# AGA CLINICAL PRACTICE GUIDELINE ON THE ROLE OF BIOMARKERS FOR THE MANAGEMENT OF ULCERATIVE COLITIS

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## ABSTRACT

**Background & Aims:** Biomarkers are frequently used for non-invasive monitoring and treatment decision-making in the management of patients with ulcerative colitis (UC). This American Gastroenterological Association (AGA) guideline is intended to support practitioners in decisions about the use of biomarkers for the management of UC.

**Methods:** A multi-disciplinary panel of content experts and guideline methodologists used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework to prioritize clinical questions, identify patient-centered outcomes, and conduct an evidence synthesis on the clinical performance of serum C-reactive protein, fecal calprotectin and fecal lactoferrin as biomarkers of disease activity in patients with established UC in symptomatic remission or with active symptoms. The guideline panel used the Evidence-to-Decision framework to develop recommendations for the use of biomarkers for monitoring and management of UC and provided implementation considerations for clinical practice.

**Results:** The guideline panel made seven conditional recommendations. In patients with UC in symptomatic remission, the panel suggests the use of a biomarker- and symptom-based monitoring strategy over a symptom-based monitoring strategy. In patients in symptomatic remission, the panel suggests using fecal calprotectin <150μg/g, normal fecal lactoferrin and/or normal CRP to rule out active inflammation and avoid routine endoscopic assessment of disease. In patients with UC with moderate to severe symptoms, the panel suggests using fecal calprotectin >150μg/g, elevated fecal lactoferrin or elevated CRP to inform treatment decisions and avoid routine endoscopic assessment of disease. However, in patients in symptomatic remission but elevated biomarkers, and in patients with moderate to severe symptoms with normal biomarkers, the panel suggests endoscopic assessment of disease to inform treatment decisions. In patients with UC with mild symptoms, the panel suggests endoscopic assessment of disease activity to inform treatment decisions. The panel identified the use of a biomarker-based monitoring strategy over an endoscopy-based monitoring strategy as a knowledge gap. The panel also proposed key implementation considerations for optimal use of biomarkers, and identified areas for future research.

**Conclusions:** In patients with UC, non-invasive biomarkers including fecal calprotectin, fecal lactoferrin and serum CRP can inform disease monitoring and management.

**Key words:** Inflammatory bowel disease; monitoring; endoscopic remission; treat to target; evidence synthesis

## INTRODUCTION

Inflammatory bowel diseases comprising Crohn's disease (CD) and ulcerative colitis (UC) are estimated to affect over 7 million individuals worldwide.[1, 2] UC is characterized by periods of relapsing-remitting activity, resulting in significant morbidity.[3] Up to one-in-five patients with UC may undergo definitive surgery in the form of a total colectomy, often for medically refractory disease.[4] The direct and indirect costs attributable to UC are considerable and continue to increase.[5]

There has been a paradigm shift in the management of UC over the past two decades. The therapeutic target has shifted from symptom resolution alone to a combination of symptomatic and endoscopic remission (Mayo endoscopy score [MES] 0 or equivalent) or improvement (MES 0 or 1).[6, 7] Achievement of endoscopic remission or improvement is associated with superior outcomes including lower risk of relapse, need for corticosteroids, hospitalizations, colectomy and colorectal neoplasia.[8, 9] While there are no randomized controlled trials (RCTs) in UC, indirect evidence from treatment strategy intervention trials in CD, such as CALM where a tight control strategy based on a combination of symptoms and biomarkers, was more effective than a usual care strategy targeting symptoms alone, in achieving deep remission, which in turn was associated with lower risk of disease progression and complications, surgery and hospitalization.[10, 11]

Most RCTs have relied on endoscopic evaluation to confirm resolution of bowel inflammation. Similarly, in clinical practice, endoscopic assessment of bowel inflammation after initiation of therapy is performed in 45-70% of patients.[12, 13] Despite the fact that early proactive assessment of bowel inflammation is associated with superior long-term outcomes, there is significant variability in utilization.[12] Moreover, in routine clinical practice, repeated endoscopic assessment is invasive, expensive, and may be impractical. There is an important need for understanding how non-invasive biomarkers may serve as accurate and reliable surrogates for endoscopic assessment of inflammation and if they can be more readily implemented in a UC care pathway. Finally, patients with UC may prefer alternative non-invasive tests, such as biomarkers, over endoscopy, although this preference varies depending on the diagnostic and prognostic performance of biomarkers.[14]

### Objective

The objective of this guideline is to provide guidance about the use of well-established and commonly available biomarkers as surrogate tests for endoscopic assessment of disease or in longitudinal monitoring of patients with an established diagnosis of UC. This guideline focuses on the following biomarkers: serum C-reactive protein (CRP), fecal calprotectin and fecal lactoferrin. Laboratory evaluation of diarrhea in patients with suspected UC is discussed elsewhere.[15, 16] The role of biomarkers in patients with CD will be discussed in a subsequent guideline.

### Target Audience

The target audience of these guidelines includes primary care and gastroenterology health care professionals, patients, and policy makers. These guidelines are not intended to impose a standard of care, rather they provide the basis for rational informed decisions for patients and health care professionals. Statements regarding the underlying values and preferences as well as qualifying remarks accompanying each recommendation should never be omitted when quoting or translating recommendations from these guidelines. Recommendations provide guidance for typical patients with UC; no recommendation can consider all unique circumstances that must be accounted for when making recommendations for individual patients. However, discussions about benefits and harms can be used for shared decision-making, especially for conditional recommendations in which specific tradeoffs and patient values are important to consider.

## METHODS

### Overview

This document represents the official recommendations of the AGA and was developed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework for diagnostic tests and strategies and adheres to best practices in guideline development, as outlined by the National Academy of Medicine (formerly Institute of Medicine).[17] The development of this guideline was fully funded by the AGA Institute.

## Guideline Panel Composition and Conflict of Interest

Members of the guideline and evidence synthesis panel were selected on the basis of their clinical and methodological expertise after undergoing a vetting process that required disclosing all conflicts of interest. The evidence synthesis panel consisted of 2 content experts with expertise in UC (Ananthakrishnan, Adler), a senior guideline methodologist with expertise in evidence synthesis and GRADE (Singh) and two junior guideline methodologists (Nguyen, Siddique). The guideline panel consisted of a multidisciplinary panel that included a general gastroenterologist (Weiss), gastroenterologists with expertise in IBD (Chachu, Cohen, Velayos), and guideline methodologists (Singh, Nguyen, Siddique, Sultan). A patient representative also participated in the development of the guideline recommendations. Panel members disclosed all potential conflicts of interest. Conflicts were managed according to AGA policies, the National Academy of Medicine, and Guidelines International Network standards. Guideline chair (Chachu) and co-chair/senior methodologist (Singh) had no conflict of interest. No guideline panel member was excused from participation in the process owing to disqualifying conflict. A full list of conflicts can be accessed at AGA's National Office in Bethesda, MD.

## Scope

Biomarkers are defined biological molecules that are quantifiable in a tissue or body fluid (blood, stool, and urine) that represent an underlying biological disease process. Various biomarkers have been extensively investigated in UC for several outcomes including prediction of onset, establishing diagnosis, assessing disease activity, prognosticating natural history including likelihood of colectomy, and assessing post-colectomy outcomes.[18] However, most of these studies have been small, lack replication, and utilize markers that are not readily available outside of a research setting. For inclusion, we required the biomarker to be both readily measurable in a tissue or body fluid compartment and widely available for commercial use and utilized routinely in day-to-day clinical practice for assessing disease activity or provide actionable prognostic information longitudinally. Based on these criteria, we focused on serum CRP, fecal calprotectin and fecal lactoferrin.

## Formulation of Clinical Questions

Through an iterative process, the guideline and evidence synthesis panels developed focused clinical questions deemed relevant for clinical practice that the guideline would address, related to the diagnostic performance and utility of commonly used serum and stool biomarkers in patients with established UC. From these focused questions, well-defined statements in terms of patients, intervention, comparator and outcome were defined, and these formed the framework for formulating the study inclusion and exclusion criteria and guided the literature search. The AGA Governing Board approved the final set of questions and statements in October 2021. The final focused questions and PICO questions are shown in **Table 1**.

## Search Strategy

An experienced medical librarian conducted a comprehensive search of the following databases (Ovid Medline In-Process & Other Non-Indexed Citations, Ovid MEDLINE, EMBASE, and Wiley Cochrane Library) from inception to November 21, 2021, using a combination of controlled vocabulary terms supplemented with keywords (**eTable 1**). To ensure that recent studies were not missed, searches were updated before external review. The search was limited to English language and humans. The bibliography of prior guidelines and the included references were searched to identify relevant studies that may have been missed. In addition, content experts helped identify any ongoing studies.

## Study Selection, Data Abstraction and Statistical Analysis

We included RCTs or observational studies of diagnostic accuracy which met the following inclusion criteria: (a) performed in patients with UC, (b) provided adequate description of biomarker (CRP, fecal calprotectin and/or fecal lactoferrin) with cut-off corresponding to detection of moderate to severe endoscopic activity (corresponding to MES 2 or 3), (c) with lower endoscopy as gold standard, and (d) provided sufficient data to allow estimation of diagnostic accuracy of biomarker (sensitivity, specificity) for detection of endoscopic activity. For these questions, we preferentially chose cut-offs most commonly used in clinical practice, rather than focusing on optimized cut-offs

identified in individual studies. These cut-offs were: C-reactive protein (5±5mg/L or 0.5±0.5mg/dl), fecal calprotectin (250±50μg/g, 150±50μg/g and 50±50μg/g) and fecal lactoferrin (normal or elevated based on laboratory cut-off).

From each study, we abstracted data on study, patient, biomarker, outcome and test performance data (sensitivity, specificity, prevalence of outcome of interest in study, to impute numbers of true-positive, true-negative, false-positive, and false-negative results). The paired values of sensitivity and specificity were pooled using a bivariate regression random-effects model proposed by Reitsma *et al* using STATA 14.0 software (College Station, TX).[19] Statistical assessment of heterogeneity was performed using the inconsistency index ($I^2$), which estimates what proportion of total variation across studies was due to heterogeneity rather than chance.[20]

**Outcomes of Interest and Illustrative Clinical Scenarios**

For PICOs focusing on biomarker cut-offs to either detect or rule out moderate to severe endoscopic activity, the preferred outcome was direct consequences on patient-important outcomes (i.e., implications of true positive [TP], false positive [FP], true negative [TN], false negative [FN] results for patients – see below). However, none of the studies assessed these outcomes directly, and hence, we used TP, FP, TN and FN rates as surrogate outcomes and inferred downstream consequences on patient-important outcomes.

For questions focusing on *ruling out moderate to severe endoscopic activity*, our outcome was minimizing rates of FN (i.e., patients incorrectly being labeled as being in remission, when they actually have moderate to severe endoscopic inflammation) to a level <5% in general, preferably lower, with reasonable rates of TP, FP and TN (**eFigure 1**). For questions focusing on *detecting moderate to severe endoscopic activity*, our outcome was minimizing rates of FP (i.e., patients incorrectly labeled as having moderate to severe endoscopic inflammation, when their disease is actually in remission) (**eFigure 2**). These thresholds of 5% FN and FP rate were also consistent with patient preference for choosing stool-based biomarkers over endoscopic assessment for monitoring inflammation.[14]

While sensitivity and specificity are agnostic of disease prevalence, overall TP, FP, TN and FN rates are highly dependent on pre-test probability. We derived illustrative

prevalence of moderate to severe endoscopic activity based upon combination of rectal bleeding score (RBS) and stool frequency score (SFS), two of the most commonly used patient-reported outcomes, derived from the Mayo Clinic Score (**eTable 2**). Prevalence of moderate to severe endoscopic activity (MES 2 or 3), and of endoscopic improvement (MES 0 or 1), for different combinations of RBS and SFS, at varying time points after treatment initiation/adjustment were derived from existing literature based on individual participant data from phase 2 and 3 clinical trial programs of biologic agents and small molecule inhibitors in patients with moderate to severely active UC.[21]

For our analysis, we used three illustrative scenarios:

- ***Low pre-test probability*** of having moderate to severe inflammation: These include asymptomatic patients with UC (no rectal bleeding, and normal to mild increase in stool frequency, RBS 0 and SFS 0 or 1), on stable maintenance therapy, or having recently achieved symptomatic remission after treatment adjustment. The expected prevalence of moderate to severe endoscopic inflammation in these patients is ~15%.
- ***Intermediate pre-test probability*** of having moderate to severe inflammation: These include patients with mild symptoms of UC, such as infrequent rectal bleeding (RBS 0 or 1) and/or increased stool frequency (SFS 2 or 3). The prevalence of moderate to severe endoscopic inflammation in these patients is ~50%.
- ***High pre-test probability*** of having moderate to severe inflammation: These include patients with typical symptoms of active UC with frequent rectal bleeding and significant increase in stool frequency (RBS 2 or 3 and SFS 2 or 3). The prevalence of moderate to severe endoscopic inflammation in these patients is ~85%.

**Consequences of diagnostic test results on patient-important outcomes**

Corresponding to each possible outcome (TP, FP, TN, FN), presumed downstream consequences on patient-important outcomes were considered. In using specific biomarkers either as a test replacement or triage strategy, healthcare providers and patients need to be aware of test performance and be comfortable with potential FN and FP rate with related downstream consequences. Such downstream consequences of test

results for each PICO statement and scenario have been discussed in detail in each evidence profile.

A pre-meeting questionnaire was administered to all members of the guideline panel and evidence synthesis panel to determine their *a priori* maximal tolerable FN rate and FP rate for each PICO (i.e., what level of FN and FP rate would they be willing to accept for a particular test, for their patient). As the maximally tolerable rates of FN and FP for any diagnostic strategy is highly context sensitive, we devised different clinical scenarios with corresponding downstream consequences for each PICO to arrive at fully contextualized estimates of FN and FP thresholds.

**Certainty of the Evidence**

We rated the certainty of evidence using the GRADE approach for diagnostic tests and strategies.[17] In this approach, all evidence from RCTs (comparing different diagnostic tests or cut-offs of same test) and observational diagnostic accuracy studies start at high-quality, but can be rated down for any of the following factors:

- Risk of bias in included studies (inferred based on QUADAS-2 instrument).[22]
- Indirectness (deemed present if there are important differences between the populations studied and those for whom the recommendation is intended). In this updated GRADE approach for diagnostic accuracy studies, TP, FP, TN, and FN derived from sensitivity/specificity are not considered surrogate outcomes.
- Inconsistency (deemed present if there were considerable differences between studies in the accuracy estimates that were not explained, or if cut-offs for biomarkers corresponding to endoscopic improvement for moderately to severe endoscopic activity were not pre-specified but primarily obtained post-hoc corresponding to area under the receiver operating characteristic curve).
- Imprecision (deemed present if there were wide confidence intervals for TP, FN, TN, FP rates).
- Publication bias, if strongly suspected.

Evidence profiles were developed for each intervention using the GRADEpro Guideline Development Tool (https://gradepro.org).

**Translating Evidence to Recommendations**

The guideline panel and evidence synthesis panel met face to face on May 21, 2022 to discuss the evidence and formulate the guideline recommendations. Based on the evidence-to-decision framework, the panel considered the certainty of evidence, balance of benefit and harms, patient values and preferences, and (when applicable) feasibility, acceptability, equity, and resource use. For all recommendations, the panel reached consensus. The certainty of evidence and the strength of recommendation are provided for each clinical question. As per GRADE methodology, recommendations are labeled as "strong" or "conditional". The words "we recommend" indicate strong recommendations and "we suggest" indicate conditional recommendations provide the suggested interpretation of strong and weak recommendations for patients, clinicians, and healthcare policymakers.

### Review Process

This guideline was submitted for public comment and internal review and was approved by the AGA Governing Board.

## DISCUSSION OF RECOMMENDATIONS

A summary of all the recommendations is provided in **Table 2** and discussed below. Key implementation considerations when considering using biomarkers in UC are discussed below and in **Table 3**.

### Key considerations for implementing these recommendations in clinical practice

In using a biomarker as a test replacement or triage strategy, it is critical to have a framework for understanding how each possible test result (TP, FP, TN, FN) is associated with downstream consequences and impact on patient-important outcomes (**Table 4**).

The recommendations presented in this guideline are intended to provide a framework for incorporating biomarkers in the management pathway of UC patients to inform treatment decisions. It is also critical to understand the limitations in interpreting these tests in various settings.

1. **Considerations of test performance and specificity of biomarkers:** The serum and fecal biomarkers in this guideline are not specific for UC activity. Serologic biomarkers, such as CRP, may be influenced by concurrent systemic illnesses as well as other co-existing inflammatory diseases. Fecal markers, while more specific for intestinal inflammation, are not specific for UC disease activity and may be elevated in other inflammatory diseases of the gut including infectious gastroenteritis, drug-induced colitis, etc.[23] Thus, one should also consider simultaneous evaluation for enteric pathogens in patients with UC who present with gastrointestinal symptoms; Gastrointestinal infections are detected in approximately one-third patients with UC presenting with gastrointestinal symptoms.[24, 25]
    - ***CRP, fecal calprotectin and fecal lactoferrin may be elevated because of non-intestinal sources of infection or inflammation. In patients with UC who present with elevated biomarkers and disease-related symptoms, stool testing for C. difficile and other enteric pathogens is important to help rule out other sources of GI infections.***

2. **Role of endoscopic evaluation for other indications:** This guideline includes recommendations on the use of biomarkers as a replacement strategy for endoscopy in individuals with moderate-to-severe UC, however, endoscopy is frequently performed for other indications, e.g. detection and surveillance of dysplasia in patients with long-standing UC.[26] Similarly, in patients with severe UC, particularly those who are refractory to corticosteroids, endoscopic assessment may be warranted to rule out cytomegalovirus colitis. In patients hospitalized with acute severe UC, endoscopic evaluation may be helpful to prognosticate and inform treatment.[27]
    - ***Biomarkers of inflammation have no role in dysplasia detection and surveillance, and ruling out cytomegalovirus colitis, and endoscopic evaluation is the main strategy for evaluating these. Endoscopic evaluation may be useful for prognostication in patients hospitalized with acute severe UC.***

3. **Association between treatment target and biomarker performance:** Current treatment guidelines recommend a target of endoscopic improvement (MES 0 or 1),

though more updated consensus statements recommend a target of endoscopic remission (MES 0, or equivalent).[6, 7] In a meta-analysis of 15 eligible studies, a MES of 0 was associated with a lower risk of clinical relapse (OR, 0.33; 95% CI, 0.26–0.43) compared with an MES of 1.[28] Furthermore, histologic healing may also be a superior therapeutic goal; persistent histologic activity, even in the setting of endoscopically healed mucosa, is associated with a higher risk of relapse.[28, 29] In this guideline, we focused on accuracy of biomarkers to detect moderate to severe endoscopic inflammation (MES 2 or 3), since, currently in clinical practice, these are widely accepted triggers for treatment adjustment to achieve a conventional treatment target of MES 0 or 1. Diagnostic performance of a combination of symptoms and biomarkers to detect more rigorous endpoints such as MES 0 or histologic remission was not assessed in this guideline, but are likely to have inferior performance given differences in pre-test probability. On a related note, a subset of patients with symptomatically active UC, generally with mild symptoms, may have mild inflammation on endoscopy (MES 1). The performance of biomarkers to specifically distinguish endoscopic remission (MES 0) vs. mild endoscopic activity (MES 1) is limited.

- ***Test performance of all biomarkers in this guideline reflect their ability to rule out moderate to severe endoscopic inflammation (MES 2 or 3 [or equivalent]). Biomarkers may be suboptimal for detecting more rigorous treatment targets such as endoscopic remission (MES 0) or histologic remission. Biomarkers may also be suboptimal in detecting the presence of mild endoscopic activity (MES 1) in patients with mild symptoms.***

4. **Influence of disease extent on performance of fecal biomarkers:** Elevation of fecal biomarkers such as calprotectin and lactoferrin may be influenced by the extent and location of inflamed surface. In a prospective study of patients with UC undergoing ileocolonoscopy, fecal calprotectin values demonstrated a stronger correlation with the extent of inflamed surface (r=0.86) than region of maximal severity, independent of the severity of inflammation (r=0.79).[30] In studies examining specific disease locations, the performance of fecal calprotectin in identifying active disease (MES 1-3) was weaker for proctitis (r=0.54) when compared to either left-sided colitis (r=0.75) or extensive colitis (r=0.78).[31] In other studies, fecal calprotectin was unable to

accurately identify active disease in the setting of isolated proctitis in comparison to disease more extensively involving the colon though in some studies, fecal calprotectin has demonstrated value in serially monitoring response to suppository treatment in isolated UC proctitis.[32, 33]

- ***Fecal biomarkers may be less accurate in detecting endoscopic inflammation in patients with ulcerative proctitis or limited segmental disease.***

5. **Interpreting biomarker performance for low-risk vs. high-risk treatment adjustments:** The acceptable threshold for performance of the biomarkers may differ based on the absolute and/or perceived cost and risk of the proposed interventions. A higher rate of FP may be acceptable for lower risk treatment adjustments such as optimization of dose of mesalamine, addition of topical therapy, or a brief course of steroids in individuals at low-risk for adverse effects. On the other hand, it is reasonable to accept lower FP rates for interventions that may be associated with significant cost (dose escalation of biologic therapy) or risk (change in therapy).
    - ***Application of all biomarkers in clinical practice should be guided by downstream implications, including risk of consequent treatment decisions (low-risk treatment adjustment vs. high-risk treatment adjustment). Test performance thresholds (acceptable false positive and false negative rates) may vary for patient-provider teams depending on what treatment adjustment is being considered.***

6. **Inter- and intra-assay test variability:** Variation in fecal calprotectin levels have been documented between different assays tested on the same stool sample.[34] However, the equivalence or interchangeability of calprotectin assays has not been thoroughly evaluated. Five studies directly comparing different assays identified discrepancies ranging from 2.5- to 5-fold differences between assays when each tested the same stool sample. However, most of this variability occurred at the higher end of the calprotectin range, and was reduced at the lower range of calprotectin values (which are currently proposed in this guideline).[35, 36] One study found only 8-9% variation within stool samples at lower calprotectin levels, compared to 18-33%

variation at higher calprotectin levels. Several studies also found differences ranging from 13 to 114%, using the same assay repeatedly with different stool samples from the same patient.[36-38] Other studies have found variation in results testing different regions of the same stool sample, ranging from 3-31%.[36, 38] No similar studies of variability or reproducibility of lactoferrin assays have been published.

- ***Fecal calprotectin assays may not be interchangeable and the same assay should be used for a given patient to compare results over time. Since there can be substantial within-stool, and within-day variation of fecal calprotectin measurements from a single patient, confidence in any single measurement may be limited. Hence, if there is uncertainty of results (such as borderline or unexpected results), repeat fecal calprotectin testing or endoscopic evaluation for confirmation may be required.***

7. **Inter-individual heterogeneity in biomarker responsiveness:** In addition to the accuracy and performance of the biomarker itself as a surrogate for disease activity, there is heterogeneity in the performance of the biomarker for a given patient. Among the included biomarkers, this is best exemplified for CRP.[39] In large genetic studies, the fraction of heritability attributed to CRP have been between 25-40%. In a study of 250 healthy army recruits, two polymorphisms in the CRP gene (-717G>A and +1444C>T) influenced both baseline CRP levels as well as elevation in response to vigorous exercise .[40] In an IBD cohort, patients with -717 wild-type (WT) had higher high-sensitivity CRP concentrations than those with non-WT.[41] The prevalence of non-WT status at the -717 and +1444 locations are estimated to be 10-15% with an additional 30-35% having heterozygosity at these loci. In a study of 199 subjects with active Crohn's disease, other variants in the CRP gene were also associated with lower degree of CRP elevation in the presence of specific variants.[42] Thus, it is plausible that in patients with these CRP gene variants, the performance of CRP as a biomarker may be less reliable. Therefore, in conjunction with their cross-sectional use, it is important to consider the longitudinal history of CRP elevation within each patient, focusing on their use in those individuals who have previously demonstrated a robust elevation in active inflammation. Similarly, the elevation of fecal calprotectin

and lactoferrin may be most accurate in those who have previously demonstrated an elevation. However, it is important to state that studies that have examined the performance of these biomarkers have done so in unselected cohorts, agnostic of inter-individual heterogeneity in potential for elevation of these markers. Thus, the performance of these markers as established in these guidelines remain broadly applicable across populations. The performance may indeed be better in patients where a correlation has been established between biomarkers and endoscopic activity.

- ***There are inter-individual differences in biomarker elevation in patients with intestinal inflammation, and in a subset of patients, biomarkers may correlate poorly with endoscopic activity. The overall performance and confidence in the use of biomarkers for treatment decisions in a particular patient may be higher when these biomarkers have been longitudinally observed to correlate with the patient's endoscopic disease activity (both active disease and remission).***

## GUIDELINE RECOMMENDATIONS

### PATIENTS WITH ULCERATIVE COLITIS IN SYMPTOMATIC REMISSION

**Question 1. In patients with UC in symptomatic remission, is interval biomarker-based monitoring superior to symptom-based monitoring to improve long-term outcomes?**

**Recommendation:**

***#1 In patients with UC in symptomatic remission, the AGA suggests a monitoring strategy that combines biomarkers and symptoms, rather than symptoms alone (Conditional recommendation, moderate certainty in evidence)***

**Remark:** Patients who place high value on avoiding the burden of biomarker testing, over a potentially higher risk of flare or overtreatment, may reasonably choose interval symptom-based monitoring

**Implementation considerations:**

a. Fecal biomarkers (fecal calprotectin or fecal lactoferrin) may be optimal for monitoring and may be particularly useful in patients where biomarkers have historically correlated with endoscopic disease activity.
b. A biomarker-based monitoring strategy, especially using stool-based tests however, may be inconvenient and elevated biomarkers in otherwise asymptomatic individuals may lead to high patient anxiety. The diagnostic performance of these tests in a low pre-test probability setting is suboptimal resulting in unacceptably high rates of false results.
c. It is important to think about the downstream consequences of testing and associated costs. The optimal management strategy in cases of discrepancy between symptoms and biomarkers is unclear and would generally trigger additional endoscopic testing for confirmation or repeat biomarker testing.
d. Interval biomarker monitoring may be performed every 6-12 months.

**Summary of the evidence:**

A biomarker-based monitoring strategy involves routine assessment of symptoms along with non-invasive biomarkers of inflammation to inform ongoing management in patients with UC in symptomatic remission. **eFigure 3** lays out the schematic for the proposed comparison. We did not identify any RCTs that directly compared a biomarker-based monitoring strategy vs. a symptom-based monitoring strategy. Only one RCT examined the impact of mesalamine dose escalation on reducing fecal calprotectin in patients with quiescent UC.[43] Of 52 patients with mild UC in symptomatic remission with fecal calprotectin >50μg/g, 26 patients were randomized to increasing mesalamine dose by 2.4g/d for 6 weeks vs. 26 patients who continued on a stable dose of mesalamine. In this trial, the primary endpoint of continued clinical remission with normalization of fecal calprotectin (<50μg/g) by week 6, was more likely to be achieved in those randomized to escalation of mesalamine (27% vs. 4%). However, there were no differences in time to clinical relapse by week 48. This trial did not adequately inform the focused question, given limited duration of intervention (only 6 weeks), and limited information on ongoing monitoring and optimization.

We subsequently examined cohort studies in patients with UC in symptomatic remission in which patients underwent biomarker testing, and long-term outcomes were compared between those with elevated biomarkers vs. normal biomarkers. We posited that if long-term outcomes are significantly different in patients with elevated biomarkers vs. those with normal biomarkers, then an interval biomarker-based monitoring in asymptomatic patients may inform prognosis and long-term management. We identified 17 cohort studies with 1286 patients with UC in symptomatic remission (**eFigure 4**). In these studies, fecal calprotectin was the preferred biomarker used for monitoring; 36% patients were classified as having elevated fecal calprotectin (usually >150μg/g), and 64% had normal fecal calprotectin. On median follow-up of 1 year, patients with elevated fecal calprotectin were 4.4 times more likely to have disease relapse compared with patients with normal fecal calprotectin (95% CI, 3.48-5.47), with low heterogeneity ($I^2$=24%). With an observed median annual risk of relapse of 15% in patients with UC in symptomatic remission and normal fecal calprotectin in these cohorts, estimated annual risk of relapse in patients with quiescent UC and elevated fecal calprotectin was 64% (**Table 4**).

**Benefits and Harms (Downsides)**

*Symptom-based monitoring strategy*: The potential benefit of a symptom-based monitoring strategy is the convenience of relying only on patient-reported outcomes which can be readily ascertained. However, harms related to a symptom-based monitoring strategy are higher rates of false reassurance and higher risk of disease-related complications (in patients with symptomatic remission but elevated biomarkers who are at higher risk of relapse).

*Biomarker-based monitoring strategy*: Potential benefits of a biomarker-based monitoring strategy include more accurate prognostication than symptoms alone, to facilitate optimal treatment decisions and lower the risk of disease complications. Potential harms of a biomarker-based monitoring strategy include the costs and inconvenience of sample collection, particularly stool-based tests. Additionally, elevated biomarkers in otherwise asymptomatic patients may lead to higher patient anxiety, and with high FP rates in this scenario (see question #2), would require follow-up invasive procedures or repeat biomarker testing.

**Certainty of Evidence**

When examining cohort studies comparing long-term outcomes in patients with UC in symptomatic remission with elevated vs. normal biomarkers, there was moderate confidence in effect estimates supporting the use of a biomarker-based monitoring strategy over a symptom-based monitoring strategy. Evidence was rated down for risk of bias and variability in cut-offs of fecal calprotectin (**Table 4**). There was limited data on prognostic value of other biomarkers like fecal lactoferrin and serum CRP in patients with asymptomatic UC.

**Rationale**

Using the GRADE Evidence-to-Decision framework, incorporating the potential benefits and downsides of the two strategies, and considerations of resource utilization, acceptability, feasibility and equity, the guideline panel conditionally recommended in favor of a biomarker-based monitoring strategy compared with a symptom-based monitoring strategy. Some patients, who place higher value on avoiding the burden of biomarker testing, over a potentially high risk of flares, may reasonably choose interval

symptom-based monitoring. Cost-effectiveness analyses have suggested that a symptom-based monitoring may be most cost-effective approach to implement treat to target monitoring for patients with UC receiving biologics and small molecule inhibitors.[44]

Several other factors need to be considered when deciding appropriate monitoring strategies. Most of the data on predicting risk of relapse in patients with UC in symptomatic UC was based on fecal calprotectin; as noted below, the diagnostic performance (particularly the sensitivity) of serum CRP is lower, and hence, the prognostic performance of normal CRP in patients with UC in symptomatic remission may not be as informative. Optimal management strategy in case of discrepancy between symptoms and biomarkers is unclear – the diagnostic performance of these tests in a low pre-test probability setting is suboptimal resulting in unacceptably high rates of FP or FN – and would generally trigger additional endoscopic testing for confirmation. The guideline panel felt that in patients with UC in symptomatic remission with elevated biomarkers, repeat biomarker testing may be a reasonable alternative.

**Question 2. In patients with UC in symptomatic remission, at what (A) fecal calprotectin, (B) fecal lactoferrin, and (C) serum C-reactive protein cut-off can we accurately rule out active inflammation, obviating routine endoscopic assessment?**

**Recommendations:**

***#2 In patients with UC in symptomatic remission, the AGA suggests using fecal calprotectin <150μg/g, normal fecal lactoferrin or normal CRP to rule out active inflammation and avoid routine endoscopic assessment of disease activity (Conditional recommendation, very low to low certainty in evidence)***

**Implementation considerations:**

- In patients who have recently achieved symptomatic remission after treatment adjustment in the preceding 1-3 months, fecal calprotectin <50μg/g may be preferred over <150μg/g to detect endoscopic improvement (MES 0 or 1)
- Normal CRP may be less informative to rule out moderate-to-severe active endoscopic inflammation in patients with UC in symptomatic remission, particularly in patients who have recently achieved symptomatic remission after treatment adjustment. However, if the CRP was elevated at time of initial flare, then normalization of CRP may suggest endoscopic improvement (MES 0 or 1)

***#3 In patients with UC in symptomatic remission but elevated stool or serum markers of inflammation (fecal calprotectin >150 μg/g, elevated fecal lactoferrin, elevated CRP), the AGA suggests endoscopic assessment of disease activity rather than empiric treatment adjustment (Conditional recommendation, very low certainty in evidence)***

**Implementation consideration:**

- In patients with UC in symptomatic remission but elevated biomarkers of inflammation, repeat measurement of biomarkers (in 3-6 months) may be a reasonable alternative to endoscopic assessment. However, if biomarkers are elevated on repeat evaluation, then endoscopic assessment may be warranted.

***#4 In patients with UC with mild symptoms, with normal stool or serum markers of inflammation (fecal calprotectin <150 μg/g, normal fecal lactoferrin, normal CRP), the AGA suggests endoscopic assessment of disease activity rather than empiric treatment adjustment (Conditional recommendation, very low certainty in evidence)***

**Implementation consideration:**

- In patients with UC with mild symptoms (for example, slight increase in stool frequency and/or infrequent rectal bleeding), it may be reasonable to proceed directly with endoscopic assessment rather than testing biomarkers of inflammation.
- In patients with UC with mild symptoms and normal biomarkers of inflammation who prefer to avoid endoscopic assessment or empiric treatment escalation, repeat measurement of biomarkers (in 3-6 months) may be a reasonable alternative.

**Summary of the evidence**

1. *In patients with UC in symptomatic remission (no rectal bleeding, normal or near normal stool frequency) fecal calprotectin <150±50μg/g and normal fecal lactoferrin reliably rules out active inflammation, obviating endoscopic assessment (low certainty of evidence); normal serum CRP may rule out active inflammation (very low certainty of evidence)*
2. *In patients with UC in symptomatic remission, elevated fecal calprotectin >150±50μg/g, elevated fecal lactoferrin or elevated CRP may not indicate active inflammation (very low certainty of evidence).*
3. *In patients with UC who have mild symptoms (infrequent rectal bleeding and/or increased stool frequency), fecal calprotectin <150±50μg/g, normal fecal lactoferrin or normal CRP cannot rule out active inflammation (very low certainty of evidence).*

***Diagnostic performance of fecal calprotectin*:**

The evidence synthesis team decided *a priori* to examine three diagnostic cut-offs for fecal calprotectin most frequently studied and used in clinical practice – 50μg/g, 150μg/g and 250μg/g. To account for variability in reported cut-offs in studies, we allowed for values 50μg/g above and below the cut-off. In the diagnostic testing spectrum, lower cut-offs are more sensitive, and higher cut-offs are more specific. We conducted a systematic review to identify cross-sectional and cohort studies in patients with established UC which reported the diagnostic accuracy of fecal calprotectin for detecting moderate to severe endoscopic inflammation (MES 2 or 3). From these studies, to minimize bias due to selective reporting of optimized cut-offs (as is common in diagnostic accuracy studies), we included only studies which reported diagnostic accuracy of pre-selected fecal calprotectin cut-offs or reported the performance across two or more pre-determined cut-offs. Using this approach, the sensitivity and specificity of fecal calprotectin cut-off of 50±50μg/g was 78% (95% CI, 66-86) and 57% (95% CI, 40-72), respectively, based on 11 cohorts; corresponding sensitivity and specificity of 150±50μg/g cut-off (12 cohorts) was 71% (95% CI, 62-78) and 69% (95% CI, 62-75), respectively, and of 250±50μg/g cut-off (9 cohorts) was 67% (95% CI, 53-78) and 73% (95% CI, 65-80), respectively (**eFigure 5)**.

Low pre-test probability (asymptomatic patients with RBS 0 and SFS 0 or 1, with 15% prevalence of moderate to severe inflammation): In applying these cut-offs to a low pre-test probability scenario, approximately 3.3%, 4.3% and 5.5% patients (FN rate) with fecal calprotectin <50μg/g, <150μg/g and <250μg/g, respectively, may be misclassified as having endoscopic improvement (MES 0 or 1) when they actually have moderate to severe endoscopic activity (MES 2 or 3) (**Table 5A**). In contrast, elevated fecal calprotectin >50μg/g, >150μg/g and >250μg/g in this low pre-test probability scenario, had significantly high rates of being FP (36.6%, 26.4% and 23%, respectively), i.e., a significant proportion of patients who have endoscopic improvement (MES 0 or 1) may be incorrectly classified as having moderate to severe endoscopic activity.

Intermediate Pre-test Probability (patients with mild symptoms of UC, such as infrequent rectal bleeding [RBS 1], or increased stool frequency [SFS 2 or 3], with 50% prevalence of moderate to severe inflammation): In an intermediate pre-test probability scenario, approximately 11%, 14.5% and 18.5% patients (FN rate) with fecal calprotectin <50μg/g, <150μg/g and <250μg/g, respectively, may be misclassified as having endoscopic improvement (MES 0 or 1) when they actually have moderate to severe endoscopic activity (MES 2 or 3) (**Table 5A**). In contrast, elevated fecal calprotectin >50μg/g, >150μg/g and >250μg/g in this intermediate pre-test probability scenario, had significantly high rates of being FP (21.5%, 15.5% and 13.5%, respectively), i.e., a significant proportion of patients who have endoscopic improvement may be incorrectly classified as having moderate to severe endoscopic activity.

***Diagnostic performance of fecal lactoferrin*:** The evidence base for fecal lactoferrin was more limited. We identified 9 studies reporting the diagnostic accuracy of fecal lactoferrin for detecting moderate to severe endoscopic inflammation (defined as MES 2 or 3 in 4 studies, and MES 1, 2 or 3 in 5 studies). Studies reported performance of only a single lactoferrin cut-off within a range of 7.25-10μg/g – the commercial assay reports lactoferrin as positive (elevated) or negative, corresponding to a cut-off of 7.25μg/g. At this cut-off, the sensitivity and specificity of fecal lactoferrin for detecting endoscopic inflammation was 83% (95% CI, 72-90) and 75% (95% CI, 59-87), respectively (**eFigure 6**).

Low pre-test probability (asymptomatic patients with RBS 0 and SFS 0 or 1, with 15% prevalence of moderate to severe inflammation): In applying this cut-off to a low pre-test probability scenario, approximately 2.6% patients (FN rate) with normal fecal lactoferrin (<7.25μg/g) may be misclassified as having endoscopic improvement (MES 0 or 1) when they actually have moderate to severe endoscopic activity (MES 2 or 3) (**Table 5B**). In contrast, elevated fecal lactoferrin (>7.25μg/g), in this low pre-test probability scenario, had significantly high rates of being FP (21.2%), i.e., 21.2% patients who have endoscopic improvement may be incorrectly classified as having moderate to severe endoscopic activity.

Intermediate pre-test probability (patients with mild symptoms of UC, such as infrequent rectal bleeding [RBS 1], or increased stool frequency [SFS 2 or 3], with 50% prevalence of moderate to severe inflammation): In an intermediate pre-test probability scenario, (approximately 8.5% patients (FN rate) with fecal lactoferrin <7.25μg/g may be misclassified as having endoscopic improvement when they actually have moderate to severe endoscopic activity (**Table 5B**). In contrast, elevated fecal lactoferrin (>7.25μg/g), in this intermediate pre-test probability scenario, had significantly high rates of being FP (12.5%).

***Diagnostic performance of serum CRP*:** We identified 15 studies reporting the diagnostic accuracy of serum CRP for detecting moderate to severe endoscopic inflammation. Studies reported performance of only a single CRP cut-off with a range of 1.2-7.3 mg/L. Summary sensitivity and specificity of elevated CRP for detecting endoscopic inflammation was 63% (95% CI, 50-75) and 77% (95% CI, 67-84) (**eFigure 7**).

Low pre-test probability (asymptomatic patients with RBS 0 and SFS 0 or 1, with 15% prevalence of moderate to severe inflammation): In applying this cut-off (elevated CRP, generally >5mg/L) to a low pre-test probability scenario, approximately 5.5% patients (FN rate) with normal CRP (<5mg/L) may be misclassified as having endoscopic improvement when they actually have moderate to severe endoscopic activity (**Table 5C**). In contrast, elevated CRP (>5mg/L), in this low pre-test probability scenario, had significantly high rates of being FP (19.5%), i.e., 19.5% patients who have endoscopic

improvement may be incorrectly classified as having moderate to severe endoscopic activity.

Intermediate pre-test probability (patients with mild symptoms of UC, such as infrequent rectal bleeding [RBS 1], or increased stool frequency [SFS 2 or 3], with 50% prevalence of moderate to severe inflammation): In an intermediate pre-test probability scenario, approximately 18.5% patients (FN rate) with normal CRP (<5mg/L) may be misclassified as having endoscopic improvement when they actually have moderate to severe endoscopic remission (MES 2 or 3) (**Table 5C**). In contrast, elevated CRP (>5mg/L), in this intermediate pre-test probability scenario, had significantly high rates of being false positive (11.5%).

**Certainty of Evidence**

There was no direct evidence comparing how different biomarker cut-offs and accompanying treatment decisions impact downstream patient-important outcomes, however, we did not rate down for indirectness since the presence of moderate to severe endoscopic activity is a close surrogate for unfavorable patient outcomes, and an indication for treatment adjustment.

***Fecal calprotectin:*** There was low certainty of evidence supporting the use of fecal calprotectin cut-offs of <50μg/g and <150μg/g to rule out moderate to severe endoscopic inflammation in a low pre-test probability setting, and very low certainty of evidence supporting the use of fecal calprotectin cut-offs of <250μg/g in this scenario (**Table 5A**). Evidence was rated down for inconsistency due to selective inclusion of studies reporting specific cut-offs and high heterogeneity for summary sensitivity/specificity, and for imprecision since 95% CI of the maximal tolerable FN rate was 5%; evidence for the cut-off of <250μg/g was further rated down for very serious imprecision since the point estimate is higher than the maximal tolerable FN rate.

In contrast, in the intermediate probability scenario, there was very low certainty of evidence supporting the use of any proposed fecal calprotectin cut-off to rule out moderate to severe endoscopic inflammation, due to unacceptably high rates of FN (very serious imprecision, since the point estimate crossed the FN threshold of 5%) and selective inclusion of studies and heterogeneity in summary sensitivity/specificity

(inconsistency) (**Table 5A**). Similarly, in the low and intermediate probability scenario, there was very low certainty of evidence supporting the use of any proposed cut-off of elevated fecal calprotectin to rule in moderate to severe endoscopic inflammation, due to unacceptably high rates of FP (very serious imprecision) and inconsistency.

***Fecal lactoferrin:*** There was low certainty of evidence supporting the use of normal fecal lactoferrin to rule out moderate to severe endoscopic inflammation in a low pre-test probability setting (**Table 5B**). Evidence was rated down for very serious inconsistency due to selective reporting of cut-offs in studies optimized for best performance and high heterogeneity for summary sensitivity/specificity. In contrast, in the intermediate probability scenario, there was very low certainty of evidence supporting the use of normal fecal lactoferrin to rule out moderate to severe endoscopic inflammation, due to unacceptably high rates of FN (very serious imprecision) and inconsistency. Similarly, in the low and intermediate probability scenario, there was very low certainty of evidence supporting the use of elevated fecal lactoferrin to rule in moderate to severe endoscopic inflammation, due to unacceptably high rates of FP (very serious imprecision) and inconsistency (**Table 5B**).

***Serum CRP:*** There was very low certainty of evidence supporting the use of normal CRP to rule out moderate to severe endoscopic inflammation in a low pre-test probability setting (**Table 5C**). Evidence was rated down for inconsistency due to selective reporting of cut-offs in studies optimized for best performance and high heterogeneity for summary sensitivity/specificity, and for very serious imprecision since the point estimate is higher than the maximal tolerable FN rate. Similarly, in the intermediate probability scenario, there was very low certainty of evidence supporting the use of normal serum CRP to rule out moderate to severe endoscopic inflammation, due to unacceptably high rates of FN (very serious imprecision) and inconsistency (**Table 5C**). In the low and intermediate probability scenario, there was very low certainty of evidence supporting the use of elevated CRP to rule in moderate to severe endoscopic inflammation, due to unacceptably high rates of FP (very serious imprecision) and inconsistency.

**Rationale**

In using non-invasive biomarkers as a triage strategy to determine need for endoscopy and ongoing management, healthcare providers and patients need to be aware of test performance and the downstream consequences of potential FN and FP rates. The guideline panel and evidence synthesis team determined *a priori* a maximal tolerable FN threshold of 5% for patients with UC in symptomatic remission. However, the team deemed that there may be circumstances where patients and providers may be willing to accept higher rates of FN, depending on risk of downstream consequences, particularly the nature of treatment adjustment, and emphasize the importance of shared decision-making.

For ease of implementation in clinical practice, the guideline panel felt choosing a single fecal calprotectin cut-off (<150μg/g) that is broadly applicable across a wide range of clinical scenarios is preferable, rather than reporting multiple different cut-offs for different scenarios. There may be circumstances, such as patients who may have recently achieved symptomatic remission after treatment adjustment in the preceding 1-3 months, a lower fecal calprotectin <50μg/g may be more accurate than <150μg/g to rule out the presence of moderate-to-severe active inflammation. It is important to note that in children 2-years of age or younger, a higher threshold for fecal calprotectin may be needed due to wider range of normal calprotectin in young children.[45, 46]

The guideline panel did not compare the performance of different non-invasive biomarkers, due to variable cut-offs for each test. Stool-based tests may be more sensitive for intestinal inflammation, compared with serum CRP; however, CRP has the convenience of being a blood test. The panel noted that fecal calprotectin has been well-studied and was able to study the performance of different cut-offs to adequately ascertain performance. In contrast, fecal lactoferrin had a limited evidence base with limited studies on different cut-offs. Similarly, CRP had a limited evidence base despite being very commonly measured in clinical practice. There may be circumstances where the performance of CRP may be suboptimal. For example, in patients who have recently achieved symptomatic remission after treatment adjustment, normal CRP may be less informative, exceeding the FN threshold. However, if the CRP was elevated at time of initial flare, then normalization of CRP may suggest endoscopic improvement. The panel did not study proprietary tests which are not widely available or indicated for use in UC.

## PATIENTS WITH SYMPTOMATICALLY ACTIVE ULCERATIVE COLITIS

**Question 3. In patients with symptomatically active UC, is an evaluation strategy that combines biomarkers and symptoms superior to symptom-based evaluation for making treatment adjustments?**

**Recommendation:**

***#5 In patients with symptomatically active UC, the AGA suggests an evaluation strategy that combines biomarkers and symptoms, rather than symptoms alone to inform treatment adjustments (Conditional recommendation, low certainty in evidence)***

**Remark:** Patients, particularly those with severe symptoms, who place a high value on avoiding the burden of biomarker testing, over a potentially higher risk of inappropriate overtreatment, may reasonably choose symptom-based evaluation for treatment decisions.

**Summary of the Evidence:**

A biomarker-based evaluation strategy involves checking non-invasive biomarkers of inflammation in patients with symptomatically active UC, to inform ongoing management; in contrast, symptom-based evaluation would involve treatment decisions being driven solely based on symptoms. We did not identify any RCTs that directly compared a biomarker-based evaluation strategy vs. symptom-based evaluation for patients with symptomatically active UC. Recognizing that presence of moderate to severe endoscopic inflammation, in conjunction with symptoms, is a key trigger for treatment decisions, we used ability of symptoms alone vs. symptoms + biomarkers to detect presence of moderate to severe inflammation on endoscopy as a surrogate outcome to inform decision-making and improve patient outcomes. In a prior pooled analysis of 6 clinical trials of biologic agents and tofacitinib in 2,586 patients with moderate to severely active UC, authors examined the cross-sectional prevalence of moderate to severe endoscopic inflammation (based on MES 2 or 3) in patients with varying combinations of cardinal symptoms of UC (RBS and SFS components of Mayo clinic score).[21] In this analysis, 85-

90% patients with RBS 2 or 3 and SFS 2 or 3 had moderate to severe inflammation on endoscopy (**eTable 1**). This suggests a false positive rate of 10-15%, i.e., 10-15% patients with typical symptoms suggestive of active UC may be in endoscopic remission or have only mildly active disease, such that relying on symptoms alone may lead to potentially unnecessary treatment adjustments (such as adding corticosteroids, escalating or switching therapies). As shown in subsequent analyses (please see question 4 below), in patients with typical symptoms suggestive of active UC (RBS 2 or 3 and SFS 2 or 3), presence of elevated biomarkers of inflammation decreases the FP rate to <5%; i.e., less than 5% patients with symptoms and elevated biomarkers will actually have only mild inflammation or be in endoscopic remission, resulting in acceptably low rates of unnecessary treatment adjustments.

**Benefits and Harms (Downsides)**

*Symptom-based evaluation strategy*: Potential benefit of a symptom-based monitoring strategy is the convenience of relying only on patient-reported outcomes, and faster decision-making. However, harms related to relying only on symptoms are higher rates of inappropriate treatment adjustments/overtreatment and treatment-related complications (in case of 10-15**%** patients with symptoms suggestive of UC but who may be in endoscopic remission or have only mildly active disease).

*Biomarker-based evaluation strategy*: Potential benefits of a biomarker-based evaluation strategy is more accurate prognostication than symptoms alone, to facilitate optimal treatment decisions and avoid overtreatment. Potential harms of a biomarker-based evaluation strategy are the costs and inconvenience of sample collection, particularly stool-based tests, and potential delays in treatment which happen due to the extra step of test completion.

**Certainty of evidence**

In the absence of randomized trials, we relied on cross-sectional studies, with indirect comparisons and surrogate outcome (presence of moderate to severe endoscopic inflammation), with somewhat imprecise estimates with a variety of biomarkers. Hence, there was low confidence in effect estimates supporting a biomarker-based evaluation strategy over symptom-based evaluation in patients with UC with active symptoms.

**Rationale**

Using the GRADE Evidence-to-Decision framework, the guideline panel conditionally recommended in favor of a strategy that combines biomarkers and symptoms compared with a symptom-based evaluation alone in patients with symptomatically active UC. The panel recognized that adding an extra step of biomarker testing in patients with symptomatically active UC may potentially delay treatment for patients, particularly those with limited access to healthcare resources. The panel recognized the value of shared decision-making in these patients; some patients, particularly those with severe symptoms, who place high value in avoiding burden of biomarker testing, may reasonably choose symptom-based evaluation for treatment decisions, acknowledging potentially higher risk of inappropriate overtreatment with symptom-based evaluation alone. This may be particularly true if treatment decisions are considered low risk by the treating provider-patient team.

Optimal management strategy in case of discrepancy between symptoms and biomarkers is unclear. In patients with typical symptoms suggestive of UC, normal biomarkers may not exclude lack of moderate to severe inflammation, and endoscopic assessment may be a preferred approach. However, in a subset of patients, non-invasive biomarkers do not correlate with endoscopic inflammation. In this setting, shared decision-making on empiric treatment adjustment with a 10-15% rate false positivity of symptoms alone may be acceptable, particularly when access to endoscopy is limited (which may lead to delay in treatment initiation) and treatment adjustments being considered are low risk.

**Question 4. In patients with symptomatically active UC, at what (A) fecal calprotectin, (B) fecal lactoferrin and (C) serum C-reactive protein cut-off can we accurately diagnose active inflammation, obviating routine endoscopic assessment?**

**Recommendations:**

***#6 In patients with UC with moderate to severe symptoms suggestive of flare, the AGA suggests using fecal calprotectin >150μg/g, elevated fecal lactoferrin or elevated CRP to rule in active inflammation and inform treatment adjustment and avoid endoscopic assessment solely for establishing presence of active disease (Conditional recommendation, very low to moderate certainty in evidence)***

**Remark:** Patients who place greater value in confirming inflammation, particularly when making significant treatment adjustments (such as starting or switching immunosuppressive therapies) and lesser value on the inconvenience, cost, or risk of endoscopy, may choose to pursue endoscopic evaluation prior to treatment adjustment.

***#7 In patients with UC with mild symptoms, with elevated stool or serum markers of inflammation (fecal calprotectin >150μg/g, elevated fecal lactoferrin or elevated CRP), the AGA suggests endoscopic assessment of disease activity rather than empiric treatment adjustment (Conditional recommendation, very low certainty in evidence)***

**Implementation consideration:**

- In patients with UC who underwent recent adjustment of treatment in response to moderate to severe symptomatic flare, and now have mild residual symptoms, elevated stool or serum markers of inflammation may be used to inform treatment adjustments (such as dose adjustments of therapy)

**Summary of the evidence**

1. *In patients with UC with moderate to severe symptoms suggestive of flare (frequent rectal bleeding, significantly increased stool frequency), fecal calprotectin >150μg/g, elevated fecal lactoferrin and elevated CRP reliably suggest moderate to severe endoscopic inflammation, obviating routine need for endoscopic assessment (very low to moderate certainty of evidence)*

2. *In patients with UC who have mild symptoms (infrequent rectal bleeding and/or increased stool frequency), fecal calprotectin >150μg/g, elevated fecal lactoferrin and elevated CRP may not suggest moderate to severe endoscopic inflammation (very low certainty of evidence).*

3. *In patients with UC with moderate to severe symptoms suggestive of flare (frequent rectal bleeding, significantly increased stool frequency), fecal calprotectin <150μg/g, normal fecal lactoferrin or normal CRP may not suggest lack of inflammation (very low certainty of evidence).*

***Diagnostic performance of fecal calprotectin*:**

Summary sensitivity and specificity of fecal calprotectin for detecting moderate to severe endoscopic inflammation has been reported above in question 2.

*High pre-test probability scenario* (patients with typical symptoms of UC flare with frequent rectal bleeding [RBS 2 or 3] and significant increase in stool frequency [SFS 2 or 3], with 85% prevalence of moderate to severe inflammation): In applying these cut-offs in high pre-test probability scenarios, approximately 6.4%, 4.6% and 4.0% patients (FP rate) with fecal calprotectin >50μg/g, >150μg/g and >250μg/g, respectively, may be misclassified as having moderate to severe endoscopic activity (MES 2 or 3) when they actually have endoscopic improvement (MES 0 or 1) (**Table 6A**). In contrast, fecal calprotectin <50μg/g, <150μg/g and <250μg/g in this high pre-test probability scenario, had significantly high rates of being FN (18.7%, 24.7% and 31.4%, respectively), i.e., a significant proportion of symptomatic patients who have moderate to severe endoscopic activity may be incorrectly classified as having endoscopic improvement.

*Intermediate pre-test probability scenario* (patients with mild symptoms of UC, such as infrequent rectal bleeding [RBS 1], or increased stool frequency [SFS 2 or 3], with 50% prevalence of moderate to severe inflammation): In an intermediate pre-test

probability scenario, approximately 21.5%, 15.5% and 13.5% patients (FP rate) with fecal calprotectin >50μg/g, >150μg/g and >250μg/g, respectively, may be misclassified as having moderate to severe endoscopic activity (MES 2 or 3) when they actually have endoscopic improvement (MES 0 or 1) (**Table 6A**). In contrast, fecal calprotectin <50μg/g, <150μg/g and <250μg/g in this high pre-test probability scenario, had high rates of being FN (11.0%, 14.5% and 18.5%, respectively), i.e., a significant proportion of symptomatic patients who have moderate to severe endoscopic activity may be incorrectly classified as having endoscopic improvement.

***Diagnostic performance of fecal lactoferrin*:** Summary sensitivity and specificity of fecal lactoferrin for detecting moderate to severe endoscopic inflammation has been reported above in question 2.

*High pre-test probability scenario* (patients with typical symptoms of UC flare with RBS 2 or 3 and SFS 2 or 3, with 85% prevalence of moderate to severe inflammation): In applying this cut-off to a high pre-test probability scenario, approximately 3.7% patients (FP rate) with elevated fecal lactoferrin (>7.25μg/g) may be misclassified as having moderate to severe endoscopic activity (MES 2 or 3) (**Table 6B**). In contrast, normal fecal lactoferrin (<7.25μg/g), had significantly high rates of being FN (14.5%), i.e., 14.5% patients who have moderate to severe endoscopic activity are classified as being in endoscopic improvement.

*Intermediate pre-test probability scenario* (patients with mild symptoms of UC, RBS 1, or SFS 2 or 3, with 50% prevalence of moderate to severe inflammation): In an intermediate pre-test probability scenario, FP rate of elevated fecal lactoferrin was 12.5%, and FN rate of normal fecal lactoferrin was 8.5% (**Table 6B**).

***Diagnostic performance of serum CRP*:** Summary sensitivity and specificity of serum CRP for detecting moderate to severe endoscopic inflammation has been reported above in question 2.

*High pre-test probability scenario* (patients with typical symptoms of UC flare with RBS 2 or 3 and SFS 2 or 3, with 85% prevalence of moderate to severe inflammation): In applying this cut-off (elevated CRP, generally >5mg/L) to a high pre-test probability scenario, approximately 3.4% patients (FP rate) with elevated CRP (>5mg/L) may be

misclassified as having moderate to severe endoscopic activity (MES 2 or 3) (**Table 6C**). In contrast, normal CRP (<5mg/L) had significantly high rates of being FN (31.4%).

*Intermediate pre-test probability scenario* (patients with mild symptoms of UC, RBS 1, or SFS 2 or 3, with 50% prevalence of moderate to severe inflammation): In an intermediate pre-test probability scenario, FP rate of elevated CRP was 11.5%, and FN rate of normal CRP was 18.5% (**Table 6C**).

**Certainty of Evidence**

Even though there was no direct data comparing how different biomarker cut-offs and accompanying treatment decisions impact downstream patient-important outcomes, we did not rate down for indirectness since the presence of moderate to severe endoscopic activity is a close surrogate for unfavorable patient outcomes, and an indication for treatment adjustment.

***Fecal calprotectin:*** There was low certainty of evidence supporting the use of fecal calprotectin cut-offs of >150μg/g and >250μg/g to rule in moderate to severe endoscopic inflammation in a high pre-test probability setting (evidence rated down for inconsistency and imprecision), and very low certainty of evidence supporting the use of fecal calprotectin cut-offs of >50μg/g in this scenario (evidence rated down for inconsistency and very serious imprecision) (**Table 6A**).

In contrast, in the intermediate probability scenario, there was very low certainty of evidence supporting the use of any proposed fecal calprotectin cut-off to rule in moderate to severe endoscopic inflammation, due to unacceptably high rates of FP (very serious imprecision) and selective inclusion of studies and heterogeneity in summary sensitivity/specificity (inconsistency) (**Table 6A**). Similarly, in the high and intermediate probability scenario, there was very low certainty of evidence supporting the use of any proposed cut-off of normal fecal calprotectin to rule out moderate to severe endoscopic inflammation, due to unacceptably high rates of FN (very serious imprecision) and inconsistency.

***Fecal lactoferrin:*** There was very low certainty of evidence supporting the use of elevated fecal lactoferrin to rule in moderate to severe endoscopic inflammation in a high pre-test probability setting (**Table 6B**). Evidence was rated down for very serious inconsistency due to selective reporting of cut-offs in studies optimized for best

performance and high heterogeneity for summary sensitivity/specificity, and for imprecision (upper limit of 95% CI crossing 5% FP threshold). In the intermediate probability scenario, there was very low certainty of evidence supporting the use of elevated fecal lactoferrin to rule in moderate to severe endoscopic inflammation, due to unacceptably high rates of FP (very serious imprecision) and very serious inconsistency. Similarly, in the high and intermediate probability scenario, there was very low certainty of evidence supporting the use of normal fecal lactoferrin to rule out moderate to severe endoscopic inflammation, due to unacceptably high rates of FN (very serious imprecision) and very serious inconsistency (**Table 6B**).

***Serum CRP:*** There was moderate certainty of evidence supporting the use of elevated CRP to rule in moderate to severe endoscopic inflammation in a high pre-test probability setting (**Table 6C**). Evidence was rated down for inconsistency due to selective reporting of cut-offs in studies optimized for best performance and high heterogeneity for summary sensitivity/specificity. In contrast, in the intermediate probability scenario, there was very low certainty of evidence supporting the use of elevated serum CRP to rule in moderate to severe endoscopic inflammation (inconsistency, very serious imprecision). Similarly, in the high and intermediate probability scenario, there was very low certainty of evidence supporting the use of normal CRP to rule out moderate to severe endoscopic inflammation, due to unacceptably high rates of FN (very serious imprecision) and inconsistency (**Table 6C**).

**Rationale**

The guideline panel and evidence synthesis team determined *a priori* the maximal tolerable FP thresholds at 5% for patients with symptomatically active UC. However, the guideline panel deemed there may be circumstances where patients and providers may be willing to accept higher rates of FP, depending on risk of downstream consequences, particularly the nature of treatment adjustment, and promoted shared decision-making with conditional recommendations. For example, in patients with typical symptoms suggestive of a flare with only modestly elevated fecal calprotectin, and there is delay in performing endoscopic assessment due to logistical issues, patients and providers may be willing initiate treatment despite test performance suggesting FP rates of >5%.

As noted earlier, for ease of implementation in clinical practice, the guideline panel felt choosing a single fecal calprotectin cut-off (>150μg/g) that is broadly applicable across a wide range of clinical scenarios is preferable, rather than reporting multiple different cut-offs for different scenarios. Higher fecal calprotectin cut-offs may have modestly lower rates of FP with modest improvement in confidence of decision-making. In patients with typical symptoms suggestive of flare, an elevated CRP had very good performance, at least comparable to fecal tests. While this may be convenient, it is important to note that stool testing to rule out *Clostridiodes difficile* and other enteric pathogens may still be required for all symptomatic patients.

**Question 4. In patients with established UC, is interval biomarker-based monitoring superior to endoscopy-based monitoring to improve long-term outcomes?**

**Recommendation:**

***#8 In patients with UC, the AGA makes no recommendation in favor of, or against, a biomarker-based monitoring strategy over an endoscopy-based monitoring strategy to improve long-term outcomes (No recommendation, knowledge gap)***

**Summary of the Evidence**

A biomarker-based monitoring strategy involves routine assessment of symptoms and non-invasive biomarkers of inflammation in patients with UC in symptomatic remission, to inform ongoing management. In this situation, normalization of biomarkers is an adequate treatment target – asymptomatic patients with normal biomarkers would continue current management without endoscopy, whereas those with elevated biomarkers would undergo endoscopy. In contrast, an endoscopy-based monitoring strategy involves routine endoscopic assessment to confirm achievement of endoscopic improvement (MES 0 or 1) or endoscopic remission (MES 0) target periodically. **eFigure 8** lays out the schematic for proposed comparison. We did not identify any RCTs that compared a biomarker-based monitoring strategy vs. an endoscopy-based monitoring strategy. Normalization of CRP and reduction of fecal calprotectin are recognized as

short-term treatment targets in managing UC in expert consensus statements, assessed early in treatment course. Early achievement of these biomarker outcomes is associated with favorable longer term outcomes including risk of relapse, as well as likelihood of achieving endoscopic improvement. However, the performance of these biomarkers in combination of symptoms may be more modest for detecting endoscopic remission (MES 0) and histologic remission, outcomes that have been associated with lower risk of clinical relapse compared with mild endoscopic activity (MES 1). Potential benefits of a biomarker-based monitoring strategy are convenience and low resource utilization due to avoidance of routine and recurrent endoscopic assessment. Potential harms of a biomarker-based monitoring strategy are insufficient assessment and suboptimal performance for achieving deeper remission endpoints such as complete endoscopic remission and histologic remission which may be associated with more favorable long-term outcomes. Hence, the guideline panel felt there is insufficient evidence to inform between the choice of a biomarker-based monitoring strategy vs. an endoscopy-based monitoring strategy in patients with UC in symptomatic remission. This was identified as a knowledge gap which warrants clinical trials.

**Limitations of Current Evidence and Future Directions**

The evidence panel identified numerous knowledge gaps in the literature where there is insufficient data to inform recommendations.

1. ***Timing of measuring biomarkers***: There were few studies that examined the accuracy and utility of serial measurements of serum or fecal biomarkers, particularly in settings where there was discordance between symptoms and biomarker values. As well, the optimal timing for this serial monitoring in either asymptomatic patients with UC or those with mild symptoms is unclear. In the post-induction setting under a treat to target paradigm, the optimal timing for measurement of biomarkers to inform treatment optimization has not been robustly established. In RCTs, biochemical response has been typically assessed 6-10 weeks after initiation of therapy. The STRIDE consensus statement provides optimal time intervals for assessment of clinical and endoscopic response to

treatment; whether serum or fecal biomarkers follow a similar trajectory or if there is benefit to earlier or more frequent assessment of biochemical response to guide therapy optimization remains a knowledge gap.[7]

2. ***Biomarker-based treat-to-target strategy in UC***: In contrast to CD where treatment strategy trials such as CALM have demonstrated that incorporating biomarker assessment as part of the treat-to-target strategy is beneficial, there is paucity of high-quality data in UC confirming the value of a similar biomarker-based treat-to-target strategy.[10] Indirect support for this is presented in the evidence synthesis above where persistent biomarker elevation despite being in symptomatic remission is associated with a higher risk of relapse, however direct evidence is lacking. Similarly, there have not been any studies comparing a biomarker-based strategy to an endoscopy-based strategy for assessment and monitoring of endoscopic remission. This was identified as a knowledge gap by the panel.
3. ***Prognostic significance of biomarkers***: The guideline was focused on the performance of biomarkers for detecting moderate to severe endoscopic activity and did not examine prognostic significance of the magnitude and persistence of biomarker elevation. Most reviewed studies presented data on individual biomarkers and only provided performance around specific cut-offs, usually optimized for that study. Consequently, management recommendations could only be made based on whether the value was above the cut-off for that biomarker but did not factor in the degree of abnormality. A single measurement demonstrating marked elevation of a biomarker may, for a given patient, carry a different prognostic implication, than a more modest elevation. For example, in individuals with mild symptoms, fecal calprotectin >2500μg/g may carry different implications for management than fecal calprotectin of 251μg/g.[47] There was insufficient data to guide nuanced decision making in this context. Similarly, combination of biomarkers (elevated CRP and an elevated fecal calprotectin) in a given clinical setting may have different management implications than a single biomarker. There are several novel biomarkers, including biomarker panels, of disease activity and prognosis that have been studied in research settings, but require more robust

clinical validation before widespread adoption. The paucity of data on this was also identified as a knowledge gap by the panel, requiring further research.

4. ***Biomarker performance in diverse populations***: Finally, the panel recognized the lack of robust data in specific clinical situations including mild UC, acute severe UC and inflammatory disorders of the pouch, and in diverse patient populations, where there exist only few studies examining the role of biomarkers to date.

**What do other guidelines say?**

There has been limited discussion on the role of non-invasive biomarkers in the management of UC in clinical guidelines. The American College of Gastroenterology society guidelines published in 2019 on the management of UC suggested fecal calprotectin as a surrogate for endoscopy *when endoscopy is not feasible or available*.[6] ECCO-ESGAR (European Crohn's and Colitis Organization and the European Society of Gastrointestinal and Abdominal Radiology) guidelines on the diagnostic assessment of IBD recognized that asymptomatic patients with elevated biomarkers of inflammation, mainly fecal calprotectin and CRP may suggest imminent flare and recommended endoscopic or radiologic evaluation.[48] In patients with clinical response to medical therapy, the guidelines recommend evaluating for mucosal healing either via endoscopy or fecal calprotectin. None of these guidelines discussed performance of specific cut-offs and downstream implications involved in decision-making which are critical to using these biomarkers in clinical practice.

**Plans for updating this guideline**

Guidelines are living products. To remain useful, they need to be updated regularly as new information accumulates. This document will be updated when major new research is published. The need for update will be determined no later than 2026 and, if appropriate, we will provide rapid guidance updates to incorporate updated recommendations as new evidence, without duplicating or creating a new comprehensive guideline.

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