Inflammatory Bowel Disease

Laboratory Support for Diagnosis and Management

Table of Contents

CLINICAL BACKGROUND

Inflammatory bowel disease (IBD) refers to a group of conditions, including ulcerative colitis (UC) and Crohn disease (CD), that involve chronic relapsing and remitting inflammation of the gastrointestinal (GI) tract. IBD affects about 0.5% of people in North America, with equal prevalence of UC and CD.[1] Although UC and CD share common symptoms (**Table 1**), they are distinct diseases and differ in the nature and location of their underlying inflammation. In UC, the inflammation affects the colonic mucosa, usually beginning in the rectum and extending proximally across a continuous region of the colon.[2] In CD, the inflammation is transmural and can affect any part of the GI tract, often in discontinuous segments.[3] In both diseases, the location and extent of inflammation determine the disease presentation, which can vary widely.[2,3]

Accurate diagnosis of IBD, including differentiation between UC and CD, is important for determining prognosis and selecting appropriate treatment. Diagnosis is based on a combination of patient history, physical examination, laboratory results, imaging (X-ray, computed tomography, and/or magnetic resonance), endoscopy, and histology. Once the diagnosis is established, early and effective management can stop the disease from progressing and prevent complications. IBD has no cure, but several treatments are available to induce and maintain remission.

Table 1. Common Symptoms of IBD

Symptom[2–4]	UC	CD
Abdominal pain	•	•
Diarrhea[a]	•	•
Fatigue	•	•
Fever	•	•
Incontinence	•	
Mucus discharge	•	
Nausea and vomiting		•
Urgency	•	
Weight loss	•	•

CD, Crohn disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

[a] Bloody in UC, with or without blood in CD.

Laboratory tests are an important component of IBD diagnosis and management. In diagnosis, laboratory tests are used to identify inflammation, rule out alternative conditions, and differentiate between UC and CD. In management, laboratory tests have a role in assessing disease activity and monitoring the safety and effectiveness of certain treatments.

This Clinical Focus provides an overview of laboratory tests useful in the diagnosis and management of IBD. This material is provided for educational purposes only and is not intended as medical advice. A physician's test selection and interpretation, diagnosis, and patient management decisions should be based on their education, clinical expertise, and assessment of the patient.

INDIVIDUALS SUITABLE FOR TESTING

- Individuals with symptoms consistent with IBD (**Table 1**)
- Individuals with IBD

TEST AVAILABILITY

Quest Diagnostics offers laboratory tests and panels that may be useful in the diagnosis and management of IBD (**Table 2**).

Table 2. Tests Available for Diagnosis and Management of IBD

Test code	Test name	Clinical use
Differential diagnosis		
70171	ANCA Screen with Reflex to ANCA Titer[a] Includes ANCA screen with reflex to C-ANCA, P-ANCA, and/or atypical P-ANCA titer	Differentiate UC and CD
16796	Calprotectin, Stool	Identify intestinal inflammation; differentiate IBD from IBS
6399	CBC (Includes Differential and Platelets)	Support diagnosis of IBD by identifying anemia and thrombocytosis
91664	*Clostridium difficile* Toxin/GDH With Reflex to PCR	Support diagnosis of IBD by ruling out *Clostridium difficile* infection
10231	Comprehensive Metabolic Panel Includes albumin, albumin/globulin ratio, alkaline phosphatase, ALT, AST, BUN/creatinine ratio, calcium, carbon dioxide, chloride, globulin, glucose, potassium, serum creatinine with eGFR, sodium, total bilirubin, and total protein	Support diagnosis of IBD by identifying hypoalbuminemia and electrolyte imbalances
4420	C-Reactive Protein (CRP)	Identify inflammation
16503	Inflammatory Bowel Disease Differentiation Panel[a,b] Includes ANCA screen with reflex to P-ANCA, C-ANCA, and atypical P-ANCA titers; myeloperoxidase antibody; proteinase 3 antibody; and *Saccharomyces cerevisiae* IgG and IgA antibodies	Differentiate UC and CD
10156	Lactoferrin, Qualitative, Stool	Identify intestinal inflammation; differentiate IBD from IBS
17321	Lactoferrin, Quantitative, Stool	Identify intestinal inflammation; differentiate IBD from IBS
8796	Myeloperoxidase Antibody (MPO)	Differentiate IBD from vasculitides
34151	Proteinase-3 Antibody	Differentiate IBD from vasculitides
10295	*Saccharomyces cerevisiae* Antibodies (ASCA) (IgA)	Differentiate UC and CD
17609	*Saccharomyces cerevisiae* Antibodies (ASCA) (IgA, IgG)	Differentiate UC and CD
10294	*Saccharomyces cerevisiae* Antibodies (ASCA) (IgG)	Differentiate UC and CD
809	Sed Rate by Modified Westergren	Identify inflammation
Monitoring disease		
16796	Calprotectin, Stool	Monitor intestinal inflammation
4420	C-Reactive Protein (CRP)	Monitor inflammation

(Continued)

Table 2. Tests Available for Diagnosis and Management of IBD *(Continued)*

Test code	Test name	Clinical use
10156	Lactoferrin, Qualitative, Stool	Monitor intestinal inflammation
17321	Lactoferrin, Quantitative, Stool	Monitor intestinal inflammation
Screening for treatment with biologics		
37616	Pre-biologic/biosimilar Screen Panel, HCV/HBV with Reflexes and QFT 1 Tube[a,b] Includes HBV surface antigen with reflex confirmation; HBV surface antibody immunity, quantitative; HBV core antibody, total, with reflex to IgM; HCV antibody with reflex to HCV RNA, PCR with reflex to Genotype, LiPA®; and QuantiFERON®-TB Gold Plus, 1 Tube	Detect HBV, HCV, and tuberculosis infections prior to starting biologic therapy
37620	Pre-biologic/biosimilar Screen Panel, HCV/HBV with Reflexes and QFT 4 Tubes[a,b] Includes HBV surface antigen with reflex confirmation; HBV surface antibody immunity, quantitative; HBV core antibody, total, with reflex to IgM; HCV antibody with reflex to HCV RNA, PCR with reflex to Genotype, LiPA®; and QuantiFERON®-TB Gold Plus, 4 Tubes, draw site incubated	Detect HBV, HCV, and tuberculosis infections prior to starting biologic therapy
Monitoring treatment with TNF blockers		
36294	Adalimumab Anti-Drug Antibody for IBD[c]	Determine presence of antibodies to adalimumab
36296	Adalimumab Level and Anti-Drug Antibody for IBD[a,c]	Determine adalimumab levels and presence of antibodies to adalimumab
36298	Adalimumab Level for IBD[c]	Determine adalimumab levels
36301	Infliximab Anti-Drug Antibody for IBD[c,d]	Determine presence of antibodies to infliximab and infliximab-dyyb (Inflectra®)
36311	Infliximab Level and Anti-Drug Antibody for IBD[a,c,d]	Determine infliximab and infliximab-dyyb (Inflectra®) levels and presence of antibodies to infliximab and infliximab-dyyb (Inflectra®)
36303	Infliximab Level for IBD[c,d]	Determine infliximab and infliximab-dyyb (Inflectra®) levels
Monitoring treatment with thiopurines		
91745	Thiopurine Metabolites[c]	Determine thiopurine metabolite levels
37742	Thiopurine S-Methyltransferase (*TPMT*) Genotype[c]	Identify patients at risk for thiopurine toxicity
18831	TPMT Activity[c]	Identify patients at risk for thiopurine toxicity

ALT, alanine aminotransferase; ANCA, anti-neutrophil cytoplasmic antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; AST, aspartate aminotransferase; BUN, blood urea nitrogen; C-ANCA, cytoplasmic ANCA; CBC, complete blood count; CD, Crohn disease; GDH, glutamate dehydrogenase; eGFR, estimated glomerular filtration rate; HBV, hepatitis B virus; HCV, hepatitis C virus; IBD, inflammatory bowel disease; LiPA, line probe assay; P-ANCA, perinuclear ANCA; PCR, polymerase chain reaction; TB, tuberculosis; TNF, tumor necrosis factor; TPMT, thiopurine S-methyltransferase; UC, ulcerative colitis.

[a] Reflex tests are performed at an additional charge and are associated with an additional CPT code.

[b] Panel components may be ordered separately.

[c] This test was developed and its analytical performance characteristics have been determined by Quest Diagnostics. It has not been cleared or approved by the US Food and Drug Administration. This assay has been validated pursuant to the CLIA regulations and is used for clinical purposes.

[d] Infliximab assays are validated for the infliximab biosimilar infliximab-dyyb (Inflectra®) with no analytical differences between these drugs.

TEST SELECTION AND INTERPRETATION

Diagnosis

IBD is ultimately diagnosed by endoscopy and histology, but laboratory tests help identify patients who should undergo these more invasive tests (**Figure**). Diagnosis often begins by ruling out infections and alternative conditions (eg, irritable bowel syndrome [IBS], ischemic colitis, infection, diverticulitis, and colon cancer). The initial workup may include a complete blood count (test code 6399) and comprehensive metabolic panel (test code 10231), which can reveal anemia, thrombocytosis, and hypoalbuminemia associated with IBD.[4,5] Stool tests for common pathogens, including *Clostridium difficile* (test code 91664), are recommended for patients with diarrhea to rule out infectious causes.[2,4–6]

Assessing inflammation is important to rule out noninflammatory conditions with similar symptoms (eg, IBS). Several biomarkers can be used to detect the inflammation associated with IBD; these include 2 serum markers, C-reactive protein (CRP, test code 4420) and erythrocyte sedimentation rate (ESR, test code 809), and 2 stool markers, calprotectin (test code 16796) and lactoferrin (test codes 10156 and 17321).

Serum markers: CRP and ESR

CRP is an acute-phase protein released from the liver in response to various inflammatory conditions. Although not specific for any one condition, it is highly sensitive for inflammation; CRP levels increase rapidly, up to 1,000-fold, at the onset of inflammation and decrease rapidly when it resolves.[7,8] CRP has moderate sensitivity and specificity for identifying IBD and differentiating IBD from IBS (**Table 3**). A meta-analysis of studies including patients with IBD or IBS and healthy controls found that a patient with a CRP level >2.7 mg/L had a ≥90% likelihood of having IBD as opposed to having IBS or being healthy,[9] though CRP levels at IBD diagnosis are usually much higher than that (medians: 20 mg/L in UC, 40 mg/L in CD).[8]

ESR is a measure of the speed at which red blood cells (RBCs) fall through a sample of blood. Inflammation causes red blood cells to aggregate more, which increases ESR, but elevated ESR is not specific to inflammation and can be caused by other conditions (eg, pregnancy, anemia).[8,10] ESR has moderate sensitivity and specificity for identifying IBD, but poor sensitivity and specificity for differentiating IBD from IBS (**Table 3**).[9]

CRP and ESR may not be elevated in all patients with IBD, especially in those whose disease activity is mild.[6,11] For this reason, testing ESR and CRP in combination can be useful,[8,10] as one marker may be elevated and the other normal in up to 38% of patients.[11] Thus, elevated levels of either marker are consistent with IBD, but normal levels do not rule it out.

Stool markers: calprotectin and lactoferrin

Calprotectin and lactoferrin are proteins released by neutrophils into the intestinal lumen when the intestines are inflamed. Thus, high levels of calprotectin and lactoferrin in stool indicate intestinal inflammation.

Calprotectin testing has high sensitivity and specificity for diagnosing IBD (**Table 3**) and is recommended by the American College of Gastroenterology (ACG) for differentiating between IBD and IBS.[5] High levels of calprotectin are consistent with IBD whereas low levels virtually exclude it; a meta-analysis found that a patient with a calprotectin level <40 µg/g had a <1% probability of having IBD as opposed to having IBS or being healthy.[9]

Fewer studies have assessed the utility of lactoferrin, but the available data suggests that it has comparable accuracy to calprotectin for diagnosing IBD (**Table 3**).[14,15] Similar to calprotectin, a normal level of lactoferrin indicates absence of intestinal inflammation, whereas a high level (medians: 1,100 µg/g in UC, 44 µg/g in CD[8]) is consistent with IBD.

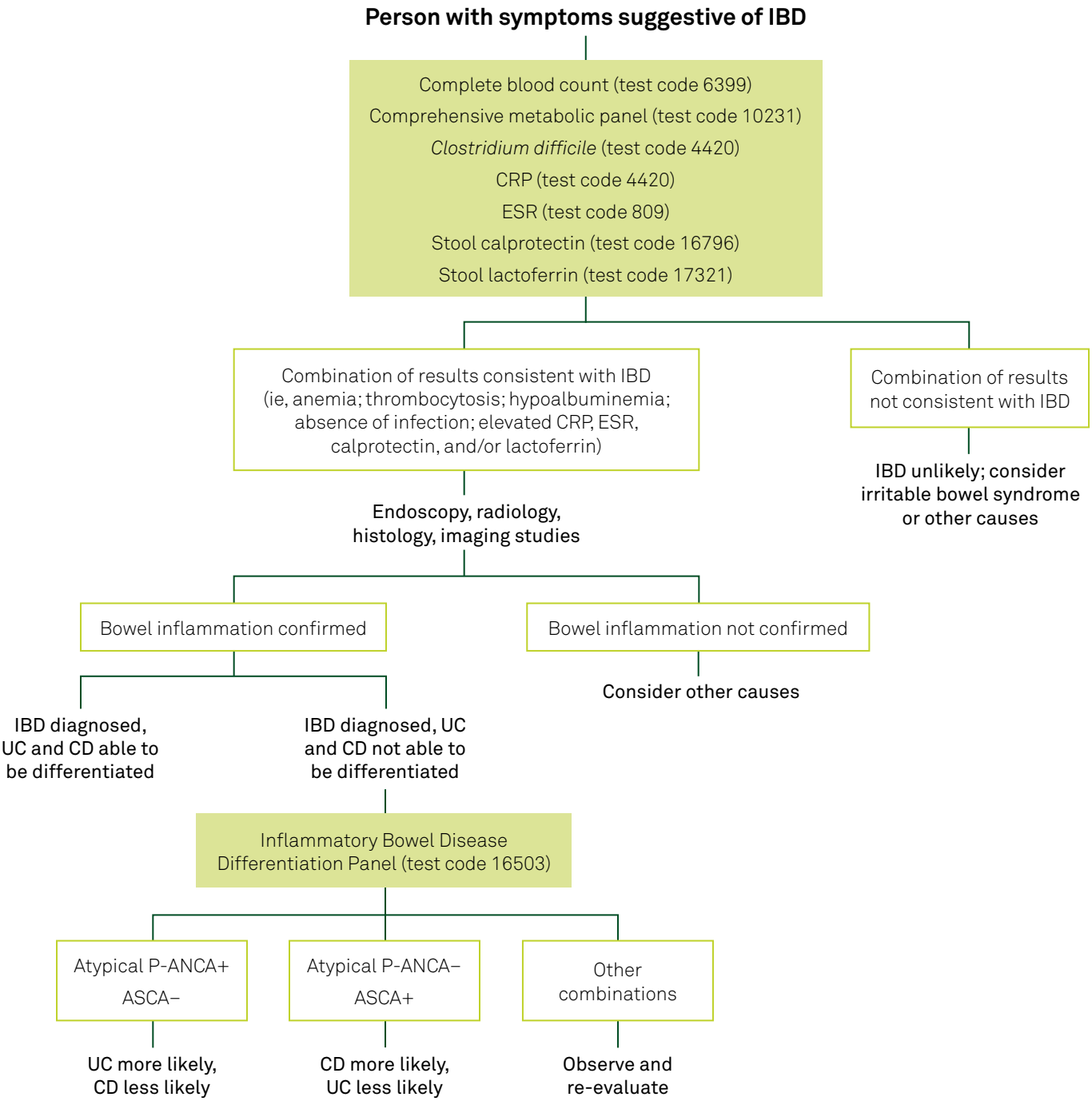
Although stool markers are more specific than serum markers for bowel inflammation, they cannot distinguish between the causes of such inflammation; GI infections and colorectal cancer, for example, are also associated with bowel inflammation and may cause elevated calprotectin and lactoferrin levels.[16] Additionally, for reasons that are not completely understood, stool markers are generally more accurate for diagnosing UC than CD.[6,15]

Differentiation of UC and CD

Differentiating UC from CD is an important part of IBD diagnosis with implications for prognosis and treatment selection. In most cases, differentiation is made with findings from endoscopy and histology. However, in some patients (1%-20% of adults and 4%-22% of children[17]), these findings are equivocal, and a definitive diagnosis cannot be made. This condition is known as indeterminate colitis (IC) or IBD-unclassified (IBD-U). For these patients, testing for certain antibodies involved in IBD inflammation—anti-neutrophil cytoplasmic antibodies (ANCAs, test code 70171) and anti-*Saccharomyces cerevisiae* antibodies (ASCAs, test codes 10294, 10295, 17609)—may provide support for differentiation.

ANCAs are autoantibodies that react to antigens in neutrophils. ANCAs can be detected by indirect immunofluorescence, which can identify the ANCA pattern, and immunoassay, which can detect specific antibodies.

Figure. Differential Diagnosis of Inflammatory Bowel Disease



ASCA, anti-*Saccharomyces cerevisiae* antibodies; CD, Crohn disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; P-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; UC, ulcerative colitis.

This figure was developed by Quest Diagnostics based in part on references 5, 6, and 21. It is provided for informational purposes only and is not intended as medical advice. Test selection and interpretation, diagnosis, and patient management decisions should be based on the physician's education, clinical expertise, and assessment of the patient.

Table 3. Sensitivity and Specificity of Laboratory Tests for IBD

Clinical context, test[12-14]	Sensitivity, % (95% CI)	Specificity, % (95% CI)
Diagnosis: IBD vs non-IBD		
CRP	63 (51-73)	88 (80-93)
ESR	66 (58-73)	84 (80-88)
Stool calprotectin	88 (83-92)	80 (69-88)
Stool lactoferrin	82 (72-89)	95 (88-98)
Diagnosis: IBD vs IBS		
CRP	75 (66-80)	60 (41-68)
ESR	55 (44-66)	47 (33-65)
Stool calprotectin	97 (91-99)	76 (66-84)
Stool lactoferrin	78 (75-82)	94 (91-96)
Diagnosis: UC vs CD[a]		
ANCA (for UC)	55 (53-58)	89 (87-90)
ASCA (for CD)	53 (51-56)	89 (87-91)
Detect active IBD		
CRP	49 (34-64)	92 (72-96)
Stool calprotectin	85 (82-87)	75 (71-79)
UC	87 (85-89)	77 (74-80)
CD	82 (80-84)	72 (69-75)
Stool lactoferrin	82 (73-88)	79 (62-89)
UC	81 (64-92)	82 (61-93)
CD	82 (73-88)	71 (63-78)
Predict IBD relapse		
Stool calprotectin		
Within 3 months	100	70
Within 12 months	69	75
Stool lactoferrin		
Within 3 months	100	62
Within 12 months	62	65

ANCA, anti-neutrophil cytoplasmic antibodies; ASCA, anti-*Saccharomyces cerevisiae* antibodies; CD, Crohn disease; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; UC, ulcerative colitis.

[a] Among patients with established diagnoses.

Common patterns and their associated antibodies include[18]

- Cytoplasmic (C-ANCA): associated with proteinase-3 (PR3) antibodies
- Perinuclear (P-ANCA): associated with myeloperoxidase (MPO) antibodies
- Atypical P-ANCA: associated with various antibodies other than PR3 and MPO

The various ANCA patterns tend to be characteristic of certain diseases. Whereas the cytoplasmic and perinuclear patterns are associated with small-vessel vasculitides, the atypical P-ANCA pattern is associated with IBD and relatively specific for UC (**Table 4**).[18] ASCAs, on the other hand, are antibodies to brewer's yeast and are relatively specific for CD.[18]

Given their specificities for the 2 diseases, ANCA and ASCA testing may be useful for differentiating UC from CD when conventional criteria cannot.[20] Used in this way, their diagnostic utility is greatest when both antibodies are tested (test code 16503)[7,18]: the combination of atypical P-ANCA–positive and ASCA–negative (P-ANCA+/ASCA–) results suggests UC, while the opposite combination (P-ANCA–/ASCA+) suggests CD.[19,21]

Although antibody testing is commonly used to help differentiate UC from CD,[22] only a few prospective studies have assessed its diagnostic accuracy in patients with IBD-U, and their results varied.[22,23] Additionally, ANCA and ASCA testing may be less useful in Asian (including Chinese, Japanese, and South Korean) patients, as they are less likely than White patients to have these antibodies.[24]

Management

Laboratory tests are useful throughout the management of IBD. The inflammatory biomarkers used in diagnosis are also used to monitor disease activity in established IBD. For some patients and treatment classes, laboratory tests can also aid in treatment selection and optimization.

Assessing disease activity

Disease activity is monitored closely in the management of IBD to guide treatment selection, assess response to treatment, and monitor for relapse.[3,5,6] The gold standard for assessing disease activity in IBD is endoscopy,[25,26] but repeated endoscopies are impractical, as they are invasive, expensive, and uncomfortable.[27] Other methods, including clinical assessment, radiology, and laboratory tests, are therefore important adjunctive tools.[5,28] In particular, biomarkers of inflammation are routinely used to assess IBD disease activity and can be used as alternatives to endoscopy in certain contexts.[5,27,28]

Levels of CRP, calprotectin, and lactoferrin correlate with endoscopic disease activity in IBD,[5,6,8,16,29] and tests for these markers have moderate to high diagnostic accuracy for detecting active IBD (**Table 3**). CRP testing has high specificity but low sensitivity for IBD disease activity,[30] as patients with mild disease activity may have false-negative results,[6,11] but CRP levels ≥5 mg/dL are consistent with moderate to severe disease activity.[30] As in diagnosis, calprotectin and lactoferrin testing are more accurate than CRP testing for assessing disease activity, and elevated levels of these markers are consistent with active disease.[30]

Guidelines from the ACG and American Gastroenterological Association (AGA) state that CRP, calprotectin, and lactoferrin testing have roles in assessing disease activity in UC and CD.[5,6,27] Additionally, recent guidance from the AGA allows using these biomarkers to obviate routine endoscopies in certain patients with UC.[27] These guidelines suggest the following:

- For patients with UC in symptomatic remission, normal levels of CRP, calprotectin (suggested cutoff: <150 µg/g), or lactoferrin are sufficient to rule out active inflammation and obviate endoscopy.
- For patients with UC and moderate to severe symptoms, elevated levels of CRP, calprotectin, or lactoferrin are sufficient to rule in active inflammation and likewise obviate endoscopy.
- For patients with UC and mild symptoms, endoscopy is still suggested in most cases, regardless of biomarker status.

Table 4. Antibody Prevalence in IBD and Healthy People

Antibody[19]	UC	CD	Healthy people
Atypical P-ANCA	50%-67%	6%-15%	<11%
ASCA	4%-14%	40%-60%	<5%

ASCA, anti-*Saccharomyces cerevisiae* antibodies; CD, Crohn disease; IBD, inflammatory bowel disease; P-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; UC, ulcerative colitis.

Because biomarkers are not perfectly accurate, their suitability to assess disease activity—especially in place of endoscopy—depends largely on a patient's pretest probability of active disease and the clinical implications of a false-negative or false-positive result.[27,30] Additionally, as in diagnosis, stool biomarkers tend to be less accurate at assessing disease activity in CD than UC.[10,31,32]

Assessing response to therapy

As markers of disease activity, inflammatory biomarkers can be used to assess response to IBD treatment when tested serially. CRP and calprotectin levels tend to decrease or normalize after the start of treatment in patients who respond positively to treatment.[8,10] For example, in a study of patients with UC and CD starting biologic therapy with infliximab, a decrease in calprotectin level by 80% or to 50 µg/g by week 2 was associated with endoscopic remission at week 10.[33] Similarly, biomarker testing during the course of treatment can indicate long-term response, such that patients with lower levels of CRP and calprotectin during treatment are more likely to maintain their response.[10,25,34] In a study of 87 patients with UC on maintenance therapy with infliximab, all those in sustained deep remission after 1 year (n=30) had maintained calprotectin levels <40 µg/g throughout the year.[35]

In a treat-to-target management approach, inflammatory biomarker levels may also serve as treatment goals themselves. A recent update to the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE-II) Initiative by the International Organization for the Study of IBD recommends using normalization of CRP and calprotectin (suggested cutoff: <100-250 µg/g) as an intermediate treatment target in IBD.[36] This recommendation, based on new evidence, represents a change from the original (2015) STRIDE guidance, in which biomarkers were not recommended as treatment targets.

Monitoring for relapse

For patients with IBD in remission, calprotectin and lactoferrin testing can be used to monitor for disease relapse and may be able to detect it before symptoms appear (**Table 3**). Prospective studies of patients with IBD in remission have found that calprotectin levels increase about 3 months before relapse becomes otherwise apparent.[35,37]

Serial measurements may predict relapse with better accuracy than isolated measurements. In one prospective study, calprotectin >300 µg/g across 2 measurements, 1 month apart, had 100% specificity for disease relapse within 12 months.[35] In another study, patients whose calprotectin levels doubled in 3 months had a doubled risk of relapse within the next 3 months.[37]

Therapeutic drug monitoring

Laboratory tests are used before and during IBD treatment to guide treatment selection and dosing and to monitor for adverse effects. Routine testing includes complete blood count and comprehensive metabolic panel,[4] but certain drug classes may require additional testing. For patients starting treatment with biologics and/or biosimilars, screening for viral hepatitis and tuberculosis is recommended.[4,5,38] For patients treated with TNF blockers and thiopurines, drug analyte testing (therapeutic drug monitoring) may be used to optimize safety and effectiveness.

TNF blockers

Tumor necrosis factor (TNF) blockers, such as adalimumab (Humira®), infliximab (Remicade®), and the infliximab biosimilar infliximab-dyyb (Inflectra®), can be used to treat UC and CD.[39–41] While some patients respond positively to these drugs, up to 80% may be refractory to treatment,[42] showing either nonresponse during induction (primary failure) or response during induction followed by loss of effectiveness (secondary failure).

When TNF blocker treatment fails, a clinician may need to consider adjusting dose or dosing intervals, switching to a different TNF blocker, or switching to a non-TNF blocker. Strategies for addressing treatment failure include

- Empiric dose escalation: increasing the dose (eg, from 5 mg to 10 mg) as a first response to failure
- Testing-based strategy: using drug and/or drug antibody levels to guide therapy changes

A testing-based strategy is suggested by the AGA when control of IBD is suboptimal.[43] Additionally, testing-based strategies are generally more cost-effective than empiric strategies and have been associated with beneficial health outcomes in patients receiving infliximab, such as higher rates of response and endoscopic remission and fewer flares requiring clinical care.[44,45]

With a testing-based strategy, measuring TNF blocker drug levels can help differentiate treatment failure–related issues that are pharmacokinetic (PK, absence of detectable drug) from those that are pharmacodynamic (PD, presence of drug but lack of effectiveness). Drug levels are typically assessed just before administration of the next dose to determine whether trough levels are therapeutic or subtherapeutic.[42] The AGA suggests target trough concentrations of ≥7.5 µg/mL for adalimumab and ≥5 µg/mL for infliximab in patients with active IBD on maintenance therapy.[43] Therapeutic trough levels generally indicate PD issues related to TNF-

independent disease. Subtherapeutic trough levels, on the other hand, can indicate different types of issues, depending on whether anti-drug antibodies (ADAs) have formed.[42,46]

ADAs, which are reported to form in up to 83% of patients treated with TNF blockers,[47] can cause subtherapeutic trough levels and reduce treatment effectiveness by (1) forming ADA-drug complexes that accelerate drug clearance and (2) directly preventing the drug from binding TNF.[42,46] Adalimumab or infliximab ADA levels ≥10 AU indicate detectable serum levels, whereas levels <10 AU are considered "not detected" and suggest that treatment failure is not caused by ADAs. Quest assays measure total (ie, free and bound) ADA. Some enzyme-linked immunosorbent assay (ELISA)-based tests for adalimumab or infliximab ADAs are susceptible to inaccurate results caused by cross-reactivity with rheumatoid factor (RF), but the ADA ELISAs developed by Quest are not affected by the presence of RF.

Testing for drug levels will indicate bioavailability, whereas testing for ADAs can help differentiate causes of insufficient bioavailability. Therefore, appropriate test selection varies as follows:

- Measuring only drug levels (test codes 36298, 36303) may be appropriate if sequential testing is preferred to concurrent testing.
- Measuring only ADA levels (test codes 36294, 36301) may be appropriate if insufficient bioavailability has already been established.
- Measuring both drug and ADA levels (test codes 36296, 36311) may help identify the cause of treatment failure more quickly.

Patients with subtherapeutic drug trough levels who test negative for ADAs may have nonimmune PK issues, such as poor adherence to treatment or accelerated drug clearance caused by nonimmune mechanisms.[42,46,47] Nonimmune PK issues can therefore be managed by addressing adherence issues, increasing the dose, or shortening the dosing interval.[42,47] However, for patients with subtherapeutic trough levels who test positive for ADAs, the cause of treatment failure is likely to be immune-mediated, and switching to a different TNF blocker may be more effective than increasing the dose.[42,47] Therefore, testing for ADAs in addition to drug levels can help determine which changes in treatment approach are most appropriate (**Table 5**).

Thiopurines

Thiopurines, including 6-mercaptopurine (Purinethol®) and its prodrug, azathioprine (Imuran®), can be used to treat UC and CD.[48,49] However, thiopurines are not appropriate for all patients and may be ineffective or have adverse effects in those who use them. To optimize safety and effectiveness, pretreatment testing and therapeutic drug monitoring may be appropriate for patients starting or receiving thiopurine treatment.

Although most patients can safely use thiopurines, a small number of people are unable to safely metabolize them and may experience serious harm.[43] Testing for thiopurine S-methyltransferase (TPMT) status can identify these patients and is suggested by the ACG and AGA before starting thiopurine treatment.[5,43] TPMT is the primary enzyme in thiopurine metabolism, and its level of expression affects a patient's risk for adverse effects to thiopurines.[50] Most people have a normal level of TPMT activity, but 1 in 10 have low TPMT

Table 5. Interpretation of Results in Patients With TNF Blocker Treatment Failure[a]

	ADAs not detected[42,46,47]	ADAs detected
Drug levels subtherapeutic	• Suggests insufficient bioavailability caused by nonimmune PK or adherence issues • Consider increasing the dose or addressing adherence issues	• Suggests insufficient bioavailability caused by immunogenicity • Consider switching to a different TNF blocker
Drug levels therapeutic	• Suggests PD issue caused by TNF-independent disease • Consider switching to a non-TNF treatment	• Rare situation that may be caused by a false-positive result or nonfunctional ADAs • Consider retesting or testing for neutralizing antibody by cell-based assay

ADA, anti-drug antibody; PD, pharmacodynamic; PK, pharmacokinetic; TNF, tumor necrosis factor.
[a] Test interpretation for infliximab assays applies to both infliximab and infliximab-dyyb.

activity, which increases the risk of thiopurine toxicity, and 1 in 300 have very low or no TPMT activity, which greatly increases this risk.[50]

TPMT status can be tested by phenotype (a blood test for TPMT activity level, test code 18831) or genotype (a genetic test for variants in *TPMT*, the gene that encodes TPMT, test code 37742). The AGA suggests using TPMT status to determine appropriate use of thiopurines, as follows[51,52]:

- Patients with normal enzyme activity level (>12 nmol 6-MMP/hr/mL RBC) or a normal genotype (0 non-functional variants) can start on a standard thiopurine dose.
- Patients with low activity (4-12 nmol 6-MMP/hr/mL RBC) or a heterozygous genotype (1 non-functional variant) should start on a low dose with gradual, closely monitored escalation to a normal dose.
- Patients with very low enzyme activity (<4 nmol 6-MMP/hr/mL RBC) or a homozygous genotype (2 non-functional alleles) should not use thiopurines.

For patients already receiving thiopurine treatment, thiopurine metabolite testing can guide dose adjustments and identify thiopurine toxicity. The AGA therefore suggests metabolite testing (test code 91745) for thiopurine-treated patients with active disease or adverse effects that may be related to thiopurine toxicity.[43] Thiopurine metabolites include 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine (6-MMP), but individuals differ in how much of each they produce, and these differences can affect their therapeutic response and risk for adverse effects.[52] Levels of 6-TGN, the therapeutic metabolite, must be high enough to be effective, but excessive levels of either metabolite can lead to various toxicities, including myelosuppression (excessive 6-TGN) and hepatoxicity (excessive 6-MMP).[52] Widely used target levels for metabolite monitoring are 230-450 pmol/8x10^8 RBCs for 6-TGN and <5,700 pmol/8x10^8 RBCs for 6-MMP.[43] Test interpretation and management strategies may depend on the indication for testing (ie, active disease or suspected toxicity) (**Table 6**).

Table 6. Interpretation of Results in Thiopurine-Treated Patients With Active IBD or Possible Thiopurine Toxicity

6-TGN level (pmol/8x10^8 RBCs)	6-MMP level (pmol/8x10^8 RBCs)	Interpretation[51–53]
Undetectable	Undetectable	• Suggests poor adherence • Consider addressing adherence issues
Low (<230)	Normal (<5,700)	• Suggests a subtherapeutic dose • Consider increasing the dose
Low (<230)	High (≥5,700)	• Suggests hypermethylation (risk of hepatotoxicity) • Consider using an alternative dosing strategy[a] or switching to a non-thiopurine treatment
Normal (230-450)	Normal (<5,700)	• Suggests a therapeutic dose • For active IBD, consider switching to a non-thiopurine treatment • For suspected toxicity, consider other causes
High (>450)	Any	• Suggests a supratherapeutic dose (risk of myelosuppression and/or hepatotoxicity) • For active IBD, consider switching to a non-thiopurine treatment • For suspected toxicity, consider reducing the dose

6-TGN, 6-thioguanine nucleotide; 6-MMP, 6-methylmercaptopurine; IBD, inflammatory bowel disease.

[a] Alternate dosing strategies for hypermethylation include (1) dose-splitting (administering half the daily dose twice daily) and (2) reducing the thiopurine dose and adding allopurinol.[52,53]

References

1. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet*. 2018;390(10114):2769-2778. doi:10.1016/s0140-6736(17)32448-0
2. Ungaro R, Mehandru S, Allen PB, et al. Ulcerative colitis. *Lancet*. 2017;389(10080):1756-1770. doi:10.1016/s0140-6736(16)32126-2
3. Torres J, Mehandru S, Colombel JF, et al. Crohn's disease. *Lancet*. 2017;389(10080):1741-1755. doi:10.1016/s0140-6736(16)31711-1
4. Bruner LP, White AM, Proksell S. Inflammatory bowel disease. *Prim Care: Clin Off Pr.* 2023;50(3):411-427. doi:10.1016/j.pop.2023.03.009
5. Lichtenstein GR, Loftus EV, Isaacs KL, et al. ACG clinical guideline: management of Crohn's disease in adults. *Am J Gastroenterol.* 2018;113(4):481-517. doi:10.1038/ajg.2018.27
6. Rubin DT, Ananthakrishnan AN, Siegel CA, et al. ACG clinical guideline: ulcerative colitis in adults. *Am J Gastroenterol.* 2019;114(3):384-413. doi:10.14309/ajg.0000000000000152
7. Chen P, Zhou G, Lin J, et al. Serum biomarkers for inflammatory bowel disease. *Front Med.* 2020;7:123. doi:10.3389/fmed.2020.00123
8. Liu D, Saikam V, Skrada KA, et al. Inflammatory bowel disease biomarkers. *Med Res Rev*. 2022;42(5):1856-1887. doi:10.1002/med.21893
9. Menees SB, Powell C, Kurlander J, et al. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. *Am J Gastroenterol.* 2015;110(3):444-454. doi:10.1038/ajg.2015.6
10. Sakurai T, Saruta M. Positioning and usefulness of biomarkers in inflammatory bowel disease. *Digestion.* 2023;104(1):30-41. doi:10.1159/000527846
11. Turner D, Mack DR, Hyams J, et al. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) or both? a systematic evaluation in pediatric ulcerative colitis. *J Crohn's Colitis.* 2011;5(5):423-429. doi:10.1016/j.crohns.2011.05.003
12. Gisbert JP, Bermejo F, Pérez-Calle J, et al. Fecal calprotectin and lactoferrin for the prediction of inflammatory bowel disease relapse. *Inflamm Bowel Dis.* 2009;15(8):1190-1198. doi:10.1002/ibd.20933
13. Oikonomou KA, Kapsoritakis AN, Theodoridou C, et al. Neutrophil gelatinase-associated lipocalin (NGAL) in inflammatory bowel disease: association with pathophysiology of inflammation, established markers, and disease activity. *J Gastroenterol.* 2012;47(5):519-530. doi:10.1007/s00535-011-0516-5
14. Shi JT, Zhang Y, She Y, et al. Diagnostic utility of non-invasive tests for inflammatory bowel disease: an umbrella review. *Front Med.* 2022;9:920732. doi:10.3389/fmed.2022.920732
15. Wang Y, Pei F, Wang X, et al. Diagnostic accuracy of fecal lactoferrin for inflammatory bowel disease: a meta-analysis. *Int J Clin Exp Pathol.* 2015;8(10):12319-12332.
16. Jukic A, Bakiri L, Wagner EF, et al. Calprotectin: from biomarker to biological function. *Gut.* 2021;70(10):1978-1988. doi:10.1136/gutjnl-2021-324855
17. Everhov ÅH, Sachs MC, Malmborg P, et al. Changes in inflammatory bowel disease subtype during follow-up and over time in 44,302 patients. *Scand J Gastroenterol.* 2019;54(1):55-63. doi:10.1080/00365521.2018.1564361
18. Zhou G, Song Y, Yang W, et al. ASCA, ANCA, ALCA and many more: are they useful in the diagnosis of inflammatory bowel disease? *Dig Dis.* 2016;34(1-2):90-97. doi:10.1159/000442934
19. Bossuyt X, Tervaert JWC, Arimura Y, et al. Revised 2017 international consensus on testing of ANCAs in granulomatosis with polyangiitis and microscopic polyangiitis. *Nat Rev Rheumatol.* 2017;13(11):683-692. doi:10.1038/nrrheum.2017.140
20. Moiseev S, Tervaert JWC, Arimura Y, et al. 2020 international consensus on ANCA testing beyond systemic vasculitis. *Autoimmun Rev.* 2020;19(9):102618. doi:10.1016/j.autrev.2020.102618
21. Bernstein CN, Eliakim A, Fedail S, et al. World Gastroenterology Organisation global guidelines inflammatory bowel disease. *J Clin Gastroenterol.* 2016;50(10):803-818. doi:10.1097/mcg.0000000000000660
22. Sura SP, Ahmed A, Cheifetz AS, et al. Characteristics of inflammatory bowel disease serology in patients with indeterminate colitis. *J Clin Gastroenterol.* 2014;48(4):351-355. doi:10.1097/mcg.0000000000000083
23. Joossens S, Reinisch W, Vermeire S, et al. The value of serologic markers in indeterminate colitis: a prospective follow-up study. *Gastroenterology.* 2002;122(5):1242-1247. doi:10.1053/gast.2002.32980
24. Prideaux L, Kamm MA, Cruz PD, et al. Inflammatory bowel disease serology in Asia and the West. *World J Gastroenterol.* 2013;19(37):6207-6213. doi:10.3748/wjg.v19.i37.6207
25. Plevris N, Lees CW. Disease monitoring in inflammatory bowel disease: evolving principles and possibilities. *Gastroenterology.* 2022;162(5):1456-1475.e1. doi:10.1053/j.gastro.2022.01.024
26. Rodrigues BL, Mazzaro MC, Nagasako CK, et al. Assessment of disease activity in inflammatory bowel diseases: non-invasive biomarkers and endoscopic scores. *World J Gastrointest Endosc.* 2020;12(12):504-520. doi:10.4253/wjge.v12.i12.504
27. Singh S, Ananthakrishnan AN, Nguyen NH, et al. AGA clinical practice guideline on the role of biomarkers for the management of ulcerative colitis. *Gastroenterology.* 2023;164(3):344-372. doi:10.1053/j.gastro.2022.12.007
28. Ince MN, Elliott DE. Effective use of the laboratory in the management of patients with inflammatory bowel diseases. *Gastroenterol Clin North Am*. 2019;48(2):237-258. doi:10.1016/j.gtc.2019.02.006
29. Rubio MG, Amo-Mensah K, Gray JM, et al. Fecal lactoferrin accurately reflects mucosal inflammation in inflammatory bowel disease. *World J Gastrointest Pathophysiol.* 2019;10(5):54-63. doi:10.4291/wjgp.v10.i5.54
30. Mosli MH, Zou G, Garg SK, et al. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. *Am J Gastroenterol.* 2015;110(6):802-820. doi:10.1038/ajg.2015.120

31. Rokkas T, Portincasa P, Koutroubakis IE. Fecal calprotectin in assessing inflammatory bowel disease endoscopic activity: a diagnostic accuracy meta-analysis. *J Gastrointest Liver Dis.* 2018;27(3):299-306. doi:10.15403/jgld.2014.1121.273.pti

32. Dai C, Jiang M, Sun MJ, et al. Fecal lactoferrin for assessment of inflammatory bowel disease activity. *J Clin Gastroenterol.* 2020;54(6):545-553. doi:10.1097/mcg.0000000000001212

33. De Vos M, Dewit O, D'Haens G, et al. Fast and sharp decrease in calprotectin predicts remission by infliximab in anti-TNF naïve patients with ulcerative colitis. *J Crohns Colitis.* 2012;6(5):557-562. doi:10.1016/j.crohns.2011.11.002

34. Laserna-Mendieta EJ, Lucendo AJ. Faecal calprotectin in inflammatory bowel diseases: a review focused on meta-analyses and routine usage limitations. *Clin Chem Lab Med.* 2019;57(9):1295-1307. doi:10.1515/cclm-2018-1063

35. De Vos M, Louis EJ, Jahnsen J, et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. *Inflamm Bowel Dis.* 2013;19(10):2111-2117. doi:10.1097/mib.0b013e31829b2a37

36. Turner D, Ricciuto A, Lewis A, et al. STRIDE-II: an update on the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) initiative of the International Organization for the Study of IBD (IOIBD): determining therapeutic goals for treat-to-target strategies in IBD. *Gastroenterology*. 2021;160(5):1570-1583. doi:10.1053/j.gastro.2020.12.031

37. Zhulina Y, Cao Y, Amcoff K, et al. The prognostic significance of faecal calprotectin in patients with inactive inflammatory bowel disease. *Aliment Pharmacol Ther*. 2016;44(5):495-504. doi:10.1111/apt.13731

38. Farraye FA, Melmed GY, Lichtenstein GR, et al. ACG clinical guideline: preventive care in inflammatory bowel disease. *Am J Gastroenterol*. 2017;112(2):241-258. doi:10.1038/ajg.2016.537

39. Humira® (adalimumab). Prescribing information. AbbVie Inc; 2020. Accessed November 29, 2023. https://www.rxabbvie.com/pdf/humira.pdf

40. Remicade® (infliximab). Prescribing information. Janssen Biotech Inc; 2021. Accessed November 29, 2023. https://www.janssenlabels.com/package-insert/product-monograph/prescribing-information/REMICADE-pi.pdf

41. Inflectra® (infliximab-dyyb). Prescribing information. Pfizer Inc; 2021. Accessed November 29, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/125544s018lbl.pdf

42. Albader F, Golovics PA, Gonczi L, et al. Therapeutic drug monitoring in inflammatory bowel disease: the dawn of reactive monitoring. *World J Gastroenterol.* 2021;27(37):6231-6247. doi:10.3748/wjg.v27.i37.6231

43. Feuerstein JD, Nguyen GC, Kupfer SS, et al. American Gastroenterological Association institute guideline on therapeutic drug monitoring in inflammatory bowel disease. *Gastroenterology.* 2017;153(3):827-834. doi:10.1053/j.gastro.2017.07.032

44. Kelly OB, Donnell SO, Stempak JM, et al. Therapeutic drug monitoring to guide infliximab dose adjustment is associated with better endoscopic outcomes than clinical decision making alone in active inflammatory bowel disease. *Inflamm Bowel Dis.* 2017;23(7):1202-1209. doi:10.1097/mib.0000000000001126

45. Marquez-Megias S, Nalda-Molina R, Sanz-Valero J, et al. Cost-effectiveness of therapeutic drug monitoring of anti-TNF therapy in inflammatory bowel disease: a systematic review. *Pharmaceutics.* 2022;14(5):1009. doi:10.3390/pharmaceutics14051009

46. Marsal J, Barreiro-de Acosta M, Blumenstein I, et al. Management of non-response and loss of response to anti-tumor necrosis factor therapy in inflammatory bowel disease. *Front Med.* 2022;9:897936. doi:10.3389/fmed.2022.897936

47. Lee SD, Shivashankar R, Quirk D, et al. Therapeutic drug monitoring for current and investigational inflammatory bowel disease treatments. *J Clin Gastroenterol.* 2021;55(3):195-206. doi:10.1097/mcg.0000000000001396

48. Purinethol® (mercaptopurine). Prescribing information. Teva Pharmaceutical Industries Ltd; 2020. Accessed November 29, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/009053s040lbl.pdf

49. Imuran® (azathioprine). Prescribing information. Sebela Pharmaceuticals Inc; 2018. Accessed November 29, 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/016324s039lbl.pdf

50. Ribeiro AC, Gerheim PSAS, Chebli JMF, et al. The role of pharmacogenetics in the therapeutic response to thiopurines in the treatment of inflammatory bowel disease: a systematic review. *J Clin Med.* 2023;12(21):6742. doi:10.3390/jcm12216742

51. American Gastroenterological Association. Therapeutic drug monitoring in inflammatory bowel disease clinical decision support tool. *Gastroenterology.* 2017;153(3):858-859. doi:10.1053/j.gastro.2017.07.039

52. Singh A, Mahajan R, Kedia S, et al. Use of thiopurines in inflammatory bowel disease: an update. *Intest Res.* 2022;20(1):11-30. doi:10.5217/ir.2020.00155

53. Gargallo-Puyuelo CJ, Laredo V, Gomollón F. Thiopurines in inflammatory bowel disease. how to optimize thiopurines in the biologic era? *Front Med.* 2021;8:681907. doi:10.3389/fmed.2021.681907