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## Coverage Policy

Testing for serological markers for the diagnosis or management of inflammatory bowel disease is considered experimental, investigational or unproven. Tests/test panels include, but are not limited to the following:

- anti-neutrophilic cytoplasmic antibody (ANCA), perinuclear anti-neutrophilic cytoplasmic antibody (pANCA)
- anti-saccharomyces cerevisiae antibody (ASCA)
- anti-inner membrane porin C (anti-OmpC) antibody
- anti-CBir1 flagellin (anti-CBir1) antibody
- anti-I2
- antilibranaribioside carbohydrate IgG (ALCA)
- antichitobioside carbohydrate IgA (ACCA)
- anti-synthetic mannose antibodies (AΣMA or AMCA).
- Pseudomonas-associated sequence I-2 (Anti-I2)
- Prometheus® IBD sgi Diagnostic®
- Prometheus® Crohn’s Prognostic
Testing for the measurement of antibodies to infliximab, adalimumab, or vedolizumab performed individually or as part of a test panel (e.g., Prometheus® Anser™-IFX, -ADA, -VDZ; LabCorp ECLIA) is considered experimental, investigational or unproven.

Overview

This Coverage Policy addresses serological testing for the diagnosis and management of inflammatory bowel disease (IBD).

General Background

Diagnosis of IBD and Prediction of Disease-Related Complications

Perinuclear anti-neutrophilic cytoplasmic antibody (pANCA) and anti-saccharomyces cerevisiae antibody (ASCA) are serological markers that have been proposed as tools to assist in diagnosing inflammatory bowel disease, differentiating ulcerative colitis (UC) from Crohn’s disease (CD) in patients with indeterminate colitis, and determining therapy and monitoring response to treatment. Anti-neutrophilic cytoplasmic antibody (ANCA) has been used in the diagnosis and classification of various vasculitis-associated and autoimmune disorders, and has been associated with renal manifestations of small vessel vasculitis with rapidly progressing glomerulonephritis. pANCA is an antibody directed against the cytoplasmic components of neutrophils with a perinuclear staining pattern. Serum pANCA has been reported to be present in 20–85% of patients with ulcerative colitis, and in 2–28% of patients with Crohn’s disease. Elevated levels of serum pANCA in ulcerative colitis patients are believed to be caused by pANCA production in the colonic mucosa (Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 2016; Iskandar, 2012).

Anti-saccharomyces cerevisiae antibody (ASCA) is an antibody that reacts to a component of yeast commonly found in food. ASCA has been detected in the serum of a majority of Crohn’s disease patients, but fewer ulcerative colitis patients. The origin of ASCA is not clear, nor is it known why this antibody occurs in only a subset of patients with Crohn’s disease. ASCA has been detected in approximately 39–76% of Crohn’s disease patients, and up to 15% in ulcerative colitis patients (Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 2016; Iskandar, 2012).

Several additional antibodies have been described as serological markers for IBD, including anti-outer membrane porin C (anti-OmpC) and anti-CBir1 flagellin (anti-CBir1). These antibodies are directed against luminal bacterial components seen in IBD. Anti-OmpC, directed against the outer membrane porin C of Escherichia coli, is reportedly seen more often in patients with a mixed family history of Crohn’s disease (CD) and ulcerative colitis (UC) as opposed to those with a family history of only UC. The antigens CBir1, A4-Fla2, and Fla-X are flagellin subunit proteins linked to Clostridium cluster XIVa. Anti-CBir1 is an antibody to flagellin from Clostridium species and is reported to be found in approximately 6% of UC patients and 50% of patients with CD, and may be associated with more complicated disease. Pseudomonas-associated sequence I-2 (Anti-I2 is a bacterial DNA fragment, and has been identified in lamina propria mononuclear cells of active CD patients. Anticarbohydrate antibodies have also been used in inflammatory bowel disease management, including antilaminaribioside carbohydrate IgG (ALCA), antichitobioside carbohydrate IgA (ACCA), and anti-synthetic mannoside antibodies (AΣMA or AMCA). ALCA, ACCA, and AMCA are similar to ASCA in that they are antibodies to sugars on the surface of microorganisms. ALCA and ACCA are reported to be associated with CD, and are found in 17–28% of CD patients. AΣMA is an antibody against synthetic oligomannose epitopes, and is found to be positive in 24% of patients with CD who were negative for ASCA, and had a lower sensitivity but higher specificity compared to ASCA (Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 2016; Iskandar, 2012; Bossuyt, 2006).

Combined serological testing has been proposed as a screening method for patients who present with signs and symptoms of inflammatory bowel disease, and as a method to differentiate CD from UC. The Prometheus® IBD Serology 7 was commercially available through Prometheus (San Diego, CA) as a diagnostic panel consisting of ASCA IgA, ASCA IgG, anti-CBir1, ANCA, anti-OmpC, pANCA, and DNAse-sensitive pANCA. The updated test panel, Prometheus® IBD sgi Diagnostic, combines serologic, genetic and inflammation markers in a proprietary Smart Diagnostic Algorithm, and is intended to assist in differentiating IBD vs. non-IBD and CD vs. UC in one
comprehensive test (Prometheus website). The clinical utility of this testing has not been established. Patients with negative results would still need to undergo the standard diagnostic testing for inflammatory bowel disease. Patients with a positive result would still need to undergo additional testing to distinguish Crohn’s disease from ulcerative colitis and to determine the extent of disease.

Combined serological testing has also been proposed as a method of determining the risk for disease-related complications in patients with CD. Prometheus Crohn’s Prognostic, combines proprietary serogenetic markers and serologic markers, including Anti-I2 and many of the assays included in the Prometheus® IBD sgi Diagnostic panel. The test employs a logistic regression model to provide probabilities for developing disease complications in patients diagnosed with Crohn’s disease.

There is insufficient evidence in the published medical literature to determine the role of serological testing, (whether performed as individual assays or in test panels) in the diagnosis and management of inflammatory bowel disease. There is insufficient evidence to demonstrate that the use of these tests results in improved health outcomes.

**Literature Review: Diagnosis of IBD and Prediction of Disease-Related Complications**

A prospective study (n=169 patients/523 samples) by Hamilton et al. (2017) evaluated the role of serological antibodies in predicting recurrence after Crohn's disease resection. Subjects were prospectively tested for serologic antibody presence (e.g., pANCA, ASCA, IgA/IgG, anti-OmpC, anti-CBir1, anti-A4-Fla2, anti-Fla-X) and titer perioperatively, and at six, 12 and 18 months postoperatively. Colonoscopy was performed at 18 months postoperatively. Quartile sum score (range 6-24), logistic regression analysis, and correlation with phenotype, smoking status, and endoscopic outcome were assessed. Patients with ≥ 2 previous resections were found to be more likely to be anti-OmpC positive (p=0.001). Recurrence at 18 months was associated with anti-Fla-X positivity at baseline (p=0.033) and 12 months (p=0.04). Patients who were positive (n=28) for all four 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 those who were negative (n=32) for all four antibodies (p=0.034). The baseline quartile sum score for all six antimicrobial antibodies was higher in patients with severe recurrence at 18 months, adjusted for clinical risk factors (p=0.039). It was concluded that pre-operative serologic screening may help to identify patients at increased risk for Crohn's disease recurrence.

A Hayes Medical Technology Directory report evaluated the evidence (22 studies/9130 patients) on serological assays for the diagnosis and treatment of IBD/CD. The review included systematic reviews (n=4) and primarily case-controlled studies. Of these studies, 19 evaluated the accuracy of serological assays for diagnosis of CD in various patient populations and three evaluated the accuracy of serological assays for predicting treatment response in patients with CD. The outcomes included measures of diagnostic performance (i.e., sensitivity, specificity, PPV, and NPV) for two individual serological markers, ASCA and anti-glycan-associated saccharomyces cerevisiae antibodies (gASCA). It also included a combination of ASCA or gASCA in combination with other antibodies. According to the report, the studies provided insufficient evidence to establish definitive patient selection criteria. Based on the body of low quality evidence, it was concluded that serological assays, particularly ASCA/gASCA and pANCA, have a specificity of generally ≥ 85% for diagnosis of CD, suggesting that a positive finding from such an assay may be useful for confirming this diagnosis. The sensitivity of assays with these serological antibodies was found to be too low (i.e., ≤ 65%) to be effective for identifying CD, indicating that the test is likely not useful for screening. The addition of other serological antibodies improved specificity to approximately 90%, but it was not clear which antibodies were responsible for the increased specificity and what constituted the most favorable combination of antibodies (Hayes, 2013; reviewed 2017).

Kaul et al. (2012) performed a systematic review (n=14 studies) and meta-analysis (n=9/14 studies) of the evidence evaluating the diagnostic ability of the anti-glycan antibodies (ASCA/gASCA, AMCA, ALCA, ACCA, Anti-L, Anti-C) to differentiate IBD from non-IBD and CD from UC, as well as their association with disease complications and/or need for surgery in IBD. Studies were primarily retrospective and were included if they compared the performance of at least two of the six anti-glycan antibody markers in at least one of the following outcomes: differentiating IBD from non-IBD; CD from UC; IBD-related complication; or need for IBD-related surgery. The mean age of the IBD patients ranged from 29 to 47 years, with mean duration of disease ranging from five to 12 years. For individual antibodies, ASCA was reported to have the highest diagnostic performance in differentiating conditions:
IBD versus healthy: Diagnostic odds ratio (DOR), 21.1; 95% CI, 1.8-247.3; sensitivity 44.0%; specificity 96.4%
CD versus UC: DOR, 10.2; 95% CI, 7.7-13.7; sensitivity 56.6%; specificity 88.1%
CD versus other gastrointestinal disorders: DOR, 10.3; 95% CI, 5.0-21.0; sensitivity 52.8%; specificity 90.0%
CD versus healthy: DOR, 2.7; 95% CI, 0.3-21.6; sensitivity 53.0%; specificity 70.4%

ASCA had the highest sensitivity compared to the other anti-glycan markers for diagnosis of both CD (52.8-56.6% versus 15.0-27.8%) and CD related surgery (60.2% versus 43.9-47.3%) or complications (70.8% versus 42.3-54.5%). For specificity all individual markers performed similarly (88-95%). The authors noted that although individual studies suggested that the combination of at least two markers had a better diagnostic value, this meta-analysis indicated that the combination of markers performs only slightly better than any individual marker. Limitations of this review include the retrospective design of studies included and the lack of data demonstrating improved clinical outcomes. Although results indicated that the measurement of serological antibodies may have some value in differentiating IBD conditions, additional well designed controlled studies are needed to demonstrate clinical utility and impact on health outcomes.

Anand et al. (2008) conducted a retrospective study to evaluate the diagnostic accuracy of pANCA and ASCA as single agents, and in combination, for the diagnosis of Crohn’s disease and ulcerative colitis, including cases of indeterminate colitis. Sera from 98 patients were evaluated, including 77 with Crohn’s disease, 16 with ulcerative colitis, and five with indeterminate colitis. Medical records were reviewed to obtain diagnosis, demographics, symptoms, and medications. The presence of ASCA and pANCA were detected using ELISA, and the results were compared with clinical data obtained from the medical records. A positive pANCA test alone provided a sensitivity of 50% and a specificity of 82% for ulcerative colitis. A positive ASCA test alone provided a sensitivity of 40% and a specificity of 100% for Crohn’s disease. A combination of pANCA-positive and ASCA-negative results showed a sensitivity of 50% for the diagnosis of ulcerative colitis, and a combination of ASCA-positive and pANCA-negative results provided a sensitivity and specificity of 32% and 100%, respectively for the diagnosis of Crohn’s disease. Eighty percent of indeterminate colitis patients showed serology results consistent with ulcerative colitis. The authors concluded that this combination of serological markers provides generally high specificity, but the low sensitivity, especially in terms of Crohn’s disease, precludes the possibility that they can replace currently available tools used for inflammatory bowel disease diagnosis and management. The authors also stated that these markers may prove beneficial in the management of indeterminate colitis.

A retrospective study by Sabery and Bass (2007) evaluated the use of serologic markers as a screening tool compared with elevated erythrocyte sedimentation rate and anemia in patients referred to a gastroenterology clinic for suspected inflammatory bowel disease. Patients were divided into four categories: ulcerative colitis, Crohn’s disease, indeterminate colitis, and noninflammatory bowel disease. Patients were categorized based on clinical evaluation by board-certified pediatric gastroenterologists. A total of 227 patients had inflammatory bowel disease serology (IBD First Step and Confirmatory System, Prometheus Laboratories) performed between September 2002 and September 2004. A total of 40 children (19%) were found to have inflammatory bowel disease. Overall, serological testing for inflammatory bowel disease had 60% sensitivity and 92% specificity. A positive laboratory test for anemia or an elevated erythrocyte sedimentation rate had 83% sensitivity, and a combination of anemia and elevated erythrocyte sedimentation rate had 96% specificity. The positive predictive value of serological testing was 60% compared to 79% in patients with anemia and elevated erythrocyte sedimentation rate. The positive predictive value of serological testing in the subgroup of patients without rectal bleeding (n=139) was only 35% compared to 60% using routine tests. Nearly a third of positive serologic tests were in patients with no demonstrable inflammatory bowel disease. The authors concluded that the measurement of the combination of elevated erythrocyte sedimentation rate and hemoglobin has a higher positive predictive value and is more sensitive and more specific than commercial serologic testing.

Dubinsky et al. (2006) conducted a prospective case series to examine the association of immune responses to microbial antigens with disease behavior and to determine the influence of immune reactivity on disease progression in pediatric CD patients. Serological testing for expression of ASCA, anti-outer membrane protein C (anti-OmpC), anti-12, and anti-CBir1 flagellin was performed in a blinded fashion by ELISA. Associations between immune responses and clinical phenotypes were evaluated. A total of 58 patients developed internal
penetrating and/or stricturing (IP/S) disease after a median follow-up of 18 months. Anti-OmpC (p<0.0006) and anti-12 (p<0.003) were associated with IP/S disease. The frequency of IP/S disease increased with increasing numbers of immune responses (p trend=0.002). The chance of developing IP/S disease was highest in patients who were positive for all four immune responses. The presence and/or magnitude of ASCA and CBir1 did not significantly influence disease behavior, however. The authors concluded that immune responses to an increasing number of microbial antigens are associated with complicating IP/S disease in pediatric CD patients, and serum immune responses predict a more rapid progression from uncomplicated to complicated disease. The authors stated that further studies in large independent cohorts will be important to validate the clinical applicability of these findings.

Reese at al. (2006) conducted a meta-analysis to assess the diagnostic precision of ASCA and pANCA in inflammatory bowel disease. Sensitivity, specificity and likelihood ratios (LR) were calculated for different test combinations for Crohn’s disease, ulcerative colitis and for inflammatory bowel disease compared with controls. A total of 66 studies/4019 patients were included. The ASCA+ with pANCA– test offered the best sensitivity for Crohn’s disease (54.6%) with 92.8% specificity and an area under the ROC (receiver operating characteristic) curve, area under the receiver operating characteristic curve (AUC) of 0.85 (LR + = 6.5; LR – = 0.5). Sensitivity and specificity of pANCA + tests for UC were 55.3% and 88.5%, respectively (AUC of 0.82; LR + = 4.5, LR – = 0.5). Sensitivity and specificity were improved to 70.3% and 93.4%, respectively, in a pediatric subgroup when combined with an ASCA test. The authors concluded that ASCA and pANCA testing are specific but not sensitive for CD and UC. The authors stated ASCA and pANCA testing may be useful for differentiating UC from CD in the pediatric population, but this needs to be the subject of further research.

A prospective multicenter study conducted by Joosens et al. (2002) evaluated the value of ASCA and pANCA to increase diagnostic accuracy in categorizing indeterminate colitis. A total of 97 patients with indeterminate colitis from three centers were analyzed for pANCA and ASCA and followed up prospectively. A definitive diagnosis was reached using conventional techniques for 31 of 97 patients. The authors reported that a positive ASCA and negative pANCA predicted Crohn’s disease in 80% of patients with indeterminate colitis, and a negative ASCA and positive pANCA predicted ulcerative colitis in 63.3% of patients with indeterminate colitis. A total of 48.5% of patients did not show antibodies against ASCA or pANCA, and most remained diagnosed with indeterminate colitis. Because only 31 patients had a confirmed diagnosis and only 21 of these patients were included in an evaluation of specificity and sensitivity, it is difficult to draw conclusions regarding the accuracy of serological testing in this study.

Dubinsky (2001) conducted a prospective study of pediatric patients to determine if accuracy of diagnosing IBD vs. functional childhood disorders was improved by the use of modified assays for pANCA and ASCA, with enzyme-linked immunosorbent assay test (ELISA) cut-off values maximized to increase sensitivity. ASCA, ANCA and pANCA profiles were obtained from 128 children undergoing diagnostic evaluation for IBD. Investigators were blinded to clinical diagnoses. Sensitivity of the modified assays for diagnosing IBD was 81% compared to 69% for the traditional tests, but specificity in terms of diagnosing IBD was lower, at 72% vs. 95%. The authors concluded that the incorporation of sequential noninvasive testing into a diagnostic strategy may avoid unnecessary and costly evaluations and facilitate clinical decision-making when the diagnosis of IBD in children is uncertain. The study was limited by small numbers in each group and a lack of distinction between UC and CD.

Measurement of Serum Levels and Antibodies to Infliximab (ATI) and Adalimumab
Biologic therapies for IBD include tumor necrosis factor (TNF) antagonist therapy (e.g., infliximab, adalimumab), and anti-integrin antibodies (e.g., vedolizumab, natalizumab) (Farrell and Peppercorn, 2018). TNF antagonists or blockers bind to the TNF-alpha, and block its interaction with the cell surface TNF receptors. TNF is a naturally occurring cytokine that is involved in normal inflammatory and immune responses. Infliximab is an intravenously administered chimeric (i.e., combination of non-human and human genetic material) monoclonal antibody to tumor necrosis factor-alpha, and may be used in selected patients for the treatment of moderate to severe ulcerative colitis (UC) or Crohn’s disease (CD). Some patients do not respond to initial therapy, and a percentage of patients who do respond to initial therapy become unresponsive over time. It has been suggested that this loss of response may be due to the production of antibodies to infliximab. Infusion reactions to infliximab may also occur, and are typically associated with antibodies to infliximab, also referred to as HACA (human antichimeric antibodies).
Antibodies to infliximab (ATI) are less likely to occur in patients treated with glucocorticoids or immune modulators. Delayed hypersensitivity reactions although unusual, may occur two to twelve days after an infusion, and high ATI appear after such reactions, but are not necessarily found before reinfusion. Long delays between infusions are considered to be a significant risk factor for delayed hypersensitivity. Delayed hypersensitivity is less common when a standard induction regime is used and an immune modulator is administered concurrently. Options for treatment of diminished response therefore include decreasing the interval between doses or increasing the dose, and if necessary, changing to a different anti-TNF agent.

Adalimumab is a fully human monoclonal antibody, administered subcutaneously, and may be used in the treatment of moderate to severe CD. Antibodies to the drug may also occur with adalimumab; with formation of antihuman antibodies (HAHAs). There is no consensus on the clinical significance of the presence of antidrug antibodies, but with episodic therapy there is an association between lower infliximab serum levels when ATI formation is highest, and a decreased response rate to adalimumab in patients with HAHAs (Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease, 2016).

Vedolizumab (VDZ) is a humanized anti-alpha-4-beta-7 integrin monoclonal antibody used in patients with active moderate to severe Crohn’s disease or ulcerative colitis. VDZ is administered intravenously and specifically targets the \( \alpha_4 \beta_7 \) integrin that is selectively expressed on gut-homing T lymphocytes. The drug is used in patients with IBD who have had an inadequate response with, lost response to, or were intolerant to inhibitors of tumor necrosis factor-alpha (TNF-alpha) blocker or immunomodulator; or had an inadequate response with, were intolerant to, or demonstrated dependence on corticosteroids (Farrell and Peppercorn, 2018).

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; however, laboratories offering such tests as a clinical service must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA) and must be licensed by CLIA for high-complexity testing. Laboratory methods used to evaluate serum concentrations of anti-drug antibodies include enzyme-linked immunosorbent assay (ELISA), non-radiolabeled homogeneous mobility shift assay (HMSA), and radioimmunoassay (RIA). ELISA is simple to perform but is associated with false-positive results due to binding to other antigens and is limited in that the assay cannot measure ATI in the presence infliximab. RIA is more sensitive and specific than ELISA but is also more complex. HMSAs can detect most antibodies and are considered an improvement over other testing methods because it is able to measure serum antibodies with infliximab present. None of these assays correlate seamlessly with one another (Hayes, 2015; reviewed 2017).

Prometheus® Laboratories offers non-radiolabeled, fluid phase HMSA tests for identifying serum antibodies. Prometheus® Anser IFX is a quantitative infliximab monitoring assay designed to measure infliximab (IFX) and antibodies to infliximab (ATI) levels. The test is intended to provide clarity on factors contributing to a patient's loss of response and to guide treatment decisions. A similar test, Prometheus® Anser ADA, measures serum adalimumab (ADA) and antibodies to adalimumab (AMA) levels. The Prometheus® Anser VDZ measures serum concentration of vedolizumab (VDZ) and antibodies to vedolizumab. Laboratory Corporation of America (LabCorp) (Burlington, NC) has developed an in-house electrochemiluminescence immunoassay (ECLIA) test for monitoring serum anti-Infliximab antibody levels. The ECLIA test has a lower limit of detection than ELISA and can also measure antibodies in the presence of serum IFX. However, no clinical validation data are available with this assay, and so its current use in clinical practice is uncertain (Vaughn, Sandborn, and Cheifetz, 2015).

There is insufficient evidence in the published medical literature to determine the role of measurement of antibodies to infliximab, adalimumab or vedolizumab, whether performed separately or combined with testing of blood levels, in the management of inflammatory bowel disease. There is insufficient evidence to demonstrate that the use of these tests results in improved health outcomes compared to usual clinical management.

**Literature Review: Measurement of Serum Levels and Antidrug Antibodies (e.g., Infliximab)**

Freeman et al. (2017) conducted a systematic review and meta-analysis on the accuracy of antitumor necrosis factor (anti-TNF) and antibodies to anti-TNF to predict loss of response or lack of regaining response in patients with anti-TNF managed Crohn’s disease (n=31). The included studies consisted of patients with Crohn’s disease treated with infliximab or adalimumab. Studies with mixed Crohn’s and ulcerative colitis populations were included if the proportion of Crohn’s patients was at least 70%. Studies reporting clinical status (i.e., response or
lack of response) as an outcome were eligible for inclusion. Studies were heterogeneous with respect to the type of test used, criteria for establishing response/lack of response, population examined and results. Meta-analytic summary point estimated for sensitivity and specificity were 65.7% and 80.6% for infliximab trough levels and 56% and 79% for antibodies to infliximab, respectively. Pooled positive and negative predictive values ranged between 70% and 80% implying that between 20% and 30% of both positive and negative test results may have been incorrect in predicting loss of response. Author-noted limitations were insufficient data for subgroup analyses and many of the studies had a high risk of bias. The review concluded that these tests have modest predictive accuracy for clinical status. Additional studies are required before the clinical utility of the tests can be reliably evaluated.

A Hayes published a Search and Summary on Anser ADA (Prometheus Laboratories Inc.) for monitoring adalimumab treatment of inflammatory bowel disease. The review included six abstracts (a prospective comparative study, a validation study, a technical review and prospective uncontrolled studies). Hayes concluded there is insufficient published evidence to assess the safety and/or impact on health outcomes or patient management for the use of Anser ADA for monitoring adalimumab treatment in patients with IBD (Hayes, 2017).

A Hayes Technology Brief evaluated the evidence (n=13 studies) on the utility of antidrug antibodies for monitoring patients treated receiving infliximab for IBD. The report included RCTs (n=2), prospective and retrospective cohort studies (n=9), and retrospective cross-sectional studies (n=2). Most of the studies were found to be of poor quality. Sample sizes in studies ranged from 69-573 patients with follow-up periods of 12 weeks to 48 months. Outcomes included concentrations, titers, or presence of ATI or infliximab at trough using ELISA, RIA, or HMSA and ATI-free survival. It was determined that the overall low to very-low-quality body of evidence was insufficient to support a conclusion as to whether or not assessment of ATI is warranted to guide infliximab treatment of patients with IBD. Although some evidence was from RCTs, only a single poor-quality RCT was specifically designed to determine whether knowledge of ATI is helpful in guiding patient management. Most of the evidence reviewed originated from observational studies. Many of these studies did not assess patients using objective criteria such as clinical activity index scores or endoscopic evaluation. According to the Hayes Technology Brief, the evidence is equivocal on whether ATI are associated with clinical outcomes in patients with IBD who are treated with infliximab (Hayes 2015; reviewed 2017).

Moore et al. (2016) performed a systematic review and meta-analysis of the evidence (n=22 studies/3483 patients) evaluating the association between serum levels of infliximab at various thresholds and clinical outcomes in IBD. Controlled trials and observational studies were included that reported outcomes [clinical, mucosal, CRP, colectomy] in patients who were treated with IFX for UC or CD, and grouped these outcomes according to mean/median IFX levels, or according to a cut-off threshold level of IFX. Studies that only measured TNF-binding capacity, not serum drug levels or did not report clinical outcomes of IFX therapy or serum IFX levels were excluded. The primary outcome measure was clinical remission defined as absence of clinical symptoms in patients who had responded to IFX. Secondary outcomes were relative risk of remission, endoscopic remission, or colectomy, according to a threshold serum IFX level. Mean levels of serum CRP above and below a specified level of serum IFX were also compared. Meta-analysis of five studies demonstrated a significant difference in mean serum IFX levels between remission and non-remission patients (p<0.001). Comparisons were made from pooled remission rates (n=7 studies) between patients with an IFX level < 2 μg/ml, and those with a level > 2 μg/ml. Analysis of remission rates from raw data in these studies showed that patients with an IFX level greater than 2 μg/ml were more likely to be in remission than those with an IFX level < 2 μg/ml (p<0.001). Patients with an IFX level > 2 μg/ml were also more likely to achieve endoscopic remission (p=0.004) than patients with levels < 2 μg/ml. The authors noted that cumulatively these data may imply that patients with low trough IFX levels experience worse outcomes than patients with higher levels, and thus interventions to identify and address this are warranted. However a considerable overlap in the range of drug levels in ‘remitters/relapsers’ was observed, suggesting that serum IFX levels alone do not explain clinical status in most patients. Acknowledged limitations include the heterogeneity of patient populations, assays, and outcome measures and the retrospective, uncontrolled design of studies. Further prospective studies analyzing the clinical effectiveness of adjustments of IFX dosing to trough levels are needed to support the use of routine evaluations of serum IFX levels in practice.
A systematic review and meta-analysis was conducted by O'Meara et al. (2014) to provide a pooled estimate of the risk of infusion reactions according to patients' ATI status and to analyze the relationship of immunomodulators (e.g., methotrexate) to this risk. Eight studies (1351 patients) met the inclusion criteria; seven of the eight studies had a high risk of bias in at least one quality domain. The cumulative data indicated that in patients with ATI compared to those without ATI, there was a higher risk ratio (RR) of any acute infusion reaction (RR 2.4; 95% CI 1.5-3.8, p<0.001) and severe infusion reactions (RR 5.8, 95% CI 1.7-19), p=0.004. The authors noted that there was statistical heterogeneity among the studies that implies that the summary RR should be interpreted with caution. Patients who were prescribed immunomodulators during maintenance therapy had a reduction in the risk of ATI development (RR 0.6, 95% CI 0.4-0.9, p=0.02) and infusion reactions (RR 0.6, 95% CI 0.4-0.8, p<0.001).

Nanda et al. (2013) conducted a meta-analysis of studies that reported clinical outcomes and infliximab levels according to the antibodies to infliximab (ATI) status in patients treated for ulcerative colitis (UC) or Crohn's disease (CD) (13 studies, 1378 patients). Included studies consisted of controlled trials, observational studies, and cohort studies. The pooled risk ratio of loss of clinical response to infliximab in patients with IBD who had ATI was 3.2 (95% confidence interval [CI]: 2.0-4.9, p<0.0001) when compared to patients without ATI. This effect estimate was primarily based on CD patients (n=494). In patients with UC (n=86) with ATI, there was a non-significant risk ratio of loss of response of 2.2 (95% CI: 0.5-9.0, p=0.3). The authors noted limitations of the analysis, including heterogeneity among the studies in methods of ATI detection and clinical outcomes reported, a high risk of bias in at least one quality domain in each study, and the fact that a funnel plot suggested publication bias.

Paul et al. (2013) conducted a prospective case series to evaluate the relationship between infliximab (IFX) trough levels and antibodies to infliximab (ATI) and mucosal healing in 52 patients with IBD (34 with CD and 18 with UC). According to the authors, accumulating evidence indicates that mucosal healing may change the natural course of the disease by decreasing the need for surgery and reducing hospitalization. Consecutive patients receiving IFX (5mg/kg) treatment who were developing secondary failure to IFX were included. IFX trough levels, antibodies to IFX concentrations, C-reactive protein levels, and fecal calprotectin were measured prior to IFX optimization and at week eight. On the day of the first IFX optimization, a proctosigmoidoscopy was performed and was repeated at week eight in patients with UC. After IFX dose intensification, half of the CD and UC patients achieved mucosal healing. Increase in IFX trough levels (called “delta IFX” in micrograms per milliliter) was associated with mucosal healing in both groups (p=0.001). A delta IFX >0.5 ug/ml was associated with mucosal healing (sensitivity 0.88; specificity 0.77; p=0.0001, area under the receiver operating characteristic curve, 0.89). The only factor associated with mucosal healing after IFX optimization was a delta IFX >0.5 ug/ml (likelihood ratio=2.02, 95% confidence interval, 1.01-4.08, p=0.48) the authors stated that because of small sample size, these results need to be confirmed in studies including a higher number of patients.

Lee et al. (2012) conducted a meta-analysis to determine the prevalence of ATI in patients receiving infliximab, the effect of immunosuppressants on the prevalence of ATI, the effect of ATI on the prevalence of infusion reactions and the effect of ATI on the rates of remission (18 studies/3326 patients). The prevalence of ATI was 45.8% when episodic infusion of infliximab was given and 12.4% when maintenance infliximab was given. Infusion reaction rates were significantly higher in patients with ATI (relative risk: 2.07; 95% CI, 1.61-2.67). Immunosuppressants resulted in a 50% reduction in the risk of developing ATI (p<0.00001). The presence or absence of ATI did not affect the rates of clinical remission. The authors stated that further analysis is required to determine whether loss of response is dependent on the titer of ATI.

Afif et al. (2010) conducted a retrospective review to evaluate the clinical utility of measuring HACA and infliximab concentrations in patients with IBD (n=155). Medical records were reviewed for patients for whom HACA and infliximab concentrations had been measured, to determine whether the result affected clinical management. Indications for testing included loss of response to infliximab (49%), partial response after initiation of infliximab (22%) and possible autoimmune/delayed hypersensitivity reaction (10%). Antibodies to infliximab (ATI) were identified in 35 patients (23%), and therapeutic drug concentrations in 51 patients (33%). In HACA-positive patients, change to another anti-TNF agent resulted in a complete or partial response in 92% of patients, and dose escalation resulted in a response rate of 17%. The authors concluded that measurement of HACA and infliximab concentration impacts management and is clinically useful, and that a prospective randomized trial should be conducted to confirm these findings.
Cassinotti and Travis (2009) conducted a systematic review to evaluate the incidence of ATI in CD and their impact on the efficacy and safety of infliximab. The authors stated that the observation that Infliximab use is associated over time with loss of response and infusion reactions has led to the presumption that this is due to immunogenicity, and that ATI are the principal cause. The authors stated that the mechanisms for ATI development are poorly understood, and the incidence depends on multiple patient-specific and treatment-related analytical and clinical factors. The review demonstrated that the presence of ATI is weakly and variably associated with clinical response or infusion reactions, but not with reactions relevant to clinical decision making. The authors stated that enormous variation in the methods of reporting ATI and immunogenicity of infliximab make almost any interpretation possible from different studies, but few have clinical relevance. The authors concluded that there is no clear evidence that ATI have an impact on efficacy or safety, nor is there a need to measure them in clinical practice.

Professional Societies/Organizations

**American College of Gastroenterology (ACG):** The ACG clinical guideline on the management of Crohn’s disease in adults stated that the routine use of serologic markers of IBD to diagnose Crohn’s disease is not indicated. Anti-glycan antibodies are more prevalent in Crohn’s disease, however they have a low sensitivity which makes their use in diagnosis less helpful (Lichtenstein et al., 2018).

The ACG clinical guideline on ulcerative colitis in adults, updated in 2010, stated that pANCA have been identified in 60–70% of UC patients but are also found in up to 40% of patients with CD. These pANCA-positive CD patients typically have a clinical phenotype resembling left-sided UC patients, so ANCA detection alone is of little value in distinguishing between UC and CD. The low sensitivity of pANCA for the diagnosis of UC prevents it from serving as a useful diagnostic tool. These assays may be useful, however, in the occasional patient in whom no other clinical or pathologic features allow a differential diagnosis between UC and Crohn’s colitis (Kornbluth et al., 2010).

**North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the Crohn’s and Colitis Foundation of America (CCFA):** The NASPGHAN and CCFA jointly developed a consensus conference report on differentiating UC from CD in children and young adults (Bousvaros, et al., 2007). The report stated that the value of serology in a patient with IC remains a topic of study, and further research should examine, among other areas, the role of surrogate laboratory markers (genetics, serology, microbiology) in distinguishing these entities. A proposed algorithm to assist clinicians in differentiating UC from CD does not include serological testing.

Recommendations for testing for the measurement of antibodies to infliximab or adalimumab are not included in any of the above guidelines.

**American Gastroenterological Association (AGA):** The AGA Institute guideline on therapeutic drug monitoring in inflammatory bowel disease suggested that reactive therapeutic drug monitoring can be used to guide treatment changes in adults with active IBD being treated with anti-TNF agents. This is a conditional recommendation based on very low quality of evidence with very little confidence in the effect estimate. The AGA did not include a recommendation for the use of routine proactive therapeutic drug monitoring (Feuerstein et al., 2017).

**Use Outside the U.S.**
A 2016 National Institute for Health and Clinical Excellence (NICE) guidance evaluated the efficacy of ELISA kits for the therapeutic monitoring of TNF-alpha inhibitors in Crohn’s disease. The assessment included patients who lost response to initial treatment as well as those who maintained treatment response, as this subset of patients may continue to receive the same of TNF-alpha inhibitor dosage when decreased dosing might be equally effective. Patients who did not respond to treatment during the induction phase were not considered in the analysis. It was concluded that although the testing shows promise, there is insufficient evidence to recommend routine adoption (NICE, 2016).

The third European evidence-based consensus on the diagnosis and management of ulcerative colitis, part 1, definitions and diagnosis includes the following statement:
• The routine clinical use of genetic or serological molecular markers is not recommended for the classification of ulcerative colitis

The authors noted that the most studied serological markers associated with UC, include the pANCA and ASCA. Positive pANCA serology is found in up to 65% of patients with UC and in less than 10% of patients with CD. Due to the sensitivity of these markers, their routine use is not justified (Magro et al., 2017).

The third European evidence-based consensus on the diagnosis and management of Crohn’s disease, stated that genetic or serological testing is currently not recommended for routine diagnosis of CD. In a discussion of initial laboratory investigations, the authors state that currently available serologic testing may be used as an adjunct to diagnosis, but the accuracy of the available tests (ASCA, ANCA) is such that they are unlikely to be useful in routine diagnosis, and are ineffective at differentiating colonic Crohn’s disease from ulcerative colitis. Other serologic markers, including anti-OmpC and CBir1 have not yet been shown to help in differentiating CD from UC. The authors also note that despite advances in Crohn’s disease genetics, there are currently no genetic tests which are recommended routinely for diagnoses. In regards to the loss of response to an anti-TNF agent, it was recommended to use dose optimization. If dose optimization is not successful, switching to a different anti-TNF was recommended. Where available, measurement of anti-TNF trough levels and anti-drug antibodies could be used to guide therapy (Gamollón et al., 2017).

**Coding/Billing Information**

**Note:** 1) This list of codes may not be all-inclusive.
   2) Deleted codes and codes which are not effective at the time the service is rendered may not be eligible for reimbursement.

Considered Experimental/Investigational/Unproven when used to report testing for serological markers for the diagnosis or management of inflammatory bowel disease:

<table>
<thead>
<tr>
<th>CPT® Codes</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>81401</td>
<td>Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat) NOD2 (nucleotide-binding oligomerization domain containing 2) (eg, Crohn’s disease, Blau syndrome), common variants (eg, SNP 8, SNP 12, SNP 13)</td>
</tr>
<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
</tr>
<tr>
<td>82397</td>
<td>Chemiluminescent assay</td>
</tr>
<tr>
<td>83516</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; qualitative or semiquantitative, multiple step method</td>
</tr>
<tr>
<td>83520</td>
<td>Immunoassay for analyte other than infectious agent antibody or infectious agent antigen; quantitative, not otherwise specified</td>
</tr>
<tr>
<td>84999</td>
<td>Unlisted chemistry procedure</td>
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<tr>
<td>86021</td>
<td>Antibody identification; leukocyte antibodies</td>
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<tr>
<td>86140</td>
<td>C-reactive protein;</td>
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<tr>
<td>86255</td>
<td>Fluorescent noninfectious agent antibody; screen, each antibody</td>
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<tr>
<td>86256</td>
<td>Fluorescent noninfectious agent antibody; titer, each antibody</td>
</tr>
<tr>
<td>86671</td>
<td>Antibody; fungus, not elsewhere specified</td>
</tr>
<tr>
<td>88346</td>
<td>Immunofluorescence, per specimen; initial single antibody stain procedure</td>
</tr>
<tr>
<td>88350</td>
<td>Immunofluorescence, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)</td>
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</tbody>
</table>

Considered Experimental/Investigational/Unproven when used to report testing for the measurement of antibodies to infliximab, adalimumab, or vedolizumab individually or as part of a test panel:
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<tr>
<th>CPT® Codes</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>84999</td>
<td>Unlisted chemistry procedure</td>
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</table>


References


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