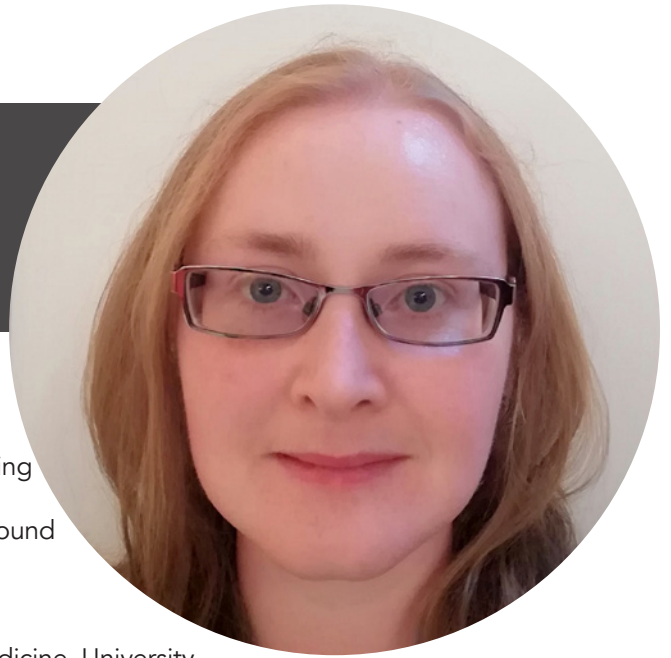


CATHERINE R. ROWAN

MB BCH BAO, MD



Dr. Catherine Rowan, is an Advanced IBD Fellow currently based in the University of Calgary. She completed her gastroenterology training in Ireland and was subsequently appointed Advanced IBD Fellow in Mount Sinai, New York. Her clinical interests include intestinal ultrasound and clinical therapeutics.

Affiliations:

Calgary IBD Unit, Department of Medicine, Cumming School of Medicine, University of Calgary, Alberta

RICHARD J.M. INGRAM

MD



Dr. Richard Ingram is Clinical Lead and Associate Director of the Calgary IBD Unit, and an Assistant Professor of Gastroenterology at Foothills Medical Centre and the University of Calgary. He completed his internal medicine and gastroenterology training and PhD in the United Kingdom. His advanced IBD training was completed at St. Mark's Hospital, UK and the University of Calgary. He is certified by IBUS in intestinal ultrasound. His clinical interests include patient-centred care and education, clinical nutrition and dietary interventions, and dysplasia management.

Affiliations:

Calgary IBD Unit, Department of Medicine, Cumming School of Medicine, University of Calgary, Alberta

TODAY AND TOMORROW: THE USE OF BIOMARKERS IN INFLAMMATORY BOWEL DISEASE

Introduction

Biomarkers play important roles in clinical care for people with inflammatory bowel diseases (IBD) (**Box 1**). Biomarkers are also central to the development of new therapies and as endpoints in their evaluation.

The recommendations from the STRIDE-II study emphasize the central role of clinical indices and biomarkers such as fecal calprotectin (FC) and C-reactive protein (CRP) in the management of Crohn's disease (CD) and ulcerative colitis (UC).¹

This review will focus on the established roles for FC and CRP, emerging roles for alternative and composite biomarkers, limitations of current biomarkers, and unmet needs in the field. This is an evolving area, with recent clinical practice guidelines from the American Gastroenterological Association in UC. In addition, updates are expected from the European Crohn's and Colitis Organisation on their multi-society guideline for IBD monitoring.²

Established roles for biomarkers

There are several roles for biomarkers in clinical care for IBD including diagnosis, assessing disease activity, monitoring therapeutic response, predicting disease recurrence, and mucosal healing. The best-established biomarkers are FC and CRP.

Fecal calprotectin – the cornerstone inflammatory bowel disease biomarker

FC is the cornerstone biomarker in IBD (**Box 2**).

Calprotectin is a soluble cytosolic calcium- and zinc-binding protein, which is produced mainly by neutrophils and granulocytes at sites of inflammation, and to a lesser extent by monocytes, macrophages, and epithelial cells.

FC can be used during diagnosis to help distinguish non-inflammatory conditions from IBD in patients with gastrointestinal (GI) symptoms. Repeated FC testing is more accurate in identifying patients who warrant endoscopic evaluation compared to a single measurement.³ FC can also be used to assess and monitor disease activity and response to therapy, and to predict relapse and post-operative recurrence.⁴ FC may also have a role in risk-stratifying patients who do not have early post-operative recurrence on their initial colonoscopy. Patients with advanced post-operative recurrence (Rutgeerts i3/i4) were identified by two consecutive FC values >250 µg/g, at 4-month intervals over a 2-year period, with 100 % sensitivity and 60% specificity. However, 25% of patients with FC values <250 µg/g were found to have Rutgeerts i2 recurrence at the end of the study period, demonstrating the limitations of this biomarker.⁵

FC is a useful marker in UC and in CD regardless of disease location, including small bowel CD, though it may be less useful in isolated proctitis.^{2,6} FC can also be a useful marker in patients with pouchitis, perianal disease, and potentially in patients with an ostomy.⁴ Overall, FC measurement can help to inform the timing and choice of disease assessment by endoscopy and/or by imaging, and potentially avoid unnecessary colonoscopy/sigmoidoscopy in some patients.

Key limitations in clinical practice are patient adherence with monitoring, equitable access to assays without additional costs to patients, and timely availability of results that integrate with electronic patient records. There are also numerous GI and non-GI factors that can impact results (**Box 2**).

Most manufacturers recommend an FC threshold of 50 µg/g to define normal and abnormal values, although, in practice, the cutoff value depends on the desired outcome. Suggested threshold values are described in **Box 2**.

Practical recommendations for optimal collection, storage, and analysis of stools were proposed in a recent international consensus, in particular⁷:

- <7 days and ideally ≤3 days stool storage at room temperature prior to analysis,
- non-liquid stools provide more precise measurements,
- discontinuation of non-steroidal anti-inflammatory drugs (NSAID)s for ≥2 weeks before measurement.

Patients should be given written information on how to collect a stool sample, when and how to submit it, and ideally a pre-made testing kit (most provincial laboratory services provide this information).

FC measurement can also be performed as a point of care test or by the patient at home. There are several commercially available home-testing kits. These kits use a lateral flow-based testing method rather than ELISA, along with software to allow mobile devices to read the measurement.⁸ The benefits of home FC-testing include a more rapid result and potentially earlier changes in management of the disease. Patients using home-based FC testing kits had a significantly higher use of medical therapy than did those using standard care.⁹ However, adherence to home testing in this study was only 29%, with lower adherence seen amongst male patients. Furthermore, the accuracy of home-based testing kits can vary considerably compared to ELISA-based testing kits. For instance, when comparing three commercial kits with the laboratory performed ELISA method, the agreement was over 75% for FC measurements <500 µg/g. The rate of agreement between home kits and the ELISA method had

reduced to 19–37% for FC measurements >500 µg/g. The type of mobile device used may also impact the reliability and accuracy of measurements.⁸ These factors should be taken into consideration when interpreting results of home-based FC testing.

C reactive protein

CRP is produced by hepatocytes during an acute-phase response and has a half-life around 19 hours; therefore, it changes more rapidly with changes in disease activity than that of the other serum biomarkers.¹⁰ CRP is usually elevated in active CD and less frequently elevated in UC, apart from acute severe UC (ASUC). Although the erythrocyte sedimentation rate (ESR) is altered in both CD and UC, it is less responsive to changes in activity, and is affected by several physiological factors, such as age, sex, pregnancy, hemocrit levels, and erythrocyte size. Unlike FC, elevated CRP values are not specific to GI inflammation and can be elevated in association with a rising body mass index, though obesity also increases the risk of CD and UC.¹¹

Both CRP and ESR lack specificity and accuracy in diagnosis, though CRP has a useful negative predictive value in the context of IBD, with a probability ≤1% in a meta-analysis of 12 prospective diagnostic cohort studies.¹² CRP shows at best a weak to moderate correlation with endoscopic disease activity, and is especially poor for ulcerative proctitis, and has a limited role in predicting risk of relapse.^{13,14} Furthermore, the accuracy in predicting post-operative recurrence in CD is low.¹⁵ CRP is most useful with severe disease and penetrating/fistulizing complications, at baseline, and to monitor response to therapy. In ASUC, CRP guides therapy escalation. The Oxford Criteria includes CRP and stool frequency and can be used to predict the rate of in-hospital colectomy in patients unresponsive to intravenous steroids, albeit with less accuracy since the introduction of rescue therapy.^{16,17}

A CRP value of <5 mg/L was used alongside FC in the CALM trial as a treatment target in CD to optimize adalimumab or combination therapy to achieve tight disease control, with deep remission linked to better medium-term patient outcomes.^{18,19} This treatment strategy was also shown to be cost effective in Canada.²⁰ Most decisions to escalate therapy in the CALM trial were driven by biomarkers rather than clinical assessment, particularly by FC values ≥250 µg/g at weeks 12 and 24 rather than by CRP or FC+CRP combination therapy.²¹ In the STARDUST trial, biomarker targets of FC ≤250 µg/g and CRP ≤10mg/L were used to optimize ustekinumab dosing in CD.²² Only 30% of patients achieved biomarker targets for FC and CRP, despite 78% of patients achieving clinical remission and >30% showing biomarker response, with no significant benefit over standard of care in endoscopic improvement at 48 weeks.

Bottom line – biomarkers cannot (yet) replace endoscopy

A systematic review and external validation study that looked at non-invasive models to identify patients with

endoscopic activity of CD found that 7 of 27 identified diagnostic models could predict endoscopic endpoints in CD, and that 4 of these models showed a benefit similar to FC and CRP, which showed positive predictive values of ≥90% for mucosal disease activity.²³ However, only the Utrecht Activity Index (UAI) and TAILORIX models were able to reliably predict endoscopic healing, and 1 in 5 patients were misclassified using FC cut-off values of <100 and ≥250 µg/g.^{23,24} Ileocolonoscopy remains the gold-standard to evaluate disease activity in adults with CD. FC has utility in UC, although biomarkers may be suboptimal in confirming endoscopic healing and evaluating mild symptoms; furthermore, it is not known whether a biomarker or endoscopic strategy is superior for long-term monitoring.² In addition, biomarkers have no role in detecting dysplasia, surveillance, or excluding cytomegalovirus colitis and infection, which require endoscopy and/or microbiological evaluation.²

Emerging biomarkers and novel roles

Despite advances in therapeutics, there remains a distinct gap between our treatment goals and actual results. Biomarkers that perform beyond the established roles in diagnosis and disease activity monitoring are essential in bridging that gap. Areas where biomarkers may be particularly important include the prediction of disease course, disease phenotype, and the choice of advanced therapy.

Composite biomarkers

There is interest in developing and integrating different biomarkers into a single readout to better predict endoscopic healing and to guide decision making in research and clinical practice.²⁵ Dragoni et al. reviewed the use of panels of blood, fecal biomarkers, and drug levels, that have the potential to replace single biomarker approaches in the future.²⁶ This approach may be particularly helpful to reduce the ambiguous “grey zone” associated with biomarker readouts.¹⁰

Better utilization of readily available biomarkers is one potential strategy. The CALM trial showed that measurements of FC and CRP together were superior to FC alone in CD, though the majority of treatment escalations were driven by FC.¹⁸ The UAI included platelet count and mean corpuscular volume alongside FC, CRP, and stool frequency, although it may offer limited benefit beyond FC and/or CRP.^{23,24} In pediatric CD, the composite Mucosal Inflammation Noninvasive index (MINI) score (comprising FC, ESR, CRP and pediatric CD activity index) can predict mucosal healing in lieu of ileocolonoscopy and/or magnetic resonance enterography.²⁷ The added benefit over FC alone was particularly seen for FC concentrations 100–599 µg/g. The Portuguese DIRECT study derived risk matrices to predict CD progression, comprising the degree of elevation in FC and CRP and the presence and persistence of anemia across single or multiple visits.²⁸ Another example of potential composite biomarkers is a combination of a fecal immunochemical test (FIT) and FC, which were superior to predict clinical

relapse over 12 months in UC and might have the ability to better predict endoscopic healing.²⁹

Putative and future biomarkers

The pursuit of an ideal biomarker continues, with many candidates studied including fecal and tissue markers of intestinal inflammation, fecal volatile organic metabolites, and urinary prostaglandins.^{30,31} Serum/plasma assays for epigenetic biomarkers, especially microRNAs, glycoprotein biomarkers, and leucine-rich alpha-2-glycoprotein, amongst others, are under review.^{10,15,32}

Lactoferrin and calgranulin C (S100A12) are fecal biomarkers similar to FC. They have not demonstrated additional utility, share similar limitations as FC and are not typically used in practice. In UC, FIT has a high positive likelihood ratio and moderate negative likelihood ratio for predicting endoscopic healing.³³ In addition, FIT is less accurate than FC although it may be equivalent in predicting endoscopic disease activity, and agnostic for disease extent.^{29,34}

Other potential biomarkers include widely available laboratory results which could be seamlessly integrated into clinical practice. For instance, the platelet-to-lymphocyte ratio index showed an area under the curve (AUC) of 0.87–0.91 for moderate/severe activity and an AUC of 0.74 for mucosal healing in isolated small bowel CD against capsule endoscopy, which was superior to FC and CRP.³⁵ Neutrophil-to-lymphocyte ratio also shows promise as a biomarker of endoscopic activity and response to biologic therapy.³⁶

Susceptibility, diagnosis and predicting disease course

Genetic susceptibility plays an important role in the development of IBD, with over 230 risk alleles identified.³⁷ The NOD-2 gene is recognized as a major susceptibility gene, and over 50 genes have been associated with very early onset IBD.^{38,39}

In terms of predicting the development of IBD, serological markers such as atypical perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA) may have a role. A study of Israeli military recruits detected ASCA in approximately 30% of patients before the clinical diagnosis of CD with a mean interval between detection and diagnosis of 38 months. In addition, pANCA was found in 25% of patients subsequently diagnosed with UC.⁴⁰ The cohort in this study was small, therefore conclusions should be limited accordingly.

More recently, anti- $\alpha\beta 6$ autoantibodies were found to be significantly higher amongst patients subsequently diagnosed with UC compared with healthy controls. These autoantibodies were detected up to 10 years before diagnosis and were associated with worse outcomes such as hospitalization, colectomy, and need for biologic therapy.⁴¹

Several serological markers have been identified in IBD patients and evaluated in distinguishing UC from CD. Most

notably, pANCA and ASCA have been studied.⁴² Atypical pANCA is found mainly in UC (50–67%) and to a lesser extent in CD. However, pANCAs are also present in other inflammatory conditions such as autoimmune hepatitis and primary sclerosing cholangitis. ASCAs are typically more common in CD (40–60%) although not exclusive to CD, having been detected in UC and disease controls.

The performance of these serological markers improves when used in combination. The pattern associated with CD is ASCA+/ANCA-, and for UC is ASCA-/ANCA+. When used in this manner, ASCA and pANCA affect the post-test probability of having CD or UC. The positive likelihood ratio of ASCA+/ANCA- ranges from 6.3–11, and that for ASCA-/ANCA+ ranges from 2.9–22 across various studies.^{43–47} An important caveat is that pANCAs are frequently detected in colonic CD as in UC, thus limiting its utility as a specific marker for CD in the scenario in which such a marker would be most useful.⁴⁸

pANCAs do not distinguish or predict disease location or phenotype.⁴⁵ However, ASCA has been associated with a more complex CD phenotype and with small bowel involvement.^{43,49,50} In a pediatric cohort, seropositivity to anti-Cbir1 (flagellin), anti-outer membrane protein C, ASCA, and pANCA was associated with a complex penetrating/stricturing phenotype, and the need for surgery while higher antibody sum, as a marker of immune reactivity, was associated with rapid disease progression.⁵¹

Personalized medicine

A key unmet need in IBD is the ability to reliably predict disease course at diagnosis, and the serological markers above demonstrate the ongoing interest in this goal. Another gap in knowledge is the ability to predict response to specific therapies. Precision medicine is an elusive goal in IBD given the complexity of the condition. With respect to predicting response to existing therapies, there have been some promising steps in recent years.

The PROFILE (PRedicting Outcomes For Crohn's disease using a moLecular biomarker) study is the first biomarker-stratified trial in IBD and has recently completed follow-up to week 48.⁵² PROFILE recruited 390 adults in the UK who were recently diagnosed with CD of at least moderate activity, and were naïve to immunomodulator and anti-TNF therapies. PROFILE utilizes a peripheral blood CD8+ T-cell transcriptomic signature early after diagnosis to classify patients into IBD^{hi} and IBD^{lo} to predict disease course and risk of complications. The analysis will also compare the relative benefit of treatment strategies in each biomarker subgroup to determine if this biomarker study can identify the most appropriate therapy.

Inflammatory modules associated with response and resistance to anti-TNF therapy have been identified.^{53,54} The glycoprotein 130 family of cytokine receptors were found to be upregulated in patients with CD refractory to anti-TNF therapy and related to particular NOD-2 gene variants.⁵⁵

Several strategies have been proposed to predict response to vedolizumab, including immunoglobulin glycosylation, mucosal vascular addressin cell adhesion molecule 1 (MadCAM1) non-expression in LP endothelial cells, and increased baseline colonic mucosal eosinophil counts.⁵⁶⁻⁵⁸ Battat et al found a trend toward more rapid increases in s- α 4 β 7 concentrations in patients treated with vedolizumab who achieved clinical remission and endoscopic remission. S-MadCAM-1 concentrations declined more rapidly in this group compared to non-responders.⁵⁹ In UC patients, increased density of mucosal eosinophils was a negative predictor of response to vedolizumab.⁵⁸

Microbiome diversity and more abundant populations of *Burkholderiales* species was associated with remission in patients treated with vedolizumab.⁶⁰ Microbial analysis and development of serum profiles reflecting microbial diversity have also been explored as a way to identify patients more likely to respond to anti-cytokine therapy rather than anti-integrin therapy.⁶¹ These profiles have yet to be used in clinical practice but incorporating multi-omic data, clinical data, and microbial signatures with machine learning models may enhance our ability to accurately predict therapeutic response in the future.

Conclusion

Biomarkers are a critical component to achieving high quality care for patients with IBD. Established biomarkers complement more invasive assessments and act as useful guides to therapy. Currently available biomarkers such as FC and CRP could potentially be exploited more to our advantage as composite biomarkers that can more accurately inform treatment goals such as endoscopic remission. However, in their present form, biomarkers cannot replace essential functions of endoscopic evaluation and fall short of predicting a response to a particular advanced therapy. Biomarker development is now focusing on disease prediction and on strategies to individualize therapy decisions. Future biomarkers are likely to incorporate data from clinical, immunologic, and microbial sources to provide a more nuanced approach to IBD therapy.

Correspondence:

Richard J M Ingram

Email: richard.ingram@ucalgary.ca

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According to the World Health Organization, a biomarker is described as follows: "Almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction."⁶²

- May include molecular, histologic, radiographic, or physiologic characteristics
- Not a measure of how an individual feels, functions or survives
- Includes the categories of susceptibility/risk, diagnostic, monitoring, prognostic, predictive, response, and safety biomarkers

Box 1. What is a Biomarker?; adapted from World Health Organization, 1993

Thresholds:

- FC <50 µg/g to distinguish between IBS and possible IBD, in settings in which patients with chronic GI symptoms are being evaluated, and a high negative predictive value is needed, though FC >250 µg/g can identify ~90% of new patients who were confirmed to have IBD
- FC <100-250 µg/g as therapeutic target in CD⁶³⁻⁶⁶
- FC <150 µg/g as therapeutic target in UC

Trends in an individual patient using the same quantitative assay and correlated with endoscopic assessment(s) are more important than an absolute binary cut-off. Exact cut-offs to distinguish between IBD and IBS or between active and inactive IBD do not exist in all scenarios.

Suggested frequency of endoscopic assessments

	Remission	Active/Treatment initiation
CD	6–12 monthly (not established for CD)	3 monthly
UC	6–12 monthly (3–6 monthly if FC >150 µg/g)	3–6 monthly

Levels of FC can be affected by:

- active IBD
- inactive IBD with anastomotic ulceration attributable to surgery-related factors and local ischemia (Rutgeert's score i2a)
- perianal disease, up to FC >1000 µg/g
- medications:
 - bowel preparation for colonoscopy, up to >1000 µg/g
 - NSAIDs and Aspirin, up to ~ 500 µg/g (including NSAID-induced enteropathy)
 - proton pump inhibitors, up to 150 µg/g
- non-IBD causes of intestinal inflammation:
 - bacterial and viral GI infections, up to ~ 1000 µg/g
 - microscopic colitis, up to ~ 500 µg/g
 - radiation proctitis, up to ~ 250 µg/g
- other GI factors:
 - colonic diverticular disease, up to 60 µg/g
 - colonic polyps (including IBD-associated inflammatory polyps), up to ~120 µg/g
 - colorectal cancer, up to ~130 µg/g
 - GI bleeding, up to ~500 µg/g
 - patients ultimately diagnosed with IBS, up to ~ 300 µg/g
- non-GI and lifestyle factors:
 - age <9 years, up to ~200 µg/g
 - age >65 years, up to ~120 µg/g
 - bariatric surgery, up to ~400 µg/g
 - obesity, up to 185 µg/g
 - physical inactivity, up to 60 µg/g
 - rheumatological diseases, up to ~500 µg/g

Box 2. Fecal Calprotectin; courtesy of Catherine R Rowan, MD and Richard J M Ingram, MD

CD, Crohn's disease; CRP, C reactive protein; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; NSAIDs, non-steroidal anti-inflammatory drugs; UC, ulcerative colitis

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