specimens remain the “gold standard” methods for detecting and quantifying bowel inflammation; however, these techniques are costly, invasive, and repeated examinations are unpopular with patients. Disease activity questionnaires and laboratory ‘inflammatory markers’, although widely used, show an unreliable correlation with endoscopy and histology. New markers are needed for detecting and quantifying bowel inflammation.

Calprotectin is a calcium- and zinc-binding protein of the S100 family derived mainly from neutrophils and monocytes. It is excreted in excess in stools during IBD. Fecal calprotectin level has been reported to parallel intestinal inflammation and can predict relapse of UC (Hanai et al, 2004).

Fecal measurement of calprotectin is emerging as a tool for the differential diagnosis of inflammatory (e.g., CD, UC) from non-inflammatory gastrointestinal disease (e.g., IBS), for monitoring patients’ response to therapy and for predicting recurrence of IBD.

The available literature reports high fecal calprotectin levels in patients with active inflammation of the bowel. As such, it differs from erythrocyte sedimentation rates and other tests that are general markers of inflammation and not specific to bowel inflammation. The fecal calprotectin test, however, cannot distinguish among different causes of bowel inflammation. Several diseases other than inflammatory bowel disease, including colorectal neoplasia and gastrointestinal infection, can also increase fecal calprotectin. Because fecal calprotectin is a nonspecific marker of bowel inflammation, endoscopic workup remains crucial to determine the underlying cause of colitis.

In March 2006, the PhiCal (Genova Diagnostics) quantitative ELISA test for measuring concentrations of fecal calprotectin in fecal stool was cleared for marketing by the U.S. Food and Drug Administration (FDA) through the 510(k) process. Thus, the manufacturer was not required to provide the evidence of efficacy necessary to support a premarket approval application (PMA).

Studies have compared fecal calprotectin levels in patients with confirmed inflammatory bowel disease versus normal controls (Tibble, et al., 2000; Schoepfer, et al., 2010) or persons
with irritable bowel syndrome (Schoepfer, et al., 2007). Joishy, et al., (2008) also had a control group of children with gastrointestinal symptoms, although the causes were not specified in detail in that report. Almost all of the studies published so far have been performed in prediagnosed groups of patients or in patients in whom the major clinical problem was to distinguish between Crohn’s disease and irritable bowel syndrome. These studies did not consider the wide spectrum of other organic causes of chronic diarrhea that must be distinguished from inflammatory bowel disease.

Studies have reported correlations of fecal calprotectin levels with endoscopic, histologic, clinical and serum markers of inflammation in patients with confirmed diagnoses of inflammatory bowel disease (Tibble, et al., 2000; Bunn, et al., 2001; Gaya, et al., 2005; Sipponen, et al., 2008; Vieira, et al., 2009; Quail, et al., 2009; Fagerberg, et al., 2007). Sipponen, et al., 2010 examined correlations between fecal calprotectin and endoscopic response to therapy. Other studies (Kallel, et al. 2010; Gisbert, et al., 2009) examined correlations between fecal calprotectin levels and relapse. However, the usefulness of these findings in the management of patients with inflammatory bowel disease has not been shown. There is a lack of clinical outcome studies of the use of fecal calprotectin in lieu of a standard workup for inflammatory bowel disease. Although some authors claim that fecal calprotectin can be used to avoid invasive testing, there are a paucity of studies demonstrating an actual reductions in number of invasive tests through use of fecal calprotectin measurement.

Berni et al (2004) examined fecal calprotectin values in different pediatric gastrointestinal diseases (n = 281; age ranging from 13 to 216 months) comparing them with those obtained in healthy children (n = 76; age ranging from 13 to 209 months). These investigators concluded that fecal calprotectin is a sensitive, but not disease specific, marker to easily detect inflammation throughout the whole gastrointestinal tract. It may help in identifying an organic disease characterized by intestinal mucosa inflammation and in the differential diagnosis of functional bowel disorders. Wassell et al (2004) posited that a single measurement of fecal calprotectin may help gastroenterologists in the differential diagnosis of CD and IBS.

Tibble et al (2000) examined if measurement of intestinal permeability and inflammation could predict relapse of IBD. Forty-three patients with CD and 37 with UC in clinical remission provided a stool sample to be assayed for calprotectin and patients with CD additionally underwent a small intestinal permeability test. Relapse was defined using clinical disease activity indices. Twenty-five (58 %) patients with CD and 19 (51 %) with UC had a relapse over the 12-month period. Median calprotectin levels in the relapse groups (122 mg/L for CD, 123 mg/L for UC; normal less than 10 mg/L) differed significantly (p < 0.0001) from those of the non-relapse groups (41.5 mg/L for CD, 29.0 mg/L for UC). At 50 mg/L, the sensitivity and
specificity of calprotectin for predicting relapse in all patients with IBD were 90% and 83%, respectively. Permeability in the CD patients who relapsed (median, 0.075; normal less than 0.04) differed significantly (p = 0.004) from that in the non-relapse group (median, 0.038). At the level of 0.05, the sensitivity and specificity of permeability in predicting relapse were 84% and 61%, respectively. The authors concluded that fecal calprotectin may be useful in predicting clinical relapse of disease activity in patients with CD and UC, whereas small intestinal permeability may be a useful predictor of relapse in patients with small intestinal CD.

Although the study by Tibble et al (2000) suggested that high fecal calprotectin levels may identify IBD patients in remission who are at risk for early relapse, there are reports that there may be differences in relapse prediction in patients with CD compared to those with UC.

Costa et al (2005) examined if the predictive value of fecal calprotectin is different in CD and UC. Seventy-nine consecutive patients with a diagnosis of clinically quiescent IBD (38 CD and 41 UC) were followed for 12 months, undergoing regular clinical evaluations and blood tests. A single stool sample was collected at the beginning of the study from each patient and the calprotectin concentration was measured by a commercially available enzyme linked immunoassay. In CD, median calprotectin values were 220.1 μg/g of stool in those patients who relapsed during follow-up, and 220.5 μg/g in non-relapsing patients (p = 0.395). In UC, median calprotectin values were 220.6 μg/g and 67 μg/g in relapsing and non-relapsing patients, respectively (p < 0.0001). The multi-variate Cox regression model, after adjustment for possible confounding variables, showed a 2- and 14-fold increase in the relapse risk, respectively, in those patients with CD and UC in clinical remission who had a fecal calprotectin concentration higher than 150 μg/g. The authors concluded that fecal calprotectin proved to be an even stronger predictor of clinical relapse in UC than in CD, which makes the test a promising non-invasive tool for monitoring and optimizing therapy.

In a commentary on the role of biomarkers for predicting relapse in patients with IBD, Pardi and Sandborn (2005) stated that based on the studies of Tibble et al (2000) as well as Costa et al (2005), fecal calprotectin appears to be a relatively sensitive and specific marker of the risk of relapse for UC. It also appears to be a sensitive marker of relapse risk in CD but the data on specificity are conflicting at this juncture. However, these data need to be interpreted carefully since the number of studies is small, and in both studies using calprotectin to predict relapse risk, most patients were on medical therapy. Calprotectin may behave differently in patients who are not on therapy. Thus, before fecal calprotectin or any other biomarker of inflammatory activity in the gastrointestinal tract can be incorporated into routine clinical practice, other studies in larger and diverse groups of patients will be needed to further clarify its role.
In the guidelines for chronic diarrhea compiled at the request of the British Society of Gastroenterology, Thomas et al (2003) stated that stool markers of gastrointestinal inflammation such as calprotectin are of considerable research interest. However, these tests have not been introduced into clinical practice. Moreover, in a review on the diagnostic and therapeutic strategies in the IBD, Cremonini and Talley (2004) stated that the usefulness of fecal tests such as calprotectin to exclude organic bowel disease is not adequately established. Furthermore, the use of calprotectin is not mentioned in the practice parameters on the management of CD in adults by the American College of Gastroenterology (Hanauer et al, 2001) and the practice guideline on the management of UC by the Society of Surgery of the Alimentary Tract (2001).

D'Inca et al (2008) evaluated the role of calprotectin tests in predicting clinical relapse in IBD patients. A total of 97 patients with UC and 65 with CD in clinical remission were prospectively included in the study. A 10-g stool sample was collected for calprotectin assay. The cut-off level was set at 130 mg/kg of feces. Patients were followed-up for 1 year after the test or until relapse. The cumulative proportion of relapses was estimated by the Kaplan-Meier analysis. Statistics for equality of survival distribution were tested using the log-rank test. The calprotectin test was positive in 44 UC patients and 26 of them relapsed within 1 year, while 11 of 53 UC patients with a negative calprotectin test relapsed within the same time frame. Thirty CD patients had a positive calprotectin test and 13 of them relapsed within 1 year, as did 7 of the 35 with a negative test result. A significant correlation emerged between a positive calprotectin test and the probability of relapse in UC patients (p = 0.000). In CD patients, only cases of colonic CD showed a significant correlation between a positive calprotectin test and the probability of relapse, i.e., 6 colonic CD patients were positive for the calprotectin test and 4 relapsed (p = 0.02). The authors concluded that measuring calprotectin may help to identify UC and colonic CD patients at higher risk of clinical relapse. The author also noted that while the marker has an impressive profile in discriminating organic versus functional intestinal diseases, its role in monitoring IBD remains promising but still unclear. These investigators are currently evaluating the usefulness of calprotectin levels for predicting relapses in a more careful manner by measuring calprotectin every 3 months, and are also planning to verify the usefulness of this marker in monitoring response to treatments.

Gisbert and McNicholl (2009) noted that fecal calprotectin has been proposed as a non-invasive surrogate marker of intestinal inflammation in IBD. Close correlation between fecal calprotectin concentration and fecal leukocyte excretion quantified with (111)indium has been described. Fecal calprotectin has a good diagnostic precision for separating organic and functional intestinal diseases. However, the specificity for the diagnosis of IBD is lower than
desirable, as several diseases other than IBD -- specially colorectal neoplasia and gastrointestinal infection -- can also increase fecal calprotectin. High concentration of calprotectin in feces is a strong argument to carry out a colonoscopy in order to rule out the presence of IBD or other organic pathologies. Parallelism between fecal calprotectin levels and IBD activity has been confirmed, although this fecal marker appears to better reflect the disease activity in UC than in CD. Fecal calprotectin's capacity to predict IBD relapse is promising. It has been suggested that, in IBD patients receiving treatment, a normalization or decrease in fecal calprotectin concentrations is an accurate indicator of endoscopic healing. Greater fecal calprotectin concentration has been shown in asymptomatic first-degree relatives of patients with IBD, suggesting that there is a high prevalence of sub-clinical intestinal inflammation in them.

One of the proposed uses of this test is to evaluate patients presenting with gastrointestinal symptoms, where irritable bowel syndrome (a noninflammatory condition) and inflammatory bowel disease (Crohn's disease or ulcerative colitis) are in the differential. A metaanalysis from the Netherlands (van Rheenen, et al., 2010) examined studies of the potential use of fecal calprotectin for inflammatory bowel disease screening. The pooled analysis showed that a normal fecal calprotectin test result reduced the probability of inflammatory bowel disease in adults to 3 percent; however the confidence intervals were wide, ranging from 1 percent to 11 percent. The test did not perform as well in children, where a normal fecal calprotectin test reduced the probability of inflammatory bowel disease to 15 percent, with confidence intervals ranging from 7 percent to 28 percent. The investigators calculated false negative results in 6 percent of adults and 8 percent of children. Based upon this analysis, it has been suggested that adults with a normal fecal calprotectin can forgo endoscopy, and be treated presumptively as having irritable bowel syndrome. Based upon that assumption, the authors presenting a modeling scenario where they estimated that screening by measuring fecal calprotectin levels would result in a 67% reduction in the number of adults requiring endoscopy. This is a modeling estimate, based upon an assumption that all adults with a normal fecal calprotectin test would forgo endoscopy. Based on this analysis, there has been some support in Europe for the use of fecal calprotectin in this way. A guideline on irritable bowel syndrome from the World Gastroenterology Organisation that lists markers of inflammation ("e.g., faecal calprotectin") in the workup of persons suspected of having irritable bowel syndrome.

This metaanalysis by Van Rheem, et al. (2010) of fecal calprotectin presents a number of limitations. One has to do with the wide confidence intervals of the estimates. For adults, the upper end of the confidence interval is 11 percent, and for children, the upper end of the confidence interval is 28 percent. Thus, for adults, the actual proportion of adults with a
normal fecal calprotectin may be more than 10 percent. The wide confidence intervals are a reflection of the heterogeneity of the results of the studies included in the review. The substantial differences in the estimates of the accuracy of fecal calprotectin testing among studies means that there is substantial uncertainty on the true diagnostic performance of this test. A critique of this metaanalysis by the Centre for Reviews and Dissemination (2010) stated that the authors of the metaanalysis, while recognizing the heterogeneity of study results, failed to do a statistical analysis of the heterogeneity, and that the substantial heterogeneity of study results suggests that pooling of the results into a single metaanalysis may have been inappropriate. In reviewing the conclusions of this study, one commentator (Rex, et al., 2010) questioned whether, in the United States, given the current medical legal environment, a gastroenterologist would be willing to forgo endoscopy knowing that there may be an 11 percent chance that they will misdiagnose a person with inflammatory bowel disease as having irritable bowel syndrome. Rex (2010) stated that, in the United States, given the medical legal burden to exclude inflammatory bowel disease, gastroenterologists would be unlikely to forgo endoscopy because of this imprecision. In addition, endoscopy detects more than just inflammatory bowel disease, and can detect other conditions, such as diverticulosis, that may be in the differential or contribute to the patient's symptoms.

The authors of this metaanalysis (Van Rheem, et al., 2010) stated that the estimates of test performance for this indication have been in referral populations and not in primary care. The absence of information on the performance of the fecal calprotectin test in the primary care population was also the conclusion of a recent Cochrane review. The test performance may be very different in primary care, given the differences in disease spectrum in the primary care population compared to the population of patients that are seen in secondary or tertiary care settings. For example, the prevalence of inflammatory bowel disease would be expected to be much lower in the primary care population, and one would expect to have a higher prevalence of acute inflammatory bowel disorders that may lead to falsely positive results. Thus, the results of this metaanalysis cannot be extrapolated for use in primary care.

There is an absence of evidence to support the use of fecal calprotectin testing to guide the management of patients with inflammatory bowel disease. There are no current guidelines from leading medical professional organizations that recommend the use of fecal calprotectin for this purpose. Guidelines from the World Health Organization (2015), in a section on diagnosis of inflammatory bowel disease, state that fecal calprotectin "could be very helpful in developing countries." The guidelines state that fecal calprotectin is not necessary for diagnosis if endoscopy available, but may help select for further investigation including with endoscopy in countries with limited (medium) resources. A Crohn's disease evaluation and treatment tool from the American Gastroenterological Association (Sandborn, et al., 2014)
includes measurement of fecal calprotectin among a series of tests in the workup of inflammatory bowel disease, but has no recommendations for use of this test in guiding management. The FDA-approved labeling of products used to treat inflammatory bowel disease do not discuss the use of fecal calprotectin for this purpose. There is a lack of reliable evidence from clinical studies where fecal calprotectin is used to guide management. There is a need for clinical studies of the use of fecal calprotectin demonstrating that, compared to standard of care management without fecal calprotetin, the use of fecal calprotectin results in better clinical outcomes, fewer endoscopies, or some other important clinical outcome. Current evidence for fecal calprotectin to guide management describes the test performance in guaging inflammation in persons with inflammatory bowel disease; this type of evidence is hypothesis generating, and suggests that fecal calprotectin has promising potential for this use.

Abej, et al. (2016) sought to determine the relationship between fecal calprotectin and imaging studies and other biochemical inflammatory markers and the impact of fecal calprotectin measurements on decision-making in inflammatory bowel disease (IBD) patient management in usual clinical practice. Investigators enrolled 240 persons with inflammatory bowel disease in a cross-sectional study conducted at a single center in Canada. The correlation between fecal calprotectin values and other markers for disease activity such as serum albumin (alb), hemoglobin (Hg), and C-reactive protein (CRP) and diagnostic imaging or colonoscopy was examined. Fecal calprotectin = 250mcg/g of stool was considered a positive result indicating active IBD. A total of 183 stool samples (76.3%) were returned. Positive fecal calprotectin was associated with colonoscopy findings of active IBD ( < 0.05), low albumin ( < 0.05), anemia ( < 0.01), and elevated CRP ( < 0.01). A positive fecal calprotectin test was not significantly associated with radiologic evidence of active disease. There was no significant difference for fecal calprotectin results by outcomes on small bowel evaluation among the 21 persons with small bowel CD. Most persons (87.5%) with normal fecal calprotectin and no change in therapy remained in remission during subsequent 3 months; however, followup time was short and lacked controls. The authors noted, in addition, that "it is difficult to discern what role the FCAL result had in the clinicians not pursuing investigations or therapy changes as opposed to their management consideration being based on a composite of other testing, as well." Other limitations noted by the authors was that the same testing was not undertaken in all subjects to fully determine the correlations between the fecal calprotectin and various outcomes, especially cross-sectional imaging. The authors found that a positive fecal calprotectin often triggered a clinical response, either more investigations or a change in therapy. But, not having done imaging in all subjects, they were not able to discern how many false negative fecal calprotectin tests were represented within their sample.
Noting that there are limited studies that have looked at the physician experience and perspective of the use of fecal calprotectin, Rosenfeld, et al. (2016) conducted a survey to evaluate the perspective of gastroenterologists affiliated with the University of British Columbia, Canada, regarding the impact of fecal calprotectin on the management of patients with inflammatory bowel disease (IBD). Patients with known IBD or symptoms suggestive of IBD for whom the physician identified that fecal calprotectin would be clinically useful were recruited. Physicians completed an online “pre survey” outlining their rationale for the test. After receipt of the test results, the physicians completed an online “post survey” to portray their perceived impact of the test result on patient management. Clinical outcomes for a subset of patients with follow-up data available beyond the completion of the “post survey” were collected and analyzed. The primary outcome was the frequency with which the fecal calprotectin result resulted in a change in the management of the patient as indicated by the pre- and post- surveys. Secondary outcomes included the deferral of colonoscopies as a result of the fecal calprotectin level, the proportion of events for which the physician found the fecal calprotectin level to be useful in the care of the patient and the association of the fecal calprotectin result to subsequent clinical, endoscopic and/or radiological outcomes. The primary outcome of change in management was analyzed according to a positive result being > 250 μg/g in addition to > 100 μg/g, given the heterogeneity in the indication for fecal calprotectin in the study cohort as well as the uncertainty in the current literature relating to what is considered a “positive” and “negative” fecal calprotectin. Of 373 test kits distributed, 290 were returned, resulting in 279 fully completed surveys. One hundred and ninety patients were known to have IBD; 147 (77%) with Crohn’s disease, 43 (21%) ulcerative colitis and 5 (2%) IBD unclassified. Indications for fecal calprotectin testing included: 90 (32.2%) to differentiate a new diagnosis of IBD from irritable bowel syndrome (IBS), 85 (30.5%) to distinguish symptoms of IBS from IBD in those known to have IBD and 104 (37.2%) as an objective measure of inflammation. Survey respondents indicated that fecal calprotectin levels resulted in a change in management 51.3% (143/279) of the time which included a significant reduction in the number of colonoscopies (118) performed (P < 0.001). Overall, 97.5% (272/279) of the time, the physicians stated that they found the test sufficiently useful that they would order it again in similar situations. Follow-up data was available for 172 patients with further support for the clinical utility of fecal calprotectin provided. The authors noted that the limitations of this "pilot study" include potential bias created by patient inclusion being solely dependent on the discretion of the involved physician. The authors stated that this accordingly has contributed to a heterogeneous patient population and is reflective of the limited guidance regarding the most appropriate use of fecal calprotectin. Other limitations of the study included the lack of validation of the study survey and failure to return about 20 percent of fecal calprotectin sample. The authors noted that, finally, at the time of initial study
design, a fecal calprotectin level of 250 μg/g was considered appropriate for analysis of outcomes; however at the time of follow up analysis, a level of 100 μg/g was considered more appropriate. The authors noted the need for additional investigations in the “grey zone” between 100 and 250 μg/g.

The authors (Rosenfeld, et al., 2016) noted that this study had several drawbacks: (i) potential bias created by patient inclusion being solely dependent on the discretion of the involved physician. This accordingly has contributed to a heterogeneous patient population and was reflective of the limited guidance regarding the most appropriate use of FC at the time of study design, (ii) given this was a real-life study, there was also unavoidable potential for inclusion of patients with co-existing undiagnosed conditions that could influence the FC level such as colonic polyps or additional immunologically driven digestive disorders, (iii) more than 20% of patients did not return FC samples. However, such patients were similarly distributed across the indications for the test suggesting that this did not influence the patient’s decision to carry out the test, (iv) it is acknowledged that questionnaires were not previously validated although a degree of quality control was performed during the study to ensure that the surveys accurately reflected the clinical practice of the doctors, with initial perusal of 20 surveys, and then a further follow-up of 210 patients. The considerable duration of follow-up possible for the latter patients did enable adjustment for variation between what had been indicated by the physician on the post-survey response and what actually occurred, and (v) at the time of initial study design, a FC level of 250 μg/g was considered appropriate for analysis of outcomes, however at the time of follow-up analysis in 2015, a level of 100 μg/g was considered more appropriate. The “grey zone” between 100 and 250 μg/g and the accompanying need for additional investigations, particularly in IBD was highlighted in the recently published clinician guide to using FC to identify and monitor disease activity in IBD.

In a retrospective cohort study from Canada, El Mataray, et al. (2017) stated that "data on the impact of fecal calprotectin directing management in an IBD population, especially in children, are scarce." The investigators measured fecal calprotectin, clinical activity indices, and blood markers in children with established diagnoses of inflammatory bowel disease (IBD). Pearson correlation coefficient analysis was performed to examine association between fecal calprotectin and other markers. Decisions based on fecal calprotectin measurements were prospectively documented and participants were evaluated 3 to 6 months later. When patients were asked to bring stool samples, pediatric gastroenterologists were asked to complete a questionnaire. Other investigations such as hemoglobin, serum albumin, and CRP were known to physicians who made no changes in treatment at that time point. The clinicians were asked to decide if they would do any additional investigations (mainly colonoscopy) or any changes in therapy, based only on the fecal calprotectin results. Clinicians
were given specific options to choose from for treatment escalation in case fecal calprotectin comes back as positive. Once the fecal calprotectin results were known, any investigations and treatment changes were documented and patients were followed up for 3–6 months. Clinical activity indices were measured in the follow-up visits. A total of 115 fecal samples were collected from 77 children with IBD [median age 14, interquartile range (IQR) 11–15.6 years, 42 females, 37 with Crohn’s disease]. The authors reported that fecal calprotectin measurements had "fair" correlation with clinical disease activity indices ($r = 0.483$, $P < 0.05$), Physicians Global Assessment of disease activity (PGA) ($r = 0.40$, $P < 0.05$), erythrocyte sedimentation rate (ESR) ($r = 0.40$, $P < 0.05$), low hemoglobin ($r = -0.40$, $P < 0.05$), with less correlation with low serum albumin ($r = -0.3$, $P < 0.05$) but no correlation with c-reactive protein (CRP) ($r = 0.1$, $P = 0.3$). Correlation was limited ($r = 0.256$, $P = 0.006$) with endoscopic activity as only 14 children had colonoscopy within 4 weeks of fecal calprotectin measurement. The authors reported that only 14 (12%) out of 115 samples resulted in colonoscopy as guided by physicians’ discretion. The authors posited that this may have been when physicians felt that fecal calprotectin value was equivocal between 100 and 250, but date to that effect were not presented. Two colonoscopies were in fecal calprotectin negative and 12 in the fecal calprotectin positive group. The authors stated that, for the rest of the cohort [101 (88%) samples], decisions on treatment (escalation or no treatment) were based solely on fecal calprotectin measurements. Sixty four out of 74 (86%) samples with positive FCal measurements were associated with treatment escalation that resulted in improvements in clinical activity indices, while in the FCal negative group, 34 out of 41 (83%) measurements were associated with no change in treatment. The authors noted that limitations of the study include the retrospective design and small sample size especially for those who had colonoscopy. As colonoscopy was not performed in all subjects, the investigators were unable to discern how many false negative fecal calprotectin measurements were represented within the sample. Another limitation was a lack of measurements of fecal calprotectin in the follow-up visits after implementing changes in therapy but the study outcome was intended to be clinical activity indices. The authors concluded, however, that ‘[n]onetheless, the study adds significantly to the current literature as little is known about the impact and outcome of FCal measurements in pediatric IBD clinical practice.’

Henderson and colleagues (2014) stated that fecal calprotectin (FC) is increasingly used during the diagnosis of inflammatory bowel disease (IBD), out-performing blood markers during investigation in children. Tests that reduce endoscopy rates in children with suspected gut inflammation would be beneficial. These investigators determined the usefulness of FC in children undergoing their primary investigation for suspected IBD by systematic review and meta-analysis. An electronic search was performed with keywords relating to IBD and
calprotectin in multiple electronic resources from 1946 to May 2012; a hand search was also performed. Inclusion criteria were studies that reported FC levels before the endoscopic investigation of IBD in patients less than 18 years old. Studies were evaluated using the Quality Assessment of Diagnostic Accuracy Studies tool, and a meta-analysis was performed using a hierarchical summary receiver operating curve model. A total of 8 papers met the inclusion criteria (6 prospective and 2 retrospective case-control studies); methodological quality was determined in detail for each study. The 8 studies presented FC levels at presentation in 715 patients, 394 pediatric IBD patients, and 321 non-IBD controls. Pooled sensitivity and specificity for the diagnostic utility of FC during the investigation of suspected pediatric IBD were 0.978 (95 % confidence interval (CI): 0.947 to 0.996) and 0.682 (95 % CI: 0.502 to 0.863), respectively; the positive and negative likelihood ratios were 3.07 and 0.03, respectively. The authors concluded that FC has a high sensitivity and a modest specificity during the diagnosis of suspected pediatric IBD. Moreover, they stated that further work is required to determine the effect of FC levels on endoscopy rates and its role during the re-evaluation of those with confirmed disease.

Also, an UpToDate review on “Clinical manifestations, diagnosis and prognosis of Crohn's disease in adults” (Peppercorn, 2013) states that “Testing for fecal calprotectin may help identify patients with intestinal inflammation, though it is not routinely done in clinical practice”.

Lasson et al (2014) examined if the concentration of FC reflects the endoscopic findings 1 year after ileo-caecal resection and evaluated the variation of FC in individual patients during 6 months prior to the ileo-colonoscopy. A total of 30 patients with CD and ileo-caecal resection performed within 1 year were included. Stool samples were delivered monthly until an ileo-colonoscopy was performed 1 year post-operatively. One year after surgery the median values of FC were not significantly different between the patients in endoscopic remission (n = 17) and the patients with an endoscopic recurrence (189 (75 to 364) versus 227 (120 to 1,066) μg/g; p = 0.25). However, most patients with low values were in remission and all patients with high (greater than 600 μg/g) calprotectin values had recurrent disease. The variability of the FC concentration was most pronounced in patients with diarrhea. The authors concluded that they found no statistical difference in the concentrations of calprotectin between patients in endoscopic remission and patients with a recurrent disease 1 year after ileo-caecal resection for CD. However, among the minority of patients with low or high values, FC indicated remission and recurrence, respectively. There was significant variation of the FC concentrations over time, which affects the utility of calprotectin in clinical practice.

Noting that "the applications of FCAL in monitoring inflammation in IBD in clinical practice have not been standardized and the impact of addition of FCAL on the diagnostic and
monitoring armamentarium in IBD is not well defined”, Abej and co-workers (2016) examined the relationship between FC and imaging studies and other biochemical inflammatory markers and the impact of FC measurements on decision-making in IBD patient management in usual Canadian clinical practice. A total of 240 persons with IBD were enrolled. The correlation between FC values and other markers for disease activity such as serum albumin (alb), hemoglobin (Hg), and CRP and diagnostic imaging or colonoscopy was examined; FC greater than or equal to 250ug/g of stool was considered a positive result indicating active IBD. A total of 183 stool samples (76.3 %) were returned. The return rate in the pediatric and adult cohorts was 91 % (n = 82) and 67.3 % (n = 101), respectively (p < 0.0001). Positive FC was associated with colonoscopy findings of active IBD (p < 0.05), low albumin (p < 0.05), anemia (p < 0.01), and elevated CRP (p < 0.01). There was no significant difference for FC results by outcomes on small bowel evaluation among the 21 persons with small bowel CD. Most persons (87.5 %) with normal FC and no change in therapy remained in remission during subsequent 3 months. The authors concluded that this study represented a snapshot of how FC testing is used in monitoring disease activity in IBD in clinical practice outside the context of clinical trials. They found that in a referral population of persons with IBD, positive FC was significantly associated with abnormal endoscopy, elevated serum CRP, low serum Hb, and low serum alb. While it was a small cohort that had imaging, FC results were not significantly associated with cross-sectional imaging evidence of disease activity, likely reflecting a weaker association with small bowel disease. They stated that the findings of this study confirmed that FC can be used as a surrogate marker for disease activity in IBD. However, clinicians are placing considerable faith in the test even though its role in small bowel CD requires further research.

The authors (Abej, et al., 2016) stated that this study had one main drawbacks: the same testing was not undertaken in all subjects to fully determine the correlations between the FC and various outcomes, especially cross-sectional imaging. However, at study outset, these investigators were particularly interested in determining how clinicians would use FC results. A positive FCAL often triggered a clinical response, either more investigations or a change in therapy. Unfortunately, not having done imaging in all subjects, the authors could not discern how many false negative FC tests were represented within their sample. Nonetheless, these findings showed how much faith clinicians have placed in FC based on the medical literature since all participating clinicians were utilizing FC for the first time in their practices.

In a retrospective cohort study from Canada, El-Matary and associates (2017) examined the impact of FC measurements on decision-making and clinical care of children with IBD; FC, clinical activity indices, and blood markers were measured in children with established diagnoses of IBD. Pearson correlation coefficient analysis was performed to examine
association between FC and other markers. Decisions based on FC measurements were prospectively documented and participants were evaluated 3 to 6 months later. A total of 115 fecal samples were collected from 77 children with IBD [median age of 14 years, interquartile range (IQR) 11 to 15.6, 42 females, 37 with CD]; FC positively correlated with clinical activity indices \((r=0.481, p<0.05)\) and erythrocyte sedimentation rate \((r=0.40, p<0.05)\) and negatively correlated with Hb \((r=-0.40, p<0.05)\); 64 out of 74 (86 \%) positive FC measurements (greater than or equal to 250\(\mu\)g/g of stools) resulted in treatment escalation with subsequent significant clinical improvement while in the FC negative group, 34 out of 41 (83 \%) measurements resulted in no change in treatment and were associated with remission on follow-up. The authors concluded that based on high FC, the majority of children had treatment escalation that resulted in clinical improvement; FC measurements were useful and reliable in decision-making and clinical care of children with IBD.

The authors noted that this study had several drawbacks: (i) the retrospective design and small sample size especially for those who had colonoscopy. However, based on previous research, FC is known to strongly correlate with endoscopic disease activity and these researchers assumed that it did. Hence, the escalation of treatment in most participants with elevated FC levels regardless of whether a colonoscopy was performed or not, (ii) the study main objective was how FC measurements would impact on disease management. A positive FC most often triggered a clinical response; especially a change in therapy. As the authors did not do colonoscopy in all subjects they could not discern how many false negative FC measurements were represented within their sample. Nonetheless, these findings showed that the faith clinicians placed in the FC result was rewarded with good clinical outcomes, and (iii) a lack of measurements of FC in the follow-up visits after implementing changes in therapy but the study outcome was intended to be clinical activity indices. Nonetheless, the study added significantly to the current literature as little is known about the impact and outcome of FC measurements in pediatric IBD clinical practice.

Bar-Gil Shitrit et al (2017) prospectively evaluated the value of FC and lactoferrin to predict capsule endoscopy (CE) findings. A total of 68 consecutive patients who were referred for CE were included. Stool samples for calprotectin and lactoferrin and blood samples were collected for relevant parameters. Correlation between fecal markers and CE findings was assessed and ROC curves were built to determine the predictive values of fecal markers for the diagnosis of CD; FC data was available for all the patients and lactoferrin data for 38. Capsule endoscopy findings compatible with CD were found in 23 (33 \%) patients and 45 (67 \%) were negative for CD. The average age of the CD group was 34 compared to 46 in the non-CD group \((p=0.048)\). Median calprotectin and lactoferrin in the CD group and in the control group were 169 mg/kg versus 40 \((p=0.004)\) and 6.6mg/kg versus 1 \((p=0.051)\), respectively.
The area under the ROC curve was 0.767 for calprotectin and 0.70 for lactoferrin. A FC concentration of 95 mg/kg and fecal lactoferrin of 1.05 mg/kg had a sensitivity, specificity, PPV and NPV of 77 and 73 %, 60 and 65 %, 50 and 50 %, and 84 and 84 % in predicting CE findings compatible with CD. The authors concluded that fecal markers are simple and non-invasive surrogates for predicting CE findings compatible with CD; they stated that fecal markers can help determine which patients should be referred for CE. The authors noted, however, that the relatively low sample size limits the power of their conclusions.

Holtman and colleagues (2016) examined the diagnostic accuracy of symptoms, signs, non-invasive tests, and test combinations that can assist the clinician with the diagnosis of IBD in symptomatic children. These researchers performed a literature search of Medline and Embase. Two reviewers independently selected studies reporting on the diagnostic accuracy of tests for IBD, with confirmation by endoscopy and histopathology or clinical follow-up, in children with chronic gastrointestinal symptoms. Two reviewers independently extracted data and assessed study quality with the QUADAS-2, an evidence-based quality assessment tool for diagnostic accuracy studies. A total of 19 studies were included (n = 2,806). Symptoms (abdominal pain, diarrhea, rectal bleeding, and weight loss) had pooled sensitivities ranging from 0.48 to 0.82 and specificities ranging from 0.17 to 0.78. Of all the blood markers, CRP (9 studies) and alb (6 studies) had the best performance, with pooled sensitivities of 0.63 (0.51 to 0.73) and 0.48 (0.31 to 0.66), respectively, and specificities of 0.88 (0.80 to 0.93) and 0.94 (0.86 to 0.98). Assessment of FC (10 studies) had a pooled sensitivity of 0.99 (0.92 to 1.00) and a specificity of 0.65 (0.54 to 0.74). One limitation was that none of the studies was conducted in non-referred children. The authors concluded that in children whose pediatrician is considering an endoscopy, symptoms are not accurate enough to identify low-risk patients in whom an endoscopy can be avoided. They stated that FC, CRP, and alb findings are potentially of clinical value, given their ability to select children at low risk (negative FC test result) or high risk (positive CRP or albu test result) for IBD. Moreover, they stated that further research should examine the accuracy of sequential testing strategies and the added values of tests beyond signs and symptoms focusing on FC, CRP, and albu; and before tests or a diagnostic strategy can be recommended in non-referred, low-risk children, high-quality studies are needed in this setting.

Caccaro and associates (2016) noted that the role of FC and fecal lactoferrin has been extensively studied in many areas of IBD patients' management. The post-operative setting in both CD and UC patients has been less investigated although few promising results come from small, cross-sectional studies. Therefore, the current post-operative management still requires endoscopy 6 to 12 months after intestinal resection for CD in order to exclude endoscopic recurrence and plan the therapeutic strategy. In patients who underwent
restorative proctocolectomy, endoscopy is required whenever symptoms includes the possibility of pouchitis. There is emerging evidence that FC and fecal lactoferrin are useful surrogate markers of inflammation in the post-operative setting, they correlate with the presence and severity of endoscopic recurrence according to Rutgeerts' score and possibly predict the subsequent clinical recurrence and response to therapy in CD patients. Similarly, fecal markers show a good correlation with the presence of pouchitis, as confirmed by endoscopy in operated UC patients. Fecal calprotectin appears to be able to predict the short-term development of pouchitis in asymptomatic patients and to vary according to response to medical treatment. The authors concluded that the possibility of both fecal markers to be used in the routine clinical practice for monitoring IBD patients in the post-operative setting should be confirmed in multi-centric clinical trial with large sample set. An algorithm that can predict the optimal use and timing of fecal markers testing, the effective need and timing of endoscopy and the cost-effectiveness of these as a strategy of care would be of great interest.

Wright (2016) stated that the diagnosis and monitoring of IBD has traditionally relied on clinical assessment, serum markers of inflammation and endoscopic examination. Fecal biomarkers such as calprotectin and lactoferrin are predominantly derived from neutrophils, are easily detectable in the feces and are now established as valuable markers of intestinal inflammation. In recent years, a “treat to target” concept has emerged for the management of IBD. Adequate control of inflammation in IBD at a biochemical level is quickly becoming an important target in IBD management. Fecal biomarkers have been shown to be significantly and consistently increased in both adult and pediatric patients with IBD versus those without IBD. Fecal biomarkers are therefore useful in determining those patients with gastrointestinal symptoms who are likely to benefit from colonoscopy versus those in whom colonoscopy is likely to be normal. Fecal biomarkers correlate significantly with endoscopic disease in both CD and UC. Suggested cut-offs for FC for endoscopically active disease in IBD range from 50 to 280 μg/g. Fecal biomarkers reflect the success of treatment intensification and can help predict clinical relapse. Both FC and FL lactoferrin are accurate in the detection of post-operative endoscopic recurrence of CD, and FC may be clinically useful in predicting those patients with acute severe UC who may progress to colectomy. There are limitations to these fecal tests including a false positive rate and intra-individual variability. The authors noted that this review focused on the role of fecal biomarkers in the diagnosis, monitoring and management of IBD and how best to interpret results. They discussed the emerging role of these biomarkers in the IBD management landscape including FC-guided drug dosing and the development of home-based testing and e-health applications. They stated that fecal biomarker results must always be interpreted in a clinical context; endoscopic assessment remains the gold standard for diagnosis and monitoring of IBD.
Koulaouzidis et al (2016) noted that accurate inflammation reporting in CE is important for diagnosis and monitoring of treatment of IBD; FC is a highly specific biomarker of gut inflammation. Lewis score (LS) was developed to standardize quantification of inflammation in small-bowel (SB) CE images. In a retrospective, multi-center study, these researchers investigated correlation between LS and FC in a large group of patients undergoing CE for suspected or known small-bowel IBD, and developed a model for prediction of CE results (LS) based on FC levels. A total of 333 patients were recruited; they had small-bowel CE and FC done within 3 months. Overall, correlation between FC and LS was weak (r_s: 0.232, p < 0.001). When 2 clinically significant FC thresholds (100 and 250 μg/g) were examined, the r_s between FC and LS was 0.247 (weak) and 0.337 (moderate), respectively (p = 0.307). For clinically significant (LS greater than or equal to 135) or negative (LS less than 135) for SB inflammation, ROC curves gave an optimum cut-off point of FC 76 μg/g with sensitivity 0.59 and specificity 0.41. The authors concluded that LS appeared to show low correlation with FC as well as other serology markers of inflammation; and FC did not appear to be a reliable biomarker for significant small-bowel inflammation. Nevertheless, FC level greater than or equal to 76 μg/g may be associated with appreciable visual inflammation on small-bowel CE in patients with negative prior diagnostic work-up.

Ministro and Martins (2017) stated that over the past 30 years knowledge on fecal biomarkers (FM) has substantially increased. Nowadays these non-invasive inflammation markers are used in the daily management of IBD. The interest in investigating FM was motivated by the need of a simple, quick, disposable and less invasive marker of disease activity, which might remove the need for endoscopy when following-up with patients. These researchers reviewed the current literature for articles regarding the role of FM in IBD diagnosis, activity, flare prediction, medication and surgical treatment response as well as how FM may differ in adult and pediatric IBD patient populations. The authors stated that although FM is relevant in IBD patient follow-up, there isn't enough data regarding FM reference values for different ages, different disease subtypes, disease localization/extension or response to therapy. Serial measurements of FM for each patient may be useful in accessing relapse in most patients. FM presented more consistent results when used as a predictive tool of relapse after ileocecal surgery in CD. The authors concluded that ongoing research will clarify FM role in decision-making IBD daily practice.

An UpToDate review on “Approach to the adult with chronic diarrhea in resource-rich settings” (Bonis and Lamont, 2017) states that “Calprotectin is a zinc and calcium binding protein that is derived mostly from neutrophils and monocytes. It can be detected in tissue samples, body fluids, and stools, making it a potentially valuable marker of neutrophil activity. Fecal calprotectin levels are increased in intestinal inflammation and may be useful for
distinguishing inflammatory from non-inflammatory causes of chronic diarrhea. A meta-analysis of 13 studies of both adults and children with chronic diarrhea and/or suspected inflammatory bowel disease (IBD) who underwent endoscopy estimated sensitivity and specificity to discriminate IBD from other causes of symptoms. In adults, pooled sensitivity was 93% (95% CI 0.85 to 0.97) and pooled specificity was 96% (95% CI 0.79 to 0.99). In children and teenagers, sensitivity was similar (92%, 95% CI 0.84 to 0.96) but specificity was significantly lower (76%, 95% CI 0.62 to 0.86). The authors note that in settings with a low prevalence of IBD (such as among patients seen for abdominal pain or diarrhea in a primary care setting) the test might be most useful to help rule out IBD while in high prevalence settings (such as a gastroenterology clinic) the test might be most useful for ruling in IBD. However, test characteristics varied considerably among the studies included in the meta-analysis. Furthermore, diagnostic evaluation (including endoscopy) is sometimes needed even if IBD is not strongly suspected. Thus, fecal calprotectin can be considered as an adjunctive test in diagnostic evaluation of patients with chronic diarrhea. Other potential roles have also been proposed including in colorectal cancer screening and monitoring of activity in inflammatory bowel disease. However, its test characteristics are not yet sufficiently defined for routine clinical application for of these indications. Fecal lactoferrin (another marker of neutrophils) has also been proposed as an indicator of intestinal inflammation but its role in the evaluation of patients with chronic diarrhea remains uncertain”.

Furthermore, an UpToDate review on “Clinical manifestations, diagnosis and prognosis of Crohn disease in adults” (Peppercorn and Kane, 2017) states that “Tests for fecal calprotectin or lactoferrin may help identify patients with intestinal inflammation, though they are not routinely performed in clinical practice”.

Pharmacogenomic and Metabolic Assessment of Thiopurine Therapy

The PRO-Predict series of tests were developed by Prometheus, Inc. with the intent of providing guidance in determining therapeutic direction and predicting therapeutic response in individual patients. PRO-Predict 6MP is for 6-MP/Azathioprine remission and toxicity monitoring, PRO-Predict TPMT is for 6-MP/azathioprine response stratification, and PRO-Predict TNF is for anti-TNF response stratification. However, there is insufficient scientific evidence in the current medical literature to support the routine use of these tests in clinical practice.

Although there is a potential to regulate azathioprine and mercaptopurine according to measurement of metabolites, the optimal mode of therapeutic monitoring remains to be established through prospective clinical trials demonstrating that clinical outcomes are improved through metabolite monitoring. Current evidence is largely limited to studies
exploring correlations between metabolite levels and azathioprine and mercaptopurine toxicity and response. These studies have shown conflicting results in the strength of the relationship between metabolite levels, toxicity, and response. There are no well-designed prospective clinical studies demonstrating that azathioprine or mercaptopurine therapy based upon results of metabolite testing leads to improved clinical outcomes compared to therapy based upon clinical assessment and standard testing including routine blood counts and liver enzymes.

A significant association has been found between erythrocyte 6-thioguanine levels and the likelihood of clinical remission with azathioprine and 6-mercaptopurine (Osterman et al, 2006); however, studies of the relationship between 6-thioguanine levels and the likelihood of clinical remission have not shown consistent results (Cuffari et al. 1996; Gupta et al, 2001; Lowry et al, 2001; Belaiche et al, 2001; Gilissen et al, 2004). A similar association with clinical remission has not been found with levels of 6-methylmercaptopurine (Cuffari et al, 1996; Dubinsky et al, 2000; Gilissen et al, 2004). Despite an overall correlation with clinical response rates, clinical studies have shown a wide range of rates of response and toxicity across a range of 6-TG levels (e.g., Cuffari et al, 1996; Dubinsky et al, 2000). In addition, studies have shown that the predictive value of metabolite levels in individual patients is low (e.g., Cuffari et al, 2006; Goldenberg et al, 2004). Another limitation to using metabolites levels to guide drug dosing is that there may be substantial variation in levels between measurements in individual patients (e.g., Wright et al, 2004).

Bone marrow toxicity due to azathioprine or 6-mercaptopurine has been found to correlate with elevated levels of 6-thioguanine, and liver toxicity has been found to correlate with levels of 6-methylmercaptopurine (Ciffari et al, 1996; Dubinsky et al, 2000; Ooi et al, 2007). However, clinical studies have shown that 6-methylmercaptopurine levels are not an accurate predictor of liver toxicity in individual patients (e.g., Goldenberg et al, 2004; Shaye et al, 2007; Heneghan et al, 2006; Reinshagen et al, 2007). In addition, other adverse reactions, such as pancreatitis or leukopenia, can occur unrelated to serum metabolite levels (Cuffari et al, 1996; Ooi et al, 2007). Thus, measurement of serum metabolite levels can not replace regular monitoring for toxicity with blood counts and liver enzymes.

In addition, measurement of serum metabolite levels must be compared with commonly available standard laboratory methods. Waljee et al (2010) reported that algorithms that use age and laboratory values (CBC and chemistries) can differentiate clinical response, nonadherence, and shunting of thiopurine metabolism among patients who take thiopurines, and that this approach was more accurate than 6-TGN metabolite measurements in predicting clinical response.
A Cochrane Collaboration meta-analysis of literature on azathioprine and 6-mercaptopurine in Crohn’s disease (Sandborn et al, 2002) concluded: “Another potential area of investigation is individualization of therapy. 6-Mercaptopurine is inactivated by methylation catalyzed by thiopurine methyltransferase (TPMT) whereas the active metabolites are the 6-thioguanine nucleotides (6-TGN). The toxicity and effectiveness of 6-mercaptopurine therapy in childhood lymphocytic leukemia is inversely correlated with the activity of TPMT and directly correlated with the erythrocyte concentrations of 6TGN. Measurement of both TPMT enzyme activity and erythrocyte 6TGN (therapeutic drug monitoring) in patients with Crohn’s disease may allow individualized dosing to produce an optimal therapeutic effect while minimizing the potential for leukopenia. Colanna and Korelitz (1994) have advocated using the presence of mild leukopenia as an indicator of the appropriate dose of 6-mercaptopurine. In their recent retrospective analysis of 6-mercaptopurine therapy in 98 patients with refractory CD, they demonstrated a correlation between leukopenia and the achievement and maintenance of remission. However, another retrospective study reported that leukopenia did not predict response in patients with UC treated with 6-mercaptopurine. Prospective studies are needed to determine whether any of these attempts to optimize therapy with azathioprine or 6-mercaptopurine will be of use in routine clinical practice.”

Guidelines from the American College of Gastroenterology (2001) on management of CD state that, although there is a potential to regulate azathioprine and mercaptopurine according to measurement of metabolites, the optimal mode of therapeutic monitoring remains to be established through clinical trials. “It remains to be determined how to optimize dose and whether induction of leukopenia or therapeutic monitoring of 6-thioguanine metabolites offer improved means of assuring a long-term response.” American College of Gastroenterology guidelines on UC (2004) reached similar conclusions about the utility of metabolite monitoring. “The utility of thiopurine metabolite testing requires prospective controlled evaluation before routine use can be recommended.”

A position statement from the American Gastroenterological Association (Lichtenstein et al, 2006) provides a Grade C recommendation for the use of thiopurine metabolite monitoring in the treatment of patients with 6-MP or AZA when attempting to determine medical noncompliance. The guidelines note that thiopurine metabolite monitoring “may be helpful” for optimizing dose and monitoring for toxicity. The guidelines, however, do not provide specific recommendations for use of thiopurine monitoring for these indications. “Given the conflicting data, the retrospective nature of these studies, and the limited positive and negative predictive values for these particular uses, the utility of these tests needs prospective controlled evaluation before their routine use can be recommended.”

More recently, an American College of Gastroenterology (ACG) (Lichtenstein et al, 2009)
guideline on CD in adults stated that, although retrospective analyses indicate that
determination of 6-TGN and 6 MMP levels can be helpful to assess lack of response, patient
adherence, leukopenia, or elevations in liver enzymes, "at the present time, there are
inadequate data to recommend routine measurement of these metabolites...." The discussion
of metabolite monitoring in the supporting ACG technical review cites a European evidence
based consensus on the diagnosis and management of Crohn's disease (2006), where none of
the consensus used 6-thioguanine concentrations to adjust the dose. The discussion in the
AGA technical review also cites an American Gastroenterological Association Technical Review
(Lichtenstein, et al., 2006), which stated that “[t]hiopurine metabolite monitoring in the
treatment of patients with 6-MP or AZA is useful when attempting to determine medical
noncompliance ...”. It should be noted that this is a grade C recommendation based upon low
quality evidence (defined as “evidence based on clinical experience, descriptive studies, or
reports of expert committees”). Although thiopurine metabolite monitoring has been
advocated for assessing compliance, there are no rigorous study data on this use. The
guidelines also state that thiopurine metabolite monitoring “may be helpful” (grade C
recommendation) for optimizing dose and monitoring for toxicity. The guidelines, however, do
not provide specific recommendations for use of thiopurine monitoring for these indications.
The technical review reports that “[s]ome, but not all, recent retrospective studies have
suggested that measurement of AZA and 6-MP metabolites may be useful in dose
adjustments.... Given the conflicting data, the retrospective nature of these studies, and the
limited positive and negative predictive values for these particular uses, the utility of these
tests needs prospective controlled evaluation before their routine use can be recommended.
The utility of these metabolite markers, however, can assist an individual in determining
whether a patient is noncompliant with their immunomodulator therapy.” The technical review
also states that “Future prospective studies with measurement of dose optimization
based on clinical efficacy and remission compared with measurements of TPMT, 6-TGN, and 6-
MMP levels are still needed to better define appropriate treatment algorithms and doses in
patients with UC. These particular studies can also assess the level (if any is present) of
incremental benefit to the traditional routine of monitoring complete blood count, liver-
associated laboratory chemistries, and clinical response.” It also should be noted that the FDA-
approved labeling for Imuran (azathioprine) and mercaptopurine include recommendations
for TPMT genotyping and phenotyping, but include no recommendation for use of thiopurine
metabolites in dosing and administration.

The role of 6-TGN for assessing compliance, and in combination with 6-MMP to assess drug-
failure attributable to high methylator status has been advocated but has not
been substantiated by rigorous study data.
Prospective clinical studies are needed to determine whether outcomes are improved by tailoring individual drug regimens using azathioprine metabolite testing. A prospective, randomized controlled clinical trial found that 6-TGN-adapted azathioprine therapy did not lead to higher remission rates than standard therapy in patients with chronic active CD. Reinshagen et al (2007) reported on a prospective randomized trial that examined whether 6-TGN concentration-adapted azathioprine therapy is clinically superior to a standard dose of 2.5 mg/kg/day azathioprine in patients with CD. After 2 weeks of standard therapy, patients (n = 71) were randomized into standard (n = 32) or adapted-dose (n = 25) groups; 14 patients dropped out before randomization. In the adapted group, the azathioprine dose was adjusted to maintain 6-TGN concentrations between 250 and 400 pmol/8 x 10(8) erythrocytes (Ery). Response criteria were the number of patients in remission after 16 weeks without steroids (primary) and remission after 24 weeks, frequency of side effects, and quality of life (secondary). After 16 weeks, 14 of 32 (43.8 %) patients in the standard group versus 11 of 25 (44 %) in the adapted group were in remission without steroids (intent-to-treat analysis). After 24 weeks, 43.8 % versus 40 % were in remission. The researchers found no significant differences concerning quality of life, disease activity, 6-TGN concentrations, azathioprine dose, or dropouts due to side effects. Sixty-six patients had a wild-type TPMT genotype, with TPMT activities of 8 to 20 nmol/(mL Ery x h). Five patients (dropouts after randomization) were heterozygous, with TPMT activities less than 8 nmol/(mL Ery x h). 6-MMP concentrations greater than 5,700 pmol/8 x 10(8) Ery were not associated with hepatotoxicity. The investigators concluded that standard and adapted dosing with the provided dosing scheme led to identical 6-TGN concentrations and remission rates. The investigators reported that adapted dosing had no apparent clinical benefit for patients with TPMT activity between 8 and 20 nmol/(mL Ery x h). The investigators also found that 6-MMP monitoring had no predictive value for hepatotoxicity. Additional prospective controlled clinical studies of thiopurine metabolite monitoring have been sponsored by the National Institutes of Health (NIDDK, 2010).

Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of azathioprine, 6-mercaptopurine and thioguanine. Several studies have reported correlations between mutations in the TPMT gene with susceptibility to leukopenia from azathioprine and mercaptopurine therapy, and investigators have proposed using the results of TPMT gene mutation testing to select candidates for azathioprine therapy. However, almost all studies that have been published to date have only tested patients exhibiting toxicity for TPMT gene mutations and not a broader group of patients; thus, the sensitivity and specificity of TPMT gene mutation testing to toxicity susceptibility is unknown (Evans et al, 2001; Colombel et al, 2000). In those studies where all patients were tested for TPMT gene mutations prior to therapy, TPMT mutations were detected in only a minority of patients exhibiting drug-induced
leukopenia (Naughton et al, 1999; Dubinsky et al, 2000; Relling et al., 1999). This has led investigators to conclude that myelosuppression may be due to other factors in addition to variable TPMT therapy, and that monitoring of blood counts throughout azathioprine or mercaptopurine therapy is essential in all patients, regardless of the presence or absence of TPMT gene mutations. In addition, there are no prospective studies demonstrating improvement in outcomes by guiding azathioprine therapy or mercaptopurine therapy based on the results of TPMT gene mutation testing.

Prometheus Laboratories, manufacturer of the ProPredict Series of Tests, has obtained United States distribution rights for Imuran (azathioprine). The FDA has agreed to include information about TPMT genotyping and phenotyping in the Imuran product labeling. It should be noted that the product labeling emphasizes that TPMT testing cannot substitute for complete blood count monitoring in patients receiving Imuran.

A position statement from the American Gastroenterological Association (Lichtenstein et al, 2006) notes that information about TPMT testing is included in azathioprine product labeling. The position statement explains that “[c]urrent Food and Drug Administration (FDA) recommendations suggest that individuals should have thiopurine methyltransferase (TPMT) genotype or phenotype assessed before initiation of therapy with AZA [azathioprine] or 6-MP [6-mercaptopurine] in an effort to detect individuals who have low enzyme activity (or who are homozygous deficient in TPMT) in an effort to avert AZA or 6-MP therapy and thus avoid potential adverse events.” The position statement notes, however, that individuals who have intermediate or normal TPMT activity (wild type or heterozygotes) need measurement of frequent complete blood counts (as above) in addition to TPMT assessment because these individuals may still develop myelosuppression subsequent to use of azathioprine or 6-mercaptopurine.

Guidelines from the British Society of Gastroenterology (2004) stated that TPMT testing is not necessary. The guidelines state that research has shown that the majority (77 %) of inflammatory bowel disease patients with azathioprine (AZA)-induced bone marrow suppression did not carry a TPMT mutation. The guidelines noted that evidence that TPMT activity predicts other side effects or outcome is limited. The guidelines noted that TPMT testing “cannot yet be recommended as a prerequisite to therapy, because decades of experience has shown clinical AZA to be safe in UC [ulcerative colitis] or CD [Crohn’s Disease].”

Guidelines from the American College of Gastroenterology (Kornbluth and Sachar, 2004) are in agreement. “6-MP and its prodrug azathioprine are both metabolized by thiopurine methyltransferase (TPMT), and enzyme that exhibits variation as a result of a genetic polymorphism of its alleles and this enzyme can now be measured by commercial
laboratories. Approximately 0.3% of the general population have low to absent enzyme activity, 11% have intermediate, and 89% have normal to high levels of activity. However, only about a quarter of cases of leukopenia in practice are associated with one of these genetic polymorphisms. Therefore, prospective studies of dose-optimization based on measurements of TPMT, 6-TG, or 6-MP levels to monitor clinical response are still needed before the routine use of these assays can be recommended as providing much incremental benefit to the traditional routine of monitoring the CBC, liver associated laboratory chemistry abnormalities, and clinical response.”

Teml et al (2007) summarized clinical pharmacological aspects of thiopurines in the treatment of chronic IBD. These investigators noted that on the basis of an excellent phenotype-genotype correlation for TPMT, genotyping has become a safe and reliable tool for determination of an individual's phenotype. Based on several cost-benefit analyses, assessment of TPMT activity is recommended prior to thiopurine therapy in patients with IBD. They also stated that although the therapeutic response appears to be related to 6-TGN concentrations above a threshold of 230 to 260 pmol/8 x 10(8) red blood cells, currently therapeutic drug monitoring of 6-TGN can be recommended only to estimate patients' compliance.

In a cross-sectional study, Firooz et al (2008) examined the role of TPMT activity in the safety and effectiveness of azathioprine in the treatment of pemphigus vulgaris. A total of 139 patients were included in this study. The TPMT activity in red blood cells was measured using high-performance liquid chromatography. Severe adverse effects were defined as those judged serious enough that azathioprine therapy be discontinued. These investigators also evaluated the relationship of clinical response and TPMT concentration in 52 patients who had been treated with a combination of prednisolone and azathioprine for at least 1 year, and the clinical response was considered favorable if there was no recurrence of pemphigus vulgaris in the first year of treatment. The median activity of TPMT was 44.7 ng/ml/h (interquartile range of 28.7 ng/ml/h). Eleven patients (7.9%) had low TPMT activity (TPMT-HL), 127 patients (91.4%) had normal TPMT activity (TPMT-HH), and 1 patient (0.7%) had supra-normal enzyme activity; TPMT activity was noted in all patients. Serious adverse effects occurred in 14 patients (10.1%). There was no relationship between development of adverse effects and TPMT activity (p = 0.29). Eleven patients with low TPMT activity had been treated with azathioprine for a mean (SD) of 10.2 (4.1) months. Only 1 patient exhibited serious adverse effects. The TPMT enzyme activity was not different in 28 patients with unfavorable clinical response compared with 24 patients with favorable clinical response (p = 0.09). The authors concluded that larger prospective studies are needed to determine the clinical relevance of TPMT activity and to determine accurate azathioprine dosing guidelines based on TPMT
A study by Heneghan et al (2006) of patients with autoimmune hepatitis receiving azathioprine found that advanced fibrosis, rather than TPMT genotype or phenotype, predicted drug toxicity. In addition, the investigators found that measurement of 6-TG and 6-MMP levels was not useful. The investigators retrospectively evaluated data on 6-TG and 6-MMP levels and on TPMT genotyping and enzyme activity in patients with autoimmune hepatitis who received azathioprine therapy with or without prednisone. From 1995 through 2001, 86 patients began courses of azathioprine; to maintain remission, 26 received azathioprine alone, 19 received prednisone alone, 26 received azathioprine plus prednisone, 9 received alternative therapies (mycophenolate mofetil, sirolimus, or cyclosporine), and the rest received no therapy. TPMT genotyping was performed and 6-TG and 6-MMP levels were measured in all patients. Twenty-two patients had at least 1 episode of azathioprine-related toxicity. Neither TPMT genotype nor enzyme activity predicted such episodes, although cirrhosis was more common among patients who developed azathioprine toxicity than among those who did not (p = 0.043). No threshold levels of 6-TG or 6-MMP predicted remission.

Cao et al (2009) identified the TPMT gene polymorphisms in Chinese IBD patients and studied the relationship between TPMT status and thiopurine-related toxicity in these patients. A total of 189 IBD patients, 87 with CD and 102 with UC, and 273 healthy controls were enrolled. All subjects were from the Han Chinese ethnic group. Polymorphisms in TPMT*2, *3A, *3B and *3C were analyzed using allele-specific polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism. Direct sequencing was used to confirm the mutation results. Exons of the TPMT gene from patients who suffered from azathioprine-induced toxicity were amplified and sequenced to detect TPMT mutations. No TPMT*2, *3A or *3B mutant alleles were detected. The allele frequency of TPMT*3C in the IBD group was 1.59 %, which was similar to that of the healthy control group (1.59 % versus 1.47 %, p = 1.000). Forty-three patients were treated with azathioprine therapy, 4 experienced myelotoxicity, and 1 experienced hepatotoxicity, so the incidence of drug toxicity was 11.7 % (5/43). No TPMT*2, *3A, *3B or *3C polymorphisms were detected in these 5 patients. After directly sequencing the exons of the TPMT gene in these 5 patients, a synonymous single-nucleotide polymorphism (TPMT*1S), which does not alter the encoded amino acid, was found in 3 patients. The authors concluded that TPMT*3C seemed to be a unique variant allele in this Han Chinese population. The overall frequencies of variant TPMT alleles in this population were lower than those in Caucasians, but thiopurine toxicity in Han Chinese IBD patients is not low. The authors reported that factors other than TPMT polymorphisms may be responsible for the development of toxicity. Similarly, Takatsu et al (2009) found that adverse reactions to azathioprine can not be predicted by thiopurine S-
methyltransferase genotype in Japanese patients with inflammatory bowel disease.

There is no indication for repeat pharmacogenomic testing for TPMT, as a person’s TPMT genotypic or phenotypic status remains unchanged over one’s lifetime.

**NOD2/CARD15 Genotyping for Crohn’s Disease**

Nucleotide-binding oligomerization domain (NOD) proteins are cytosolic proteins that include principal regulators of apoptosis such as the apoptotic protease activating factor 1. NOD proteins have also been described as intra-cellular activators of the caspase and nuclear factor-kappaB (NF-kappaB) signaling pathways. In particular, NOD1, NOD2, cryopyrin, and ICE protease-activating factor (ICE refers to interleukin-1b converting enzyme that is also known as caspase-1) have been implicated in protective immune responses against pathogens. Moreover, a large number of NOD proteins contain leucine-rich repeats (LRR), hence referred to as NOD-LRR proteins, which include human NOD2, cryopyrin, and MHC class II trans-activator (CIITA), as well as mouse neuronal apoptosis inhibitory protein 5. NOD2 participates in the signaling events triggered by host recognition of specific motifs in bacterial peptidoglycan and, upon activation, induces the production of pro-inflammatory mediators. Neuronal apoptosis inhibitory protein 5 is needed in macrophages to restrict intra-cellular growth of Legionella pneumophila, whereas CIITA plays a critical role in antigen presentation and development of antigen-specific T lymphocytes. Thus, NOD-LRR proteins appear to be involved in a diverse array of processes needed for host immune reactions against pathogens (Inohara and Nunez, 2003; Inohara et al, 2005; Eckmann and Karin, 2005).

Epidemiological data, notably concordance rates in twin pairs as well as sibling pairs, have provided support for the importance of the genetic contribution to inflammatory bowel diseases, especially in CD. Studies have found that genetic variations in several NOD-LRR proteins resulting in the abolishment of NF-kappaB signal transduction of pattern recognition receptors such as NOD/caspase recruitment domain (NOD/CARD) receptors is associated with inflammatory disease or increased susceptibility to microbial infections. The identification of the IBD1 gene on chromosome 16 as NOD2 (also known as CARD15) is an important finding. Specifically, mutations in the gene encoding NOD2 have been reported to be associated with the predisposition to CD and Blau syndrome. It is estimated that at least 5 additional genes are also involved in susceptibility to CD. These findings provided the impetus for laboratory-based studies of the molecular genetics of IBD, CD and UC. Although many issues regarding gene function and expression remain to be resolved, there is much enthusiasm that useful clinical applications may follow (Satsangi et al, 2003; Girardin et al, 2003; Carneiro et al, 2004; Philpott and Viala, 2004).
Mutations in the gene that encodes NOD2 occur in a small portion of patients with CD. Hampe et al. (2001) noted that background genetic predisposition to IBD has been shown by epidemiological and linkage studies. These researchers sequenced the coding region of the NOD2 gene and genotyped an insertion polymorphism affecting the leucine-rich region of the protein product in 512 individuals with IBD from 309 German or British families, 369 German trios (namely, German patients with sporadic IBD and their unaffected parents), and 272 normal controls. They then tested for association with CD and UC. Family-based association analyses were consistently positive in 95 British and 99 German affected sibling pairs with CD (combined p < 0.0001); the association was confirmed in the 304 German trios with CD. No association was seen in the 115 sibling pairs and 65 trios with UC. The genotype-specific disease risks conferred by heterozygous and homozygous mutant genotypes were 2.6 (95 % CI: 1.5 to 4.5) and 42.1 (4.3 to infinity), respectively. These investigators stated that the insertion mutation in the NOD2 gene confers a substantially increased susceptibility to CD, but not to UC. The lack of effect of the described mutation on UC might be due to the use of different pathways in this disorder. Also, the NF-kappaB activation is stronger in CD than UC. Moreover, the authors noted that this NOD2 frame-shift mutation is rather rare -- approximately 6.5 % of CD patients are homozygous for it. They estimated that about 18 % of the genetic risk in the population can be attributed to this mutation; and did not find any homozygote in the control group.

Ahmad and co-workers (2002) stated that mutations in the NOD2 gene have been associated with CD, but are found in only 25 % of patients. No data regarding their contribution to specific disease subtypes exist. The authors reported a detailed genotype-phenotype analysis of accurately characterized patients. A total of 244 white patients with CD recruited from a single center in the United Kingdom were studied. All patients were phenotyped and followed-up for a median time of 16 years. By using linkage disequilibrium mapping, these researchers studied 340 polymorphisms in 24 HLA genes and 3 NOD2 polymorphisms. They demonstrated that NOD2 mutations determine ileal disease only, and confirmed that alleles on specific long-range HLA haplotypes determine overall susceptibility to CD. These investigators concluded that clinical pattern of CD may be defined by specific genotypes.

There is also ethnic variation in the relationship of NOD2 genotype and clinical phenotype. Guo et al. (2004) noted that an insertion mutation at nucleotide 3020 (3020insC) in NOD2 is associated with CD. The C-insertion mutation at nucleotide 3020 (3020insC) in the LRR region results in a frame-shift in the 10th LRR followed by a premature stop codon, which is responsible for the inability to activate NF-kappaB in response to bacterial lipopolysaccharide. These researchers aimed to genotype NOD2 gene 3020insC frame-shift mutation in Chinese patients with IBD. They genotyped an insertion polymorphism affecting the leucine-rich
region of the protein product by the allele specific PCR in 74 unrelated patients with ulcerative colitis of Han nationality in Hubei Province of China, 15 patients with CD and 172 healthy individuals. No significant differences were found in the genotype and allele frequencies of the C-insertion mutation of NOD2 gene among patients with CD and UC and healthy controls. These investigators concluded that NOD2 gene 3020insC frame-shift mutation is not a major contributor to the susceptibility to both CD and UC in Chinese Han patients. Moreover, Tosa et al (2006) noted that Japanese patients with CD do not have any of the common NOD2/CARD15 variants that are associated with CD in Caucasians.

Economou et al (2004) stated that 3 variants of the NOD2 gene -- SNP8, SNP12, and SNP13 -- have been associated with CD. These investigators assessed the impact of NOD2 variants on the CD risk across diverse populations and examined possible associations with disease phenotype. In a meta-analysis, a total of 42 eligible studies contributed data on 206 comparisons. No variants were detected in Asians. In non-Jewish descent Caucasians, carriage of SNP8, SNP12, or SNP13 had an odds ratio (OR) for CD of 2.20 (95 % CI: 1.84 to 2.62), 2.99 (95 % CI: 2.38 to 3.74), and 4.09 (95 % CI: 3.23 to 5.18), respectively. For Jewish descent patients, the corresponding ORs were 1.74, 1.93, and 2.45, respectively. The OR in carriers of at least 2 alleles was 17.1 (95 % CI: 10.7 to 27.2). Large studies tended to yield more conservative estimates than smaller studies, so publication or other bias can not be excluded. Among patients with CD, carrying at least 1 high-risk variant increased slightly the risk for familial disease (OR = 1.49, (95 % CI: 1.18 to 1.87)), modestly the risk of stenosing CD (OR = 1.94, (95 % CI: 1.61 to 2.34)), and more prominently the risk of small bowel involvement (OR = 2.53, (95 % CI: 2.01 to 3.16)). The authors concluded that SNP8, SNP12, and SNP13 have differential effects on CD risk, with SNP13 having the strongest genetic effect. The authors found that these NOD2 variants may also be significant risk factors for CD phenotype, especially ileal location.

Uyar and co-workers (2006) stated that 3 common genetic variations -- R702W, G908R, and 1007fs -- on CARD15 have been shown to increase the risk for CD in Caucasian populations. The authors ascertained the frequencies of these CARD15 variants by genotyping in 56 patients with CD and 100 healthy ethnically matched controls from Turkey. Overall frequency of all 3 variants was 10.7 % in patients with CD, compared with 1.5 % in controls (OR: 7.9). Among them, the frequency of the G908R variant allele was 8 % in CD cases, compared with 0 % in controls (OR: 36.8). The allele frequencies of 3 CD-related CARD15 variants were considerably lower in the control group compared to the reported Caucasian populations. Among the described CARD15 variants, G908R confers an increased susceptibility to CD, whereas the more frequently reported associations in Europeans with R702W and 1007fs are not confirmed in this Turkish population.
In addition to studies on the role of NOD2 genetic variations in the development of intestinal strictures, questions also arise regarding whether the response to treatment or need for surgery can be predicted by genotype. Preliminary reports analyzing the association between these variants and the need for surgeries have produced inconsistent results. Alvarez-Lobos and associates (2005) examined the predictive value of NOD2 gene variants along with disease phenotypic characteristics for requirement of initial surgery and for surgical recurrence in CD. A total of 170 CD patients were included prospectively in the study and followed-up regularly for a mean of 7.4 +/- 6.1 years. Clinical characteristics of CD, time and indication for surgery, and recurrence were noted. NOD2 gene variants were determined by DNA sequencing analysis. It was reported that surgery for stricturing disease was significantly more frequent in patients with NOD2 variants in the uni-variate analysis (OR, 3.63; 95 % CI: 1.42 to 9.27), and it was required at an earlier time (p = 0.004). Only NOD2 variants (OR, 3.58; 95 % CI: 1.21 to 10.5) and stricturing phenotype at diagnosis of CD (OR, 9.34; 95 % CI: 2.56 to 33.3) were independent predictive factors of initial surgery for stricturing lesions in the multivariate analysis. Among 70 patients that required surgery, post-operative recurrence was also more frequent in patients with NOD2 variants in the uni-variate and multi-variate analysis (OR, 3.29; 95 % CI: 1.13 to 9.56), and re-operation was needed at an earlier time (p = 0.03). The authors concluded that NOD2 genotyping may have a useful clinical application as a major marker of evolution of CD, especially an early need of initial surgery due to stricturing disease and need of re-operation. They noted that although determination of the NOD2 genotype presently cannot be used to guide indications for surgery, it might define a subgroup of patients with a severe course of the disease, who may require a more aggressive therapeutic approach to prevent the appearance of complications. Moreover, these investigators stated that confirmation of these results in future studies is needed before interventional studies based on NOD2 genotyping are designed.

Fries and associates (2005) noted that a defect of gastrointestinal barrier function is considered to represent an important step in the pathogenesis of CD; but the mechanisms leading to an increased intestinal permeability (IP) are poorly understood. Since IP is influenced by pro-inflammatory mediators, it seems likely that a genetically determined abnormal immune response may lead to a loss of barrier function. In a geographic area in Southern Italy with high incidence of CD, these researchers examined IP (by means of lactulose/mannitol testing) together with the 3 main mutations of the NOD2/CARD15 and the D299G polymorphism of the toll-like receptor (TLR)-4 gene in 23 families of CD patients (patients and 1st-degree relatives). A total of 48 % of CD patients and 40 % of their healthy relatives were found to have an abnormal IP compared to 5 % of an appropriate control population (p < 0.0001). However, IP was not associated with the L1007finsC mutation of the NOD2/CARD15 or the D299G variant of the TLR-4 gene. Allele frequency of the only
L1007finsC mutation of CARD15 was significantly increased in patients (8.7 %, p < 0.003) and in relatives (8.3 %, p < 0.024) compared with controls (2.4 %), whereas the D299G variant of the TLR-4 gene was found to be increased only in relatives (8.3 %, p < 0.022), but not in patients (4.3 %) compared with the control population (1.7 %). These investigators concluded that there was no association between IP and genetic markers. These findings showed a very high proportion of healthy 1st-degree relatives to bare alterations suggested to constitute determinants of CD. Mutations of NOD2/CARD15 or TLR-4, however, do not lead to permeability defects emphasizing the importance of additional environmental and/or genetic factors for pathogenesis.

While research has been focused on trying to explain the biological role of NOD2 and how mutations can contribute to the development of CD, it is important to note that the association between NOD2 gene variants and CD is not absolute (i.e., not everyone who has one of the variants will get the disease, and not everyone with CD has an alteration in NOD2). Mascheretti and Schreiber (2005) noted that genetic testing for the NOD2/CARD15 variants has only a modest relevance in clinical practice. This is in agreement with the observations of Vermeire (2004) who stated that the relevance of NOD2/CARD15 genotyping for clinical practice is modest. The current data show that NOD2/CARD15 mutations in CD are associated with small-bowel involvement. More studies are needed to ascertain if NOD2/CARD15 mutations are also associated with a fibro-stenotic behavior of the disease. If CARD15 variants would predict a more aggressive disease course, then a more aggressive treatment may be justified in these patients after NOD2/CARD15 genetic testing. It is not clear whether NOD2/CARD15 genotyping is helpful in differentiating indeterminate colitis patients. Although CARD15 variants do not predict response to the tumor necrosis factor-alpha monoclonal antibodies, the role of the gene in response to other drugs is not known. Finally, screening unaffected relatives of CD patients is not recommended until preventive strategies are available. It is also interesting to note that although there is evidence to suggest that NOD2/CARD15 may influence individuals’ susceptibility to colorectal cancer, there is no evidence to indicate that mutations of this gene predict the clinicopathological characteristics of this disease (Roberts et al, 2006). Chamaillard and colleagues (2006) stated that the current etiologic model for IBD emphasizes an interaction between susceptibility and modifier genes along with environmental factors. Together, these lead to disease progression. However, further work should clarify the pathophysiological mechanisms leading to IBD and how innate immune signaling confers susceptibility to intestinal inflammation.

There is currently insufficient scientific evidence to support the clinical use of NOD2/CARD15 genotyping of CD. More research is needed to define more precisely the relationship between NOD2/CARD15 genotype, clinical phenotype, and the effect of ethnic variation on this
relationship. Further investigation is also needed to better understand the mechanisms involved in gene-environment interactions in the gastrointestinal tract, and to examine the role of genetic variations in the NOD2/CARD15 gene on the natural cause of the disease, response to therapies and need for surgery.

**CPT Codes / HCPCS Codes / ICD-10 Codes**

*Information in the [brackets] below has been added for clarification purposes. &nbsp;Codes requiring a 7th character are represented by "+":*

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<thead>
<tr>
<th>TPMT gene mutation assays or TPMT phenotypic assays (eg, Prometheus TPMT Genetics, Prometheus TPMT Enzyme):</th>
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<td><strong>CPT codes covered if selection criteria are met:</strong></td>
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<td>81401</td>
</tr>
<tr>
<td>82657</td>
</tr>
</tbody>
</table>

**CPT codes not covered for indications listed in the CPB:**

83789 Mass spectrometry and tandem mass spectrometry (eg, MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen

86256 Fluorescent noninfectious agent antibody; titer, each antibody

**Anti-chitobioside carbohydrate antibodies (ACCA), Anti-laminaribioside carbohydrate antibodies (ALCA), Anti-mannobioside carbohydrate antibodies (AMCA) anti-chitin IgA (Anti-C), anti-laminarin IgA (Anti-L):**

**Anti-neutrophil cytoplasmic antibodies (ANCA), anti-Saccharomyces cerevisae antibodies (ASCA), anti-out membrane porin C (OmpC) antibodies, anti-CBir1 flagellin (anti-CBir1) antibodies, 12 antibodies, and Anti-smooth muscle antibodies (ASMA):**

**CPT codes not covered for indications listed in the CPB:**

82397 Chemiluminescent assay

83516 Immunoassay for analyte other than infectious agent antibody or infectious agent antigen, qualitative or semi-quantitative; multiple step method

83518 Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, single step method (eg, reagent strip)

83519 quantitative, by radioimmunoassay (eg, RIA)
83520  Immunoassay, analyte, quantitative; not otherwise specified
86021  Antibody identification; leukocyte antibodies [ANCA antibodies]
86255  Fluorescent noninfectious agent antibody; screen, each antibody
86671  Antibody; fungus, not elsewhere specified
88350  Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)

6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine nucleotide (6-MMPN) (Prometheus Thiopurine Metabolites):

**CPT codes covered if selection criteria are met:**

82491  Chromatography, quantitative, column (e.g., gas liquid or HPLC); single analyte not elsewhere specified, single stationary and mobile phase

**CPT codes not covered for indications listed in the CPB:**

80299  Quantitation of therapeutic drug, not elsewhere specified

**Other HCPCS codes related to the CPB:**

J7500  Azathioprine, oral, 50 mg
J7501  Azathioprine, parenteral, 100 mg

**Calprotectin:**

**CPT codes not covered for indications listed in the CPB:**

83520  Immunoassay, analyte, quantitative; not otherwise specified
83993  Calprotectin, fecal

**Other HCPCS codes related to the CPB:**

J7500  Azathioprine, oral, 50 mg
J7501  Azathioprine, parenteral, 100 mg
S0108  Mercaptopurine, oral 50 mg

**NOD2/CARD15 genotyping:**

No specific code

The Prometheus IBD sgi diagnostic panel and Prometheus IBS diagnostic panel (see next entry):

(including BDNF [brain-derived neurotrophic factor], GRO-α [growth-regulated oncogene-alpha], IL1B [interleukin-1beta], NGAL [neutrophil gelatinase-associated lipocalin], CD14 gene C-260T polymorphism testing, interleukin-10-1082A/G polymorphism testing, Prometheus Crohn’s Prognostic

No specific code

TIMP-1 [tissue inhibitor of metalloproteinase-1], tTG [anti-human tissue transglutaminase IgA) and TWEAK [TNF-related weak inducer of apoptosis]:

No specific code
<table>
<thead>
<tr>
<th>No specific code</th>
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**CPT codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>82397</td>
<td>Chemiluminescent assay</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay, analyte, quantitative; not otherwise specified</td>
</tr>
<tr>
<td>86140</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>88347</td>
<td>Immunofluorescent study, each antibody; indirect method</td>
</tr>
</tbody>
</table>

*Crohn’s disease peptide antibody testing, eXalBD test (a quantitative PCR test for 6 genes [BLCAP, UBE2G1, RAP1A, CALM3 AND NONO]), Measurement of serum mannose-binding lectin, Macrophage inflammatory protein-1β (MIP-1β):*

**Interleukin-6, IL-8:**

No specific code

**CPT codes not covered for indications listing in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>83520</td>
<td>Immunoassay, analyte, quantitative; not otherwise specified</td>
</tr>
</tbody>
</table>

**ICD-10 codes not covered if selection criteria are met:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>K50.0 - K51.919</td>
<td>Regional enteritis and ulcerative colitis</td>
</tr>
</tbody>
</table>

*Myeloperoxidase antibody testing:*

**CPT codes not covered for indications listing in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>83876</td>
<td>Myeloperoxidase (MPO)</td>
</tr>
</tbody>
</table>

*Proteinase-3 antibody testing:*

**CPT codes not covered for indications listed in the CPB:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>83516</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen, qualitative or semi-quantitative; multiple step method</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay, analyte, quantitative; not otherwise specified</td>
</tr>
<tr>
<td>86021</td>
<td>Antibody identification; leukocyte antibodies</td>
</tr>
</tbody>
</table>

*Raman spectroscopy:*

No specific code

**ICD-10 codes covered if selection criteria are met:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>K50.0 - K51.919</td>
<td>Regional enteritis and ulcerative colitis</td>
</tr>
</tbody>
</table>

**ICD-10 codes not covered if selection criteria are met:**

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>K58.0 - K58.9</td>
<td>Irritable bowel syndrome</td>
</tr>
</tbody>
</table>

The above policy is based on the following references:


EEE. Thompson AI, Lees CW. Genetics of ulcerative colitis. Inflammatory Bowel Diseases. 2011;17(3):831-848.


QQQQ. Peppercorn MA, Kane SV. Clinical manifestations, diagnosis and prognosis of Crohn disease in adults. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed December 2016.


Fecal Markers of Inflammatory Bowel Disease


Turner D, Leach ST, Mack D, et al. Faecal calprotectin, lactoferrin, M2-pyruvate kinase and S100A12 in severe ulcerative colitis: A prospective multicentre comparison of predicting


UUUU. Sipponen T, Savilahti E, Kolho KL, et al. Crohn's disease activity assessed by fecal


WWWWW. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: Diagnostic meta-analysis. BMJ. 2010;341:c3369.


Bonis PAL, Lamont JT. Approach to the adult with chronic diarrhea in resource-rich settings. UpToDate Inc. Waltham, MA. Last reviewed February 2017.

Peppercorn MA, Kane SV. Clinical manifestations, diagnosis and prognosis of Crohn disease in adults. UpToDate Inc. Waltham, MA. Last reviewed February 2017.

Pharmacogenomic and Metabolic Assessment of Thiopurine Therapy


Evans WE, Hon YY, Bomgaars L, et al. Preponderance of thiopurine S-methyltransferase


BBBBBBB. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance


Reinshagen M, Schütz E, Armstrong VW, et al. 6-thioguanine nucleotide-adapted azathioprine therapy does not lead to higher remission rates than standard therapy in chronic active Crohn disease: Results from a randomized, controlled, open trial. Clin Chem. 2007;53(7):1306-1314.


**NOD2/CARD15 Genotyping**


Stein JM, Lammert F, Zimmer V, et al. Clinical periodontal and microbiologic

AETNA BETTER HEALTH® OF PENNSYLVANIA

Amendment to
Aetna Clinical Policy Bulletin Number: 0249 –
Inflammatory Bowel Disease: Serologic Markers and
Pharmacogenomic and Metabolic Assessment of
Thiopurine Therapy

There are no amendments for Medicaid.