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Review

Biomarkers in inflammatory bowel disease: a practical guide

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Jennie Clough, Michael Colwill, Andrew Poullis, Richard Pollok, Kamal Patel and Sailish Honap

***Abstract*:** Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is a costly condition in terms of morbidity and healthcare utilization, with an increasing prevalence now approaching 1% in the Western world. Endoscopic assessment of IBD remains the gold standard for diagnosis, evaluation of treatment response and determination of post-operative recurrence, but is expensive and invasive. Biomarkers can facilitate non-invasive disease assessment, with C-reactive protein and faecal calprotectin as the most widely available biomarkers in current clinical practice. This narrative review summarizes the evidence for their use in both UC and CD and offers practical guidance for healthcare providers taking into account the limitations of biomarker interpretation. We present evidence for the future use of novel biomarkers in IBD and discuss how biomarker discovery could deliver the goal of precision medicine in IBD.

Plain language summary

Biomarkers in inflammatory bowel disease: a practical guide

Inflammatory bowel disease (IBD) is a term used to describe two conditions, ulcerative colitis (UC) and Crohn's disease (CD). These two diseases cause inflammation of the bowel, which can lead to diarrhoea, abdominal pain and bleeding from the back passage. The best way of assessing how active a patient's IBD is, is by performing a camera test called a colonoscopy. However, having a colonoscopy is inconvenient, comes with some risks to the patient, and uses a lot of healthcare resources. 'Biomarkers' are proteins detectable in body fluids (such as blood, poo and urine) which can give information to medical staff about how active a patient's disease is, without the need for colonoscopy. In this article, we give guidance about how best to use these tests, and when they might not be so useful. We also discuss new biomarkers and ways in which they could be used in the future to predict which treatments patients might respond to best.

Keywords: biomarker, C-reactive protein, Crohn's disease, disease monitoring, faecal calprotectin, precision medicine, ulcerative colitis

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Correspondence to:
Sailish Honap
St George's University Hospitals NHS Foundation Trust, London, UK

School of Immunology and Microbial Sciences, King's College London, London, UK

INFINY Institute, Nancy University Hospital, Vandœuvre-lès-Nancy, France
shonap@nhs.net

Jennie Clough
St George's University Hospitals NHS Foundation Trust, London, UK

School of Immunology and Microbial Sciences, King's College London, London, UK

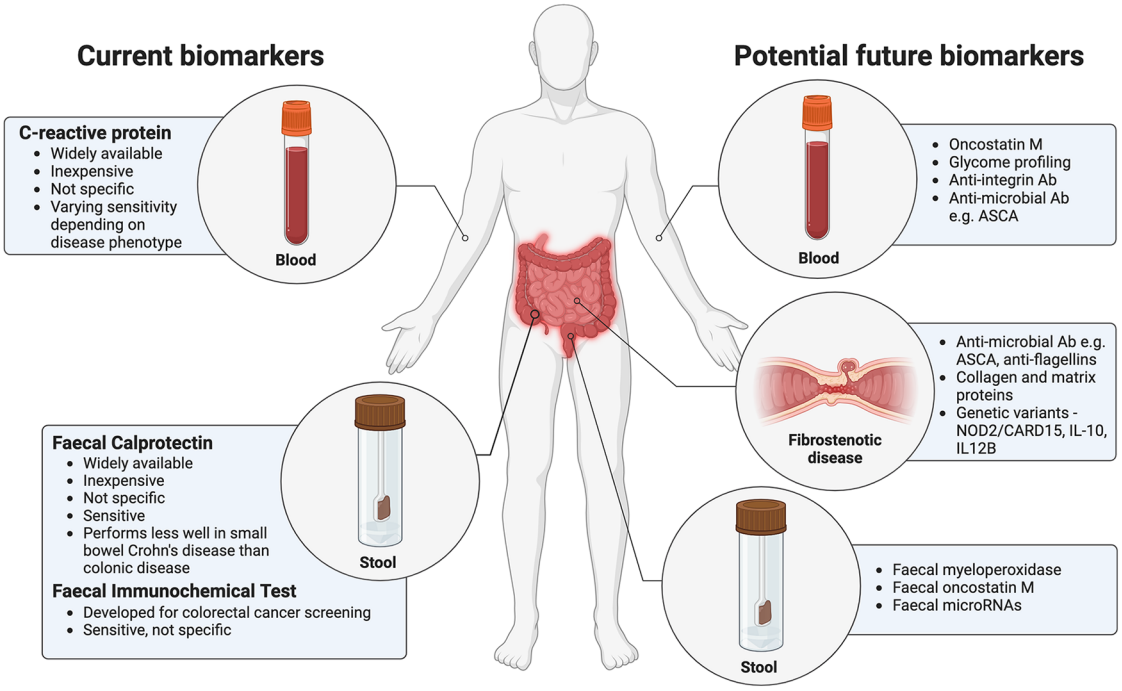
Michael Colwill
Andrew Poullis
Kamal Patel
St George's University Hospitals NHS Foundation Trust, London, UK

Richard Pollok
St George's University Hospital NHS Foundation Trust

Institute of Infection and Immunity, St George's University, London, UK

Biomarkers in inflammatory bowel disease: a practical guide

A narrative review of current and potential future biomarkers for diagnosis, prognostication and disease monitoring



Visual Abstract

Introduction

Inflammatory bowel disease (IBD) is a chronic immune-mediated disease affecting the gastrointestinal tract and comprises two main subtypes: ulcerative colitis (UC) and Crohn's disease (CD).[1,2] The exact cause of IBD remains unknown, but it is believed that in genetically predisposed individuals, an environmental trigger initiates an inappropriate intestinal immune response.[3] The overall global prevalence is expected to rise to approximately 1% in the coming decades.[4]

Currently available therapeutic agents attenuate an array of pro-inflammatory cytokines and prevent leucocyte trafficking to the site of inflammation by inhibiting sphingosine-1-phosphate receptors and integrins.[5,6] The goal of these therapies is to induce clinical and endoscopic remission, reduce the risks of complications and the need for surgical intervention, and improve the quality of life for patients. The gold standard in the assessment of IBD activity is endoscopy, usually through colonoscopy.[7] However, this procedure is costly and invasive with associated risks to the patient. Therefore, the use of biomarkers to non-invasively assess disease activity, response to therapy and recurrence of disease has become commonplace. Biomarkers are defined by the National Institute of Health as 'a characteristic that is objectively measured and evaluated as an indication of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention'.[8]

Biomarkers can be collected from sources including serum, urine, stool or tissue. Whilst the number of biomarkers available to clinicians has increased in recent years, particularly driven by the growth of metabolomics, genomics and proteomics, not all biomarkers are useful or available to the practicing clinician in everyday practice.[9–12] An ideal biomarker is sensitive and specific to the observed outcome, available without the need for invasive collection, relevant to underlying pathophysiology, responsive to treatment, useful in prognostication, cost-effective and acceptable to the patient.[13] For clinicians, there are also further considerations such as availability, turn-around time for testing and robustness of the analytic method used.

While the search for the optimal biomarker in IBD continues, the most widely available biomarkers in current clinical practice include serum and stool testing with C-reactive protein (CRP) and faecal calprotectin (FCP). The use of other faecal markers, for example faecal lactoferrin, is less widespread. CRP and FCP are frequently used by primary care clinicians to differentiate between IBD and irritable bowel syndrome (IBS)[14] and by IBD clinicians to evaluate symptoms and monitor response to therapy. There are also newer genetic biomarkers that may have a future role to play in IBD, such as the NUDT15 codon that predicts adverse effects from thiopurines.[15] Despite significant collaborative research efforts towards biomarker identification, the recent PRedicting Outcomes For Crohn's dIsease using a moLecular biomarkEr (PROFILE) trial of a blood-based biomarker did not show clinical utility in identifying patients with CD at risk of a more severe disease course at diagnosis, for example.[16,17]

All biomarkers have individual strengths and limitations, and effective clinical use requires nuance and careful interpretation. We present a narrative review of the current literature to provide a practical guide to assist clinicians in day-to-day practice and explore the future of biomarker use in IBD.

Methods

To identify relevant articles for this narrative review, a MEDLINE literature search was conducted through the PubMed platform for articles published in the English language from inception until March 2024. The following search terms were used 'inflammatory bowel disease', 'Crohn's disease', 'ulcerative colitis', 'biomarker(s)', 'C-reactive protein', and 'calprotectin'. Secondary references of the retrieved articles were reviewed to identify publications not captured by the electronic search.

C-reactive protein

CRP and the inflammatory response

First discovered in the 1930s, CRP is a pentameric acute-phase protein that is primarily synthesized in the liver but also by smooth muscle cells, lymphocytes, adipocytes, macrophages and endothelial cells.[18] In response to infectious stimuli or tissue damage, cytokines including interleukin (IL)-6 and IL-1β are produced leading to the secretion, primarily by hepatocytes, of CRP into the plasma. CRP binds to C1q molecules to activate the complement pathway, as well as binding *via* Fc receptors to IgG resulting in the release of further pro-inflammatory cytokines.[19] It also plays a role in innate immunity by binding to phosphocholine expressed on the surface of bacterial cells, activating complement-induced phagocytosis and apoptosis. Studies have also shown that circulating CRP breaks down into monomeric subunits which can exert pro-inflammatory effects through activation of monocytes, endothelial cells, platelets and neutrophils.[20] CRP serum concentrations can increase by up to 1000-fold within 24–72 h of some bacterial infections, but once the stimulus ends levels rapidly decrease over 18–20 h.[18] As well as infection and tissue damage, CRP is found to be elevated in a multitude of inflammatory conditions such as rheumatoid arthritis, some cardiovascular disease and IBD.

CRP testing is widely available in primary and secondary care, with results available within minutes to hours, acceptable to patients and cost-effective for assessing inflammation. However, CRP is limited as a biomarker in IBD by its lack of specificity, with its expression upregulated in numerous infective and inflammatory pathologies, thus limiting its usefulness in distinguishing between IBD and other differential diagnoses. Its utility in IBD is largely as an adjunct to clinical and endoscopic findings.

CRP in the diagnosis of IBD

CRP is often used in primary care to screen for underlying inflammatory pathology, and with regard to gastrointestinal symptoms, it is effective at distinguishing between inflammatory and functional disease.[21] However, a review identified that up to 25% of patients with active CD did not mount a CRP response and early work from St Mark's Hospital (UK) found CRP to be elevated in only 50% of patients with UC.[22] Genetic polymorphism has also been described as a source of inter-patient variability in CRP.[23] Exclusion of IBD, therefore, should not be made based solely on a normal CRP but in combination with clinical assessment and other markers with better sensitivity.

CRP in disease monitoring

In patients with a known diagnosis of IBD, CRP is commonly used in clinical practice to provide a non-invasive marker of disease activity. However, its accuracy varies based on many clinical factors including whether the patient suffers from UC or CD, and the extent of their disease.[24,25] The correlation between disease activity in CD and CRP is stronger in CD than in UC[26]; however, this is dependent upon the disease severity and location. A Korean study of 435 patients found that an elevated CRP was more likely to be seen in ileocolonic or colonic CD compared to patients with isolated ileal disease.[27]

Even in the setting of a normal CRP, many patients with CD have active disease. One study identified that 92.9% of patients with an elevated Crohn's Disease Activity Index (CDAI) and a normal CRP had active mucosal disease at endoscopy, although these lesions were deemed to correspond only to mildly active disease [Crohn's Disease Endoscopic Index of Severity (CDEIS) $\leqslant$6]. It has been demonstrated that a normal CRP is negatively associated with hospitalization and the need for surgery, indicating that a normal CRP is suggestive of the absence of severely active CD.[28] Conversely, asymptomatic patients with an elevated CRP (so-called 'silent IBD') are at a seven-fold higher risk of having worse disease trajectories[29] and a two-fold higher risk of hospitalization.[28] Whilst escalating CD therapy based upon a single biomarker is not an advisable strategy, it should prompt physicians to consider other modalities of disease assessment.

CRP also has utility in acute presentations of CD, when a significantly elevated CRP can be indicative of a complication such as perforation, abscess formation or peri-anal collection and may guide the need for subsequent radiological or endoscopic investigation.

The performance of CRP in assessing disease activity in UC is inferior to FCP when correlating with endoscopic appearances.[30] A 2019 study found that CRP did not correlate well with low-grade mucosal disease activity, defined as Mayo endoscopic sub-score (MES) of 0 or 1.[31] An elevated CRP was associated with MES 2–3 disease but only in left-sided or pan-colonic disease. The investigators also examined other serum biomarkers – albumin, erythrocyte sedimentation rate, white blood cell count and platelet count – but none were found to have any statistically significant correlation with MES. Therefore, whilst CRP may be useful in identifying those with moderate to severe and extensive disease, it does not have a role in proctitis or mild disease, and assessment for these patients should be based upon clinical assessment and other biomarkers such as FCP.

CRP does have a crucial role, however, in the assessment of acute severe UC (ASUC), together with clinical, radiographic and endoscopic evaluation. European Crohn's and Colitis Organisation (ECCO) guidelines state that patients with a significantly elevated CRP $>$30 mg/L in association with bloody diarrhoea with a stool frequency of $>$6/day have developed ASUC and require admission for intensive treatment with either intravenous steroids, infliximab or ciclosporin.[32] The ECCO guidelines, based upon work by Truelove and Witts and the Oxford criteria, state that non-responders or patients with a worsening clinical picture at day 3, including a static or worsening CRP, have an 85% chance of requiring colectomy during admission.[33] Whilst more recent data have suggested the rate of colectomy may not be this high,[34] a recent study found a clear correlation between CRP and deep ulceration seen at endoscopy[35] which itself represents a higher colectomy risk. The CRP:albumin ratio (CAR) has also been identified as a useful biomarker in ASUC, with a CAR $>$0.85 at day 3 of admission predictive of steroid-refractory ASUC and the need for rescue therapy.[36] Furthermore, in patients who had responded to medical management, a CAR of $>$0.37 at discharge was predictive of the need for colectomy within 12 months.[37] CRP therefore remains a key part of overall clinical assessment and decision-making regarding treatment escalation and the need for surgery in ASUC.

CRP in assessing treatment response

As above, a significant proportion of patients with CD will not mount an elevated CRP despite endoscopically and clinically active disease, and CRP is therefore not a useful monitoring tool in these patients. Overall, a persistently elevated CRP is associated with therapy failure,[38,39] whereas a fall in CRP is correlated with clinical response.[40,41] For patients in remission, a prospective 2010 study found that CRP can predict relapse but it was less sensitive and specific compared to FCP in this role.[42] It has also

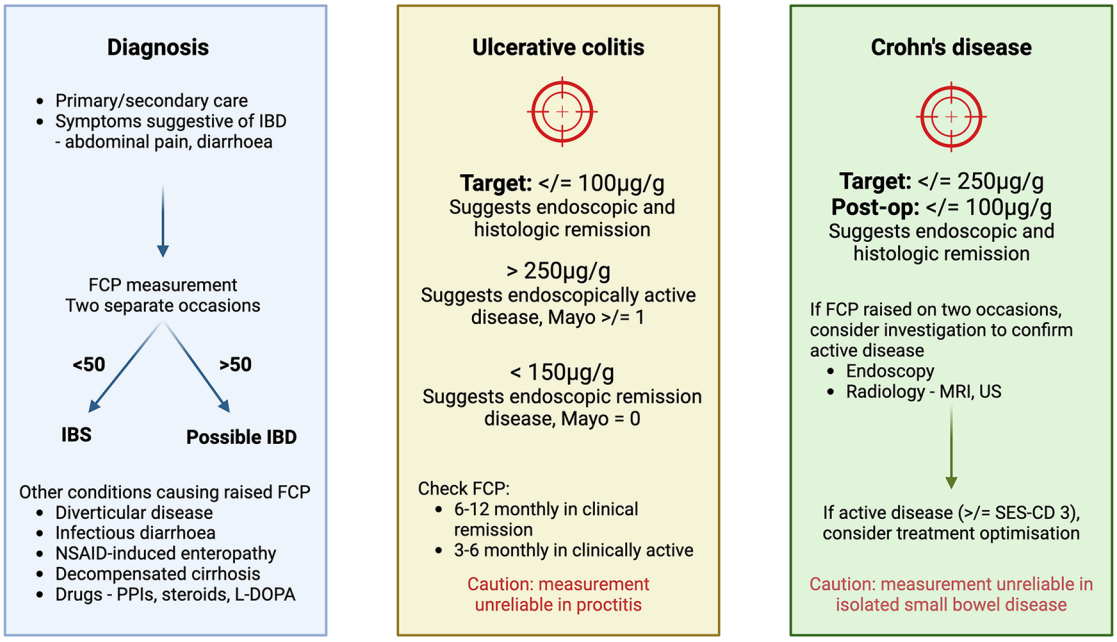


Figure 1. A guide to use and interpretation of FCP testing in clinical practice.
Source: Image created in BioRender.
IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; FCP, faecal calprotectin; MRI, magnetic resonance imaging; NSAID, non-steroidal anti-inflammatory; PPI, proton pump inhibitor; SES-CD, simple endoscopic score in Crohn's disease; US, ultrasound.

been demonstrated that even in asymptomatic individuals, persistently elevated CRP is associated with a higher rate of hospitalization.[28] A persisting CRP can therefore be an indicator that further assessment and consideration of treatment modification may be required in CD.

In patients with moderate to severe UC, data suggest that CRP correlates with response to therapy. A 3-year follow-up study of 72 patients with a partial Mayo score (PMS) of 4–9 who were initiated on infliximab found that responders, defined as a PMS ⩽2 at week 14, had a significantly lower CRP at week 2 compared to partial and non-responders.[43] CRP appears to be less effective in predicting relapse, with a study of 74 patients with clinically and endoscopically inactive UC demonstrating that remission CRP was not predictive of those who would flare.[44]

CRP in the prediction of post-operative recurrence

There is conflicting data with regard to the ability of CRP to predict post-operative recurrence in CD. A 2010 study found that persistently elevated CRP was associated with post-operative recurrence in a small cohort of 12 patients.[45] However, as discussed earlier, this persistent CRP may simply reflect a more aggressive and severe disease which is known to be a risk factor for post-operative recurrence. Conversely, a 2011 study of 24 patients with CD who were randomized to receive infliximab or placebo post-operatively found that whilst there was a general trend between elevated CRP and relapse, there was no statistical correlation between CRP and endoscopically disease recurrence as assessed by the Rutgeerts score.[46] Given this conflicting data and small study cohorts, clinical, endoscopic and radiological investigation assessment remains essential in this patient group.

Faecal calprotectin

Faecal calprotectin structure and function

First identified in the 1980s, FCP is a non-invasive biomarker measured in stool, permitting the detection of gut inflammation.[47,48] Calprotectin (CP) is part of a highly conserved family of calcium-binding S100 leucocyte proteins, composed of two monomers, S100A8 and S100A9.[49] The monomers can form heterodimers and tetramers

in a calcium-dependent manner, with each heterodimer possessing transition metal binding sites.[50] CP is found in abundance in the cytosol of neutrophils and is constitutively expressed by monocytes, dendritic cells, activated macrophages and squamous mucosal epithelium.[47] Importantly, expression of CP is induced during inflammation, with bacterial lipopolysaccharide, tumour necrosis factor-alpha (TNFα) and IL 1-beta (IL-1β) able to drive CP expression.[51,52]

The S100A8/S100A9 CP complex controls several functions involved in the control of intracellular pathways of innate immune cells, including modulation of cytoskeletal rearrangement to permit leucocyte recruitment and facilitation of arachidonic acid transport to sites of inflammation.[53] Arachidonic acid is a potent inflammatory mediator that has been associated with inflammation and tissue damage in active IBD.[54] The S100A8/S100A9 complex is readily secreted, triggering neutrophil chemotaxis and endothelial adhesion.[47] Free CP promotes the expression of both pro-inflammatory and anti-inflammatory mediators, including IL-1β, IL-6, IL-10 and TNFα,[55,56] and regulates cell proliferation, differentiation and apoptosis.[57] Once tissue damage has been initiated at the mucosal surface, CP release is perpetuated by transcriptional induction of the S100A8 and S100A9 subunits in epithelial cells.[47] During unresolved inflammation, CP itself can contribute to ongoing mucosal injury in the gut.[58]

FCP in the diagnosis of IBD

Studies in healthy individuals have identified an FCP range between 10 and 50 μg/g, although this varies slightly depending on the study population and the assay used.[59,60] With the development of FCP detection capabilities in 1992, FCP became the first stool biomarker able to discriminate between inflammatory and non-inflammatory gastrointestinal diseases.[21,61] FCP testing is inexpensive, widely available in primary and secondary care settings, and CP remains stable at room temperature in stool for at least 3 days, reducing the complexities of sample handling and transport.[62] A summary guide on the use and interpretation of FCP testing in IBD is presented in Figure 1.

FCP correlates with the number of neutrophils present in the intestinal lumen and, whilst sensitive for the detection of gut inflammation, is not able to discriminate between different inflammatory aetiologies. FCP is also elevated, for example, in infective gastroenteritis, with levels correlating with disease severity in *Salmonella, Campylobacter* and *Clostridia* infections.[63–65] Elevated FCP levels can also be seen in the setting of colonic malignancy,[66] diverticular diseases,[67] necrotizing enterocolitis,[68] graft-*versus*-host disease[69] and non-steroidal anti-inflammatory (NSAID) enteropathy.[70] High FCP values can also be seen in non-intestinal pathology, including decompensated liver cirrhosis[71] and pneumonia,[72] most likely as a consequence of altered intestinal microbiota and bacterial translocation.

Despite its lack of specificity, FCP has utility in excluding a wide range of inflammatory gut disorders. This makes it especially useful in the setting of primary care, where FCP testing is recommended in national guidelines to differentiate between IBS and IBD – conditions with significant symptom overlap.[73] A level greater than 50 μg/g on two occasions is deemed to warrant further invasive testing with colonoscopy and/or bowel imaging, with a recent meta-analysis indicating a pooled sensitivity of 85.8% and specificity of 91.7% for the diagnosis of IBD at this threshold.[74] Although higher levels (>250 μg/g) may be more suggestive of active intestinal inflammation,[75] a study examining the 12-month outcome of indeterminate FCP levels (50–249 μg/g) noted an 8% chance of developing IBD compared with 1% in those <50 μg/g.[76] However, interpretation of slightly elevated FCP concentrations should be made with care, as common drugs including proton pump inhibitors,[77] NSAIDs,[70] glucocorticoids[78] and levodopa[79] may also induce CP expression.

FCP in disease monitoring

FCP correlates well with endoscopic IBD activity, particularly in the setting of colonic inflammation, with low levels seen in patients with endoscopic and histological remission.[80,81] It has shown superiority over CRP in predicting endoscopic disease activity,[82] and is increasingly used for patients in clinical remission to predict disease relapse, and to monitor response to therapy in active disease. The Effect of Tight Control Management on Crohn's disease study

demonstrated that a treat-to-target approach based on FCP results was superior to treatment escalation based on symptoms alone,[83] and the International Organisation for the Study of Inflammatory Bowel Disease has published recommendations as part of the Selecting Therapeutic Targets in IBD consensus advising a target FCP of <150 µg/g as a goal of treatment.[7]

A challenge in devising treat-to-target strategies in IBD is the lack of evidence exploring the effect of targeting various FCP thresholds on long-term clinical outcomes, with different expert groups proposing different thresholds. Although the use of different thresholds for UC and CD cohorts offers a more nuanced approach, studies suggest that a target of <250 µg/g for both groups is a reasonable long-term strategy,[80,84] which may be more achievable for clinicians managing patients with IBD outside of specialist centres.

Evidence suggests that a reduction in FCP, as well as a target below a certain threshold, has prognostic significance, with FCP able to predict long-term clinical outcomes when measured 12 weeks after initiation of biologic treatment.[85] In a study of response to anti-TNFα, FCP <300 µg/g or a 50% decrease in FCP at weeks 12–14 was predictive of clinical and endoscopic remission.[86] Whilst individual FCP trajectories are highly heterogeneous over the longitudinal course of the disease, distinct patterns can be seen in FCP trends in patients with CD.[87] Elevated FCP trajectories were associated with a longer time from diagnosis to initiation of biologic therapy and smoking at diagnosis.

An FCP >150 µg/g can predict post-operative recurrence in CD with a sensitivity of approximately 70%,[88] although current guidelines recommend colonoscopy at 6 months post-operatively for visualization of the anastomosis and calculation of prognostic scoring (Rutgeerts score) to guide further treatment. FCP has also been shown to be a valid method of assessing disease activity in pregnant patients with IBD.[89] Many clinicians will use both CRP and FCP for the assessment of IBD activity in routine clinical practice. Research supports the combination of available biomarkers as a valid disease monitoring strategy, with a raised CRP and FCP better able to predict outcomes in infliximab-treated patients than using either marker alone.[84,90]

Limitations of FCP testing

Despite its widespread adoption in the diagnosis and monitoring of IBD, FCP has limitations. Studies have demonstrated marked intra-individual variation in FCP measurement over a few days,[91] which could hinder decision-making strategies based on an isolated sample. However, the variability appears to be greatest in subjects with high levels of CP, which may reduce the clinical relevance of such variation.[62] Factors such as diet and exercise have been shown to affect day-to-day and within-day FCP variation,[92] although FCP appears to be homogenously distributed within a stool sample and a single stool 'punch' is, therefore, an adequate sampling strategy.[91] Local policy is to request that patients submit a sample taken from the first bowel motion of the day, to minimize this variability and to ensure the concentration of FCP could be expected to be highest given its accumulation overnight.[62] Whilst most patients find FCP testing acceptable, sample return rates are highly variable.[93–95] Most studies of FCP testing have been performed in secondary care settings, limiting their applicability to primary care.

FCP is more sensitive in assessing disease activity in UC than CD[96] and is limited in its ability to accurately detect disease activity in patients with isolated ileal CD.[97] Within UC, disease extent affects FCP interpretation, with patients with proctitis exhibiting a poor correlation between FCP concentration and endoscopic activity.[98] Furthermore, a minority of patients do not appear to mount a detectable FCP increase even in the presence of endoscopically active disease,[99] and disease assessment and monitoring therefore needs to be personalized for any given patient.

Although FCP is stable within stool for up to 7 days in the presence of calcium, co-existing mucus or blood in patients with active IBD can influence FCP levels.[92] FCP may undergo oxidative cross-linking *in vivo*, increasing its susceptibility to proteolytic degradation and leading to underestimation of its true levels in commercial assays.[100] Variability can also be seen based on which assay is used for FCP measurement, with the enzyme-linked immunosorbent assay (ELISA) technique deemed to produce the most robust results.[101] However, ELISA testing is time-consuming to perform, meaning tests are often run in batches, which may delay the availability of results.[101] Automated ELISA tests are now

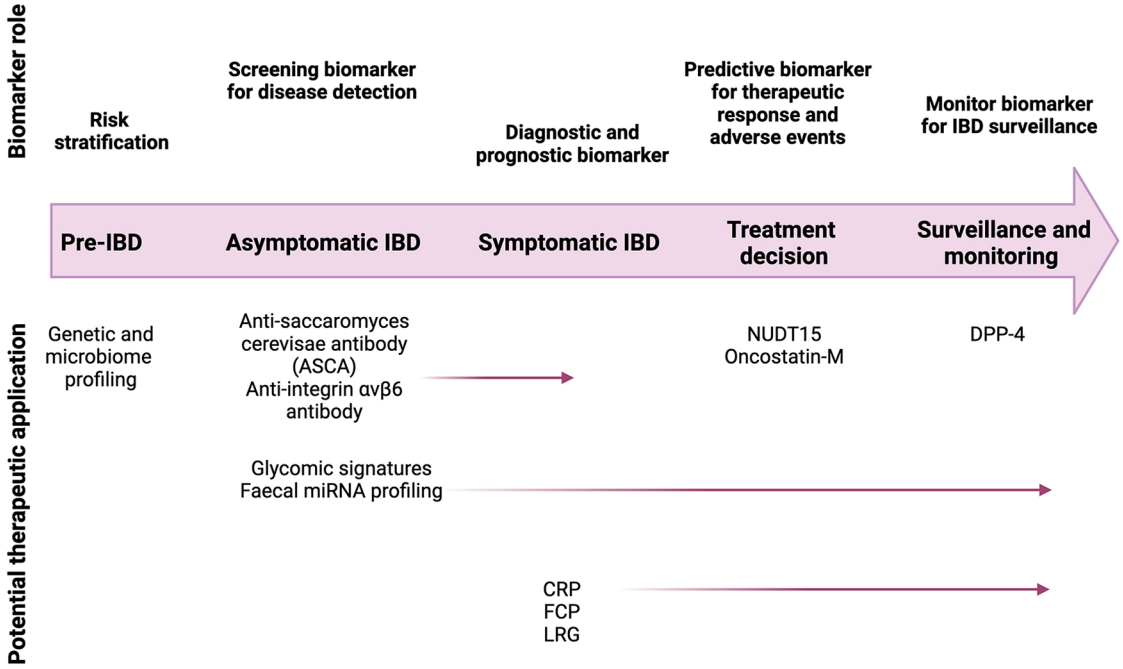


Figure 2. The potential role of biomarkers in the course of IBD, and examples of existing or novel biomarkers which could perform these roles.
Source: Figure created in BioRender.
CRP, C-reactive protein; DPP-4, dipeptidyl peptidase-4; FCP, faecal calprotectin; LRG, leucine-rich alpha-2 glycoprotein.

available permitting individual sample analysis, with data suggesting comparable accuracy to traditional ELISA testing.[102,103] Newer point-of-care (POC) and home FCP tests have been developed using lateral flow immune assays, with a sample reader able to provide either quantitative or semi-quantitative results.[30,104] POC tests have been shown to deliver rapid FCP results which correlate well with endoscopic disease activity,[30] but they are costly and their use may rely on the use of smartphone applications, limiting their accessibility to certain patient populations. Importantly, longitudinal samples from the same patient should only be compared where they have been analysed using the same FCP assay.

Faecal immunochemical testing

The faecal immunochemical test (FIT) measures faecal haemoglobin concentrations using a specific antibody and is widely used in primary care settings to predict colorectal cancer risk and the need for referral for endoscopic examination.[105,106] However, FIT is also able to sensitively detect mucosal inflammation and occult luminal blood loss, suggesting a role as a potential biomarker in IBD.[107–109] A prospective study demonstrated that a combination of FIT <100 ng/mL and FCP <250 µg/g was strongly predictive of mucosal healing, though demonstrated better performance in UC than CD.[110]

Potential advantages of FIT over FCP testing include lower cost, higher throughput and the benefit of automation over ELISA-based assays.[111] However, as with FCP testing, a limitation of FIT testing is its lack of specificity, with elevations also seen in systemic inflammatory disease and other inflammatory gastrointestinal pathologies, as well as colorectal cancer.[112] Similarly, FIT testing does not appear to perform as well in the detection of small bowel CD compared to colonic IBD.[110] This may relate to the optimization of FIT for the detection of colorectal malignancies, and it is notable that FIT is less sensitive for the detection of right-sided colonic lesions.[113]

The future of biomarkers in IBD

Of the biomarkers initially identified through research, few make it through clinical testing and validation to become available for use in clinical practice. For those that do, the timeline is long

and arduous, with an estimate of over 10 years from discovery to clinical use. Proposed biomarkers frequently do not perform as anticipated during clinical validation studies, with the recently published PROFILE study demonstrating the challenges in biomarker development and implementation.[114] Collaborative research efforts identified a 17-gene blood-based biomarker based on $CD8^+$ T-cell transcriptional signatures which were able to categorize patients into two groups associated with higher ('IBDhi') or lower ('IBDlo') risk of treatment escalation.[115] Initial validation cohorts confirmed the ability of the prognostic biomarker to identify patients at risk of a more aggressive disease course,[116] but in a larger randomized-controlled prospective CD cohort the biomarker was not able to predict patients most likely to benefit from early advanced therapy.[114]

Whilst currently available biomarkers are primarily used for differentiation of IBD from functional pathology and disease monitoring, future biomarkers could have a role in risk stratification of subjects without disease, as well as screening of asymptomatic individuals for IBD (Figure 2). Artificial intelligence (AI)-based methods are likely to expand the horizon for biomarker discovery, enabling the integration of multimodal data from existing datasets to discover new biomarkers.[117] We describe a series of novel biomarkers at various stages of the discovery pipeline, as well as newer techniques for evaluating existing biomarkers.

Novel biomarkers

Oncostatin M. Oncostatin M (OSM) is part of the IL-6 cytokine family and signals through a receptor complex to induce JAK-STAT or P13K-Akt pathway signalling, depending on the cell type and environmental conditions.[118] A role for the OSM signalling axis in the pathogenesis of IBD was first suggested with the discovery of a disease-susceptibility polymorphism within the OSM receptor locus,[119] and cytokine expression panels have identified OSM as the most highly and consistently expressed cytokine in the inflamed mucosa of patients with IBD.[120] OSM is proposed to act as an inflammatory amplifier and a driver of disease chronicity.

Newly diagnosed patients with both UC and CD demonstrate increased mucosal expression of OSM compared to control subjects, and elevated serum OSM was able to predict post-operative CD recurrence 6 months after surgery with greater accuracy than FCP.[121] Elevated colonic OSM and OSM receptor expression were associated with a worse disease prognosis in terms of the requirement to escalate biologic therapy, and high pre-treatment mucosal OSM expression was strongly associated with primary non-response to anti-TNF.[120] Interestingly, serum OSM levels were also elevated in first-degree relatives of IBD patients,[121] although further work is required to define whether this is predictive of the development of future IBD in these subjects. OSM is also detectable in faeces and has been shown to predict endoscopic disease activity both on its own and in combination with FCP.[122]

Glycome profiling. Glycans are sequences of carbohydrates conjugated to proteins and lipids. Most secreted proteins are glycosylated through post-translational modification, and glycans play an essential role in the regulation of biological processes including protein folding, immune cell migration and adhesion and pathogen recognition.[123] Studies have demonstrated that aberrant glycosylation is associated with numerous inflammatory diseases, including IBD, with the serum *N*-glycome a possible source for biomarker discovery.[124] Compared to healthy cohorts, IBD patients exhibit a significant decrease in levels of galactosylation and sialylation, as well as altered glycan complexity.[125] Glycomic signatures generated through ultra-high performance liquid chromatography from the serum of IBD patients obtained at the time of diagnosis were able to predict the need for IBD treatment escalation, with the potential for utility in guiding treatment decisions.[126] Changes to the glycosylation profile of faecal mucins are also evident in patients with CD compared to healthy controls, suggesting a role in non-invasive monitoring.[127]

Leucine-rich alpha 2-glycoprotein. Leucine-rich alpha-2 glycoprotein (LRG) is predominantly derived from neutrophils, macrophages, gut epithelial cells and hepatocytes in response to elevated TNFα, IL-1β, IL-6 and IL-22.[128] Elevated LRG levels have been reported in IBD patients with clinically and endoscopically active disease, and other inflammatory disorders including rheumatoid arthritis, systemic lupus erythematosus and primary biliary cholangitis.[129–131]

In studies in patients with UC, serum LRG levels were correlated with endoscopic disease activity but were not able to outperform standard FCP testing.[131] In patients with CD, the performance of serum LRG testing appears to be equivalent to that of CRP and FCP in identifying those with endoscopically active disease. Importantly, serum LRG could predict mucosal healing in both patients with UC and CD with normal CRP levels, suggesting a valuable role as a serum biomarker with superior performance over CRP.[131] In addition, levels of serum correlate well with active small bowel CD[132]; a situation in which FCP alone performs less well.

Faecal myeloperoxidase. Whilst FCP has been extensively studied as a biomarker, additional faecal neutrophil markers may also play a role in IBD monitoring. Myeloperoxidase (MPO) is an abundant neutrophil enzyme that plays a vital role in killing bacteria through the production of hypochlorous acid, but this can also promote inflammatory tissue damage.[133] The proposed benefit of testing faecal MPO (fMPO) is that it is not susceptible to oxidative proteolysis, which can reduce the measure of FCP in stool samples thus underestimating the inflammatory burden.[100] Elevated fMPO has been described in small historic studies, particularly in the setting of UC, but these studies demonstrated considerable variability in fMPO measurement.[134–136] A larger study ($n = 172$) including patients with both UC and CD demonstrated similar performance characteristics to FCP in the ability of fMPO to predict moderate-to-severely active IBD, with elevated fMPO levels predictive of a more severe disease course and need for treatment escalation within 12 months of follow-up.[137] Longer-term follow-up indicated that a raised fMPO is associated with long-term IBD outcomes over a 24-month period, with a combination of baseline CRP, FCP, fMPO and clinical symptom score giving the most accurate prediction of a complicated disease course.[84]

Faecal microRNAs. Micro (mi)-RNAs are small non-coding RNAs detectable in extracellular fluids. Evidence suggests that miRNA dysregulation in IBD could contribute to intestinal inflammation through increased fibrosis, activation of Nuclear factor kappa B (NF-κB) signalling and altered autophagy.[138] miRNAs are resistant to degradation and easy to detect through existing laboratory techniques, making them attractive as potential biomarkers.[139] Faecal miRNA profiling has demonstrated distinctly different composition in subjects with CD compared to healthy control subjects, with miR-223 and miR-1246 present at high levels in the stool of subjects with active IBD.[140] miR-223 can be detected in both serum and faeces and correlates well with clinical disease activity scores in CD.[141] Future work may enable the identification of specific miRNA signatures associated with specific IBD phenotypes or treatment-refractory states to guide treatment decisions.

Antibodies for the detection of IBD. Integrins are key proteins involved in cell adhesion and are comprised of an α and a β chain, with 24 combinations of chains identified.[142] The αvβ6 protein is expressed on epithelial cells, with a key role in maintaining epithelial barrier integrity.[143] Anti-integrin αvβ6 antibodies may have a role in the pathophysiology of UC, with 92% of patients with UC testing positive compared to 5.2% healthy control subjects. The presence of the αvβ6 antibody had a high specificity and sensitivity for the diagnosis of UC, with a positive correlation between antibody titre and Mayo score, suggesting it may be useful as a biomarker for both diagnosis and disease monitoring.[143] Antibodies to oligomannosidic epitopes of the yeast *Saccharomyces cerevisiae* (ASCA) have been associated with the development of CD, and when used in combination with a negative perinuclear antineutrophil cytoplasmic autoantibody test demonstrated a high sensitivity and specificity for the diagnosis of CD.[144]

Biomarkers for the prediction of fibrostenosing CD. Biomarkers which could be used singly or in combination to predict those at risk of fibrostenotic complications of CD could have clinical utility in stratifying patients most likely to benefit from aggressive treatment escalation. A systematic review identified 35 distinct markers of intestinal fibrosis, which were subsequently categorized into serum ($n = 20$), genetic ($n = 9$) and histopathology markers ($n = 8$).[145] Serum markers included anti-microbial antibodies (including ASCA) and anti-flagellins,[146,147] collagen and matrix proteins,[148,149] and miRNAs.[150] The *NOD2*/*CARD* mutations have been established as genetic variants associated with stricturing disease,[151] with additional genetic associations including IL-12B polymorphisms and IL-10 variants.[152] Whilst numerous potential markers of fibrotic CD have been identified, there is significant heterogeneity in their performance and none have yet undergone clinical validation.

Dipeptidyl peptidase 4. Dipeptidyl peptidase (DPP)-4 is nearly ubiquitously expressed and serves an essential role in many metabolic functions, including the regulation of glucose metabolism and the activation of cytokines, chemokines and neuropeptides involved in inflammation.[153] DPP-4 inhibitors, principally prescribed for glycaemic control, have been shown to suppress inflammation and alleviate oxidative stress.[154] However, the role of DPP-4 in the pathogenesis of IBD is unclear. Whilst some studies demonstrate a reduced risk of IBD in patients receiving DPP-4 inhibitors,[155] others report an increased IBD risk in association with these agents.[156] In IBD patients, serum DPP-4 appears to be inversely correlated with clinically active disease, although initial studies lacked robust endoscopic assessment.[157]

New techniques for measuring existing biomarkers

In addition to the advent of novel biomarkers to define disease activity and drug response, new patient-centred ways of measuring existing biomarkers are emerging. A wearable sensor device with the ability to measure CRP and IL-1β secreted into eccrine sweat has been developed, allowing real-time monitoring of these inflammatory biomarkers and early flare detection.[158] FCP measurement using the standard ELISA technique is frequently time-consuming, with a typical turnaround time of several days. Rapid POC tests use lateral flow chromatography to provide semi-quantitative results within 30 min and show reasonable agreement with ELISA results.[159] Newer methods in which a smartphone application scans a faecal sample to calculate FCP concentration have been proposed and validated, allowing patients to obtain an immediate FCP result in their own homes.[160] Rather than providing a stool sample, which patients can find unpleasant and inconvenient, FCP can also be measured in colonic mucous swabbed from the anus after defecation.[161] Furthermore, the use of urinary biomarkers in IBD is being explored, which may be more acceptable to patients than stool sample provision.[162]

Conclusion

Despite the promise of advances in biomarker discovery, most clinicians currently find themselves limited to the use of CRP and FCP in routine clinical practice. It is important, therefore, to understand the strengths and limitations of these commonly used biomarkers, and the guidance offered in this article is designed to support practicing gastroenterologists. Where doubt exists about variance in performance of biomarkers, or a lack of corroboration between clinical symptoms and biomarker results, endoscopy remains the gold standard tool for assessment of endoscopic healing and is still frequently used to guide treatment decisions. The growing availability of intestinal ultrasound as a POC tool for assessing disease activity is also expected to enhance decision-making in IBD care.[163,164]

A number of groups are currently underserved by existing biomarkers, including patients with isolated ileal CD, and patients with proctitis, in whom neither CRP nor FCP perform well in disease assessment and monitoring. Future research should focus on evaluation biomarkers that could accurately predict treatment response and outcomes in these populations, especially in the case of small bowel CD where disease is not easily endoscopically accessible. In addition, there is no current access outside of the research setting for biomarkers that can quantify the risk of developing IBD in a susceptible individual, nor screen for IBD detection in asymptomatic individuals.

Precision medicine, which seeks to target therapies by evaluating genetic factors and biomarkers to identify the most active inflammatory pathways in a given patient, is a major goal of future IBD care delivery.[9] Despite international research efforts, we still have some way to go before we are truly able to deliver personalized medicine in IBD – no currently available biomarker has been robustly validated in predicting response to individual advanced therapies. This represents a significant challenge in an era of ever-expanding IBD therapy, where there is little evidence to guide clinician and patient decision-making in treatment selection and sequencing. With the ever-increasing complexity of available data, AI is likely to play a valuable role in integrating genetic, transcriptomic, proteomic and metabolomic outputs to help achieve this goal,[10,12,117] driven by advances in the affordability and availability of technology.

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Not applicable.

Author contributions
Jennie Clough: Project administration; Writing – original draft; Writing – review & editing.

Michael Colwill: Writing – original draft; Writing – review & editing.

Andrew Poullis: Supervision; Writing – review & editing.

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ORCID iD
Sailish Honap https://orcid.org/0000-0001-6657-2763

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Appendix

Abbreviations

AI	artificial intelligence
ASUC	acute severe ulcerative colitis
CAR	C-reactive protein-to-albumin ratio
CD	Crohn's disease
CDAI	Crohn's disease activity index
CDEIS	Crohn's disease endoscopic index of severity
CRP	C-reactive protein
ECCO	European Crohn's and Colitis Organisation
FIT	faecal immunochemical testing
fMPO	faecal myeloperoxidase
HBI	Harvey-Bradshaw index
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IL	interleukin
JAK	janus kinase
LRG	leucine-rich alpha-2 glycoprotein
MES	Mayo endoscopic sub-score
PMS	partial Mayo score
POC	point of care
UC	ulcerative colitis
FCP	faecal calprotectin
TNFα	tumour necrosis factor alpha