



## Review Article

# Use of Biomarkers in the Management of Inflammatory Bowel Disease



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## Abstract

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of the GI tract. The etiology is unclear, and most clinical symptoms are nonspecific, making the diagnosis and prognosis of IBD challenging as there is no gold-standard diagnostic test. Both endoscopy and imaging are essential diagnostic tools for determining disease state, location, and severity. However, the high cost and invasive nature of these tests make them unrealistic for frequent assessment. Given these limitations, laboratory testing of blood and feces has proven to be a viable alternative for routine disease monitoring. To integrate more efficient and personalized treatment strategies, new studies are consistently emerging to develop minimally invasive testing that can predict disease severity and response to available treatments. The goal is to develop better predictors of disease course, response to therapy, and therapy-related adverse events, thereby establishing a more efficient and personalized treatment strategy. This review aimed to delve into existing literature to assemble a collection of currently used biomarkers that aid in monitoring treatment response, as well as highlight select novel and combined biomarkers that hold promise for future management of IBD.

## Introduction

Inflammatory bowel disease (IBD) affects about one in 200 people in developed countries and has begun to show a rising incidence in developing and newly industrialized countries.<sup>1</sup> A rising incidence has been noted in South America, Eastern Europe, Asia, and Africa as populations move from rural to urban settings, which could cause strain on healthcare systems not previously exposed to this chronic, complex, and costly disease.<sup>2,3</sup> IBD can cause a lifetime of debilitating symptoms, which frequently affect psychosocial well-being, such as limiting academic attainment, making it difficult to sustain employment, and nurturing relationships. The two major forms of IBD are Crohn's disease (CD), which can cause transmural inflammation of any region of the GI tract,<sup>4</sup> and ulcerative colitis (UC), which produces continuous mucosal inflammation in the innermost layers of the colon and rectum.<sup>5</sup>

Due to the relative unpredictability of treatment response and symptom relapses, it is imperative to have reliable and widely available methods for monitoring disease activity. It can also be chal-

lenging to differentiate between IBD and colitis of other etiologies. A recent study by Porter *et al.*<sup>6</sup> utilizes an IBD pre-disease cohort study, drawing on unique data from a multinational specimen repository entitled "Proteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects (PREDICTS)". This study combines resources and expertise to advance novel discoveries and translational research. Similar data repositories are utilized in the COMPASS and OSCCAR cohorts to collect longitudinal data on individuals with IBD.<sup>7,8</sup> A delayed or inaccurate diagnosis can adversely affect treatment by reducing treatment efficacy<sup>9</sup> which can hinder recovery and cause unnecessary harm. In the future, optimal IBD management will involve personalized treatment plans requiring better methods for predicting disease onset and response to therapy.

The purpose of this article is to review biomarkers used in IBD management, from classical biomarkers (Table 1),<sup>10–17</sup> which are well-established and widely available, to new and innovative biomarkers (Table 2), as well as biomarker panels and ratios that hold promise in directing disease management in the years to come.

**Keywords:** Inflammatory Bowel Disease; Crohn's; Ulcerative Colitis; Biomarkers; Fecal calprotectin; C-reactive protein.

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## Established biomarkers that are widely available

### Serum biomarkers

#### C-reactive protein (CRP)

CRP is one of the most ubiquitously used biomarkers given its low cost, ease of testing, and well-established protocols regard-

**Table 1. Established biomarker uses and threshold values**

	Clinical Use	Threshold Values	Special Considerations
CRP	Surveillance of disease activity, indicator of active disease, predicting clinical response	<1 mg/L - normal > 5 mg/L - sensitivity of 70% to predict IBD <sup>10</sup> > 20 mg/L - predictive of short-term relapse <sup>16</sup>	Elevation is not specific for IBD
ESR	Surveillance of disease activity, indicator of active disease	< 15–20 mm/hour - normal > 15 mm/hour - predictive of short-term relapse <sup>15</sup>	Variable based on age/sex
Vitamin D	Prediction of disease recurrence, hospitalizations, surgeries, response to anti-TNF $\alpha$ therapy	< 50 nmol/L - insufficiency <sup>12</sup> $\geq$ 50 nmol/L - supplementation goal <sup>12</sup>	Can be variable based on time of year and sun exposure
Platelets	Disease surveillance, prediction of disease severity, inflammatory mediators	$\geq 450 \times 10^9/L$ - indicative of reactive thrombocytosis <sup>13</sup>	Other dysfunctions seen in IBD include decreased mean platelet volume, increased activation in peripheral circulation, spontaneous aggregation, and mucosal microvascular thrombi <sup>13</sup>
Fecal calprotectin	Surveillance of disease activity, indicator of active disease, predicting clinical response	> 150–250 $\mu\text{g/g}$ indicative of active disease <sup>11</sup> > 140 $\mu\text{g/g}$ had 83% sensitivity and 93% specificity to predict disease recurrence <sup>16</sup> < 82 $\mu\text{g/g}$ predicted sustained clinic response to maintenance treatment on anti-TNF $\alpha$ <sup>14</sup>	Some debate about where “normal” cutoffs should be set
Fecal lactoferrin	Surveillance of disease activity, indicator of active disease, predicting clinical response	< 7.25 $\mu\text{g/g}$ indicates lack of intestinal inflammation > 125 $\mu\text{g/g}$ had a diagnostic accuracy of 65% <sup>17</sup> > 140 $\mu\text{g/g}$ had sensitivity of 67% and specificity of 71% to predict disease recurrence <sup>17</sup>	

CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; IBD, Inflammatory bowel disease; TNF, Tumor necrosis factor.

ing its usage. It is one of the body's acute-phase reactants, and its production is stimulated in hepatocytes by pro-inflammatory cytokines.<sup>18,19</sup> Its utility as an indicator of inflammation is related to its relatively short half-life of 19 hours compared to other acute-phase proteins.<sup>10</sup> Though CRP is widely used as a biomarker for IBD, its diagnostic value is limited by its lack of specificity. Elevations in CRP can also be caused by other inflammatory conditions such as autoimmune disorders, infections, and malignancies.<sup>19</sup> Thus, CRP levels cannot be diagnostic of IBD in isolation but must be interpreted along with the clinical picture.<sup>20</sup>

In the absence of inflammation, serum CRP is typically low (< 1 mg/L) but can increase over 1,000-fold in the setting of acute inflammation.<sup>9</sup> Prior studies on CD have found a significant association between CRP elevation and moderate to severe clinical activity and evidence of active disease on ileocolonoscopy. However, due to unclear causes, there has not been a strong correla-

tion between CRP levels and disease activity in UC.<sup>10,21</sup> Possible explanations include the difference in IL-6 production in UC and CRP production by mesenteric adipocytes in patients with CD.<sup>22</sup>

Conversely, normal CRP does not rule out active IBD.<sup>11</sup> Prior studies have found a subset of patients with Crohn's disease harboring genetic variations that limit CRP elevations.<sup>23</sup> Based on the ACCENT1 trial, patients with elevated baseline CRP and those whose CRP normalized by week 14 of treatment with infliximab were more likely to maintain clinical remission and treatment response. Thus, CRP can be a useful biomarker<sup>24</sup> in those whose CRP levels correspond to their disease activity.

Recent guidelines for UC management suggest monitoring CRP and fecal calprotectin in asymptomatic individuals to avoid more costly and invasive testing, such as endoscopy, for routine disease activity assessment.<sup>25</sup> Similar guidelines were released following the CALM study, which showed improved clinical and endo-

**Table 2. Novel biomarkers**

	Disease Assessment	Treatment Response	Sample Type
MadCAM-1	Elevation corresponds with inflammation	May be predictive of response to vedolizumab	Tissue biopsy or blood sample
Oncostatin M	Elevation could predict risk of IBD development	Possibly predictive of nonresponse to vedolizumab or corticosteroids	Tissue biopsy
NOD-2	Mutations could be predictive of fibrostenotic disease in CD	May predict severe ileal disease but not specific for treatment response to biologics or corticosteroids	Genetic analysis
Anti-Integrin $\alpha\text{v}\beta 6$	Useful for diagnosis and predicting disease severity in UC	Predictive of severe disease but not specific for treatment response to biologics or corticosteroids	Blood sample

CD, Crohn's disease; IBD, Inflammatory bowel disease; MadCAM-1, Mucosal addressin cell-adhesion molecule – 1; NOD2, Nucleotide oligomerization domain 2; UC, Ulcerative colitis.

scopic outcomes in patients with CD when therapy decisions were based on clinical symptoms and biomarkers rather than symptoms alone.<sup>26</sup> A large prospective observational study of CD patients in a tertiary referral center showed that asymptomatic patients with elevated CRP levels were over twice as likely to be hospitalized over a two-year follow-up period.<sup>27</sup> This study provides real-world evidence that CRP is a useful biomarker for predicting clinical outcomes in CD patients.

Salivary CRP also presents an intriguing alternative to typical serum sampling as an even more easily obtainable biomarker of IBD activity for disease tracking in select patients. Future studies are needed to establish optimal clinical applications for this alternative to venous sampling.<sup>28</sup>

## ESR

Erythrocyte sedimentation rate (ESR) is commonly tested in conjunction with CRP. It is a measure of inflammation based on how quickly erythrocytes sediment through plasma in a column. A higher sedimentation rate indicates inflammation. Like CRP, elevations in ESR are not specific to IBD and can occur in response to any inflammatory stimulus. It differs from CRP in that it peaks more slowly and takes longer to return to normal. Additionally, it does not show the same variability with UC that CRP does and tends to respond similarly to the inflammation seen in UC and in CD.<sup>19</sup>

It is important to remember that ESR can be affected by other physiological factors such as pregnancy, age, and gender, as well as changes in hematocrit seen in patients with anemia and polycythemia.<sup>19</sup> Additionally, changes in the size of erythrocytes can also affect ESR values, such as those seen in certain disease states or as a side effect of some medications.<sup>29</sup> This becomes particularly important when monitoring ESR in patients on azathioprine or 6-mercaptopurine, as these medications have been shown to cause elevated ESR despite normal CRP and no clinical evidence of active disease.<sup>30</sup>

## Vitamin D

Vitamin D is an immune modulator involved in both innate and adaptive immunity. It is primarily produced in the skin upon exposure to sunlight (UVB) or absorbed by the small intestine following food intake.<sup>12</sup> Vitamin D deficiency in IBD patients is associated with an increased risk of disease recurrence, hospitalizations, and surgeries.<sup>9</sup> Vitamin D deficiency is common in the general population due to inadequate exposure to sunlight, impaired enzymatic activation, lower bioavailability, insufficient physical activity, and smoking. In addition to these common risks, IBD patients have an increased risk of osteopenia and osteoporosis due to malabsorption of calcium and vitamin D caused by disease flares or prior surgery, dietary restrictions, and frequent use of medications that inhibit bone formation or increase bone turnover.<sup>29</sup>

Serum vitamin D levels are lower in IBD patients compared to those with IBS. In patients with CD, vitamin D levels negatively correlate with disease activity and inflammatory markers such as CRP.<sup>12</sup> Multiple studies have even suggested a role for vitamin D deficiency in the pathogenesis of CD, not simply as a consequence of the disease itself. Thus, robust supplementation of vitamin D may be of therapeutic benefit.<sup>31</sup> Similar effects have been demonstrated in UC, with vitamin D interacting with anti-inflammatory serum cytokines.<sup>32</sup> Patients with active IBD and those who have required over three months of steroid treatment should have their calcium and vitamin D levels monitored. Physicians should have a low threshold to start supplements to prevent low bone mineral

density.<sup>13</sup> Studies investigating therapeutic effects of vitamin D related to IBD are limited, and further research is needed to determine the optimal range and therapeutic potential of vitamin D.

## Platelets

Platelets are a commonly tested lab value yet are often overlooked in the evaluation of IBD patients. Evidence has increasingly shown that, in addition to their primary hemostatic function, platelets also play an active role in multiple inflammatory processes. “Reactive thrombocytosis” is now a well-established phenomenon in the setting of inflammation.<sup>33</sup> Many changes in platelet structure and function occur in IBD, especially in the setting of active disease. Inflamed bowel tissue secretes platelet activation factor, which affects circulating platelet levels and coagulation.<sup>34</sup> Compared to healthy controls, the platelets of those with IBD are more sensitive to activation, even in clinically silent disease.<sup>33</sup> Some small studies have even suggested that increased platelet counts in patients who have UC with mucosal healing could be predictive of relapse.<sup>35</sup>

Mucosal capillary thrombi have been identified in rectal biopsies of patients with CD and UC, suggesting that platelets may be involved in chronic intestinal inflammation. This finding does not appear to correlate with disease severity or the extent of inflammation in IBD patients, but these microthrombi are consistently absent in the mucosa of normal subjects.<sup>33</sup>

## Fecal biomarkers

Fecal biomarkers, primarily composed of fecal leukocyte proteins, are commonly used to assess disease severity in patients with IBD). They may be preferred over blood samples at times due to ease of sample accessibility and higher specificity for gastrointestinal inflammation.<sup>19</sup> Fecal biomarkers are likely to be the most accurate in individuals who have previously manifested an elevation and those whose biomarker activity correlates with endoscopic disease severity.<sup>25</sup>

However, studies indicate that compliance rates rarely exceed 60% for various reasons<sup>36</sup> including forgetfulness, lack of perceived benefit, and reluctance to handle feces.<sup>37</sup> Despite being an invasive procedure, blood collection is typically more readily accepted by most patients and can be completed expeditiously during routine follow-up visits. Current studies are underway to assess the viability of home fecal calprotectin tests akin to home testing for diabetes and hypertension, with patient reporting.<sup>38</sup> However, further studies supporting the utility and reliability of these tests are needed.

## Fecal calprotectin

Calprotectin is released by activated innate immune cells in response to cell damage or stress.<sup>29</sup> It belongs to the S100 family of proteins and serves to regulate protein phosphorylation, intracellular calcium regulation, and protection against oxidative cell damage within neutrophils. Its extracellular functions include antimicrobial and antifungal activities, as well as regulation of apoptosis and inflammation.<sup>14</sup>

Causes of elevated fecal calprotectin (FC) other than IBD include NSAID enteropathy, pancreatic insufficiency, alcoholic enteropathy, colorectal cancer, and microscopic colitis. Since neutrophils are relatively scarce in normal intestinal mucosa, FC levels are low in healthy individuals.<sup>39</sup> For this reason, it is also useful for distinguishing between functional and organic diseases, especially in the setting of known IBD who may also have IBS overlap symptoms despite adequate control of inflammation.<sup>40</sup> The sensitivity and specificity of FC for Crohn’s disease are 100% and

97%, respectively, compared to IBS.<sup>29,40</sup> Fecal calprotectin also has the potential to differentiate between perianal fistulas due to CD and cryptoglandular perianal fistulas,<sup>41</sup> which is a common benign anorectal disorder that is mainly managed with surgery.<sup>42</sup> A meta-analysis indicated that FC testing could reduce endoscopy by 67% in adults, although it could lead to treatment delays in 6% of patients due to false negatives when used as a screening tool for IBD.<sup>29,43</sup>

Fecal calprotectin is valuable for assessing active disease and monitoring treatment response, as FC levels decrease with mucosal healing. Persistently high levels in IBD patients in remission could predict a higher risk of disease relapse within the next 12 months.<sup>44</sup> This correlation may have a higher predictive value for UC than for CD, likely due to differences in inflammatory patterns.<sup>45</sup> In UC, an FC level  $\leq 250$   $\mu\text{g/g}$  following biologic induction was associated with a higher probability of achieving clinical, endoscopic, and histologic remission by week 52, as well as a decreased probability of colectomy within 7 years.<sup>46</sup> Elevated FC in UC patients in clinical and endoscopic remission has also been associated with the risk of relapse.<sup>47</sup>

Nevertheless, recent studies have shown that FC remains a useful tool for evaluating active disease in isolated small bowel CD with both inflammatory and stenotic disease but may not be as effective for monitoring penetrating disease.<sup>48</sup> The combined evaluation of FC, hemoglobin, and CRP at least once may improve CD monitoring and management through risk matrices.<sup>49</sup> Notably, FC from ileostomy output demonstrates high sensitivity and specificity for monitoring small bowel inflammation and disease recurrence in post-operative CD patients.<sup>50</sup>

Establishing baseline FC levels has been challenging due to differences in extraction methods and variable baseline levels among certain populations. For example, individuals from areas with poor sanitation may have elevated baseline FC levels.<sup>11</sup> Children also have a lower reference range for FC than adults.<sup>39</sup> There may also be variability depending on the time of sample collection throughout the day. Therefore, it is generally recommended to collect samples in the morning to standardize testing and reduce variability.<sup>51,52</sup> Despite these variations, most studies agree that FC levels of 150–250  $\mu\text{g/g}$  indicate active disease.<sup>11</sup>

### Fecal lactoferrin

Lactoferrin is an iron-binding glycoprotein found in neutrophil granulocytes and is activated during acute inflammation.<sup>19</sup> The diagnostic accuracy of fecal lactoferrin is similar to that of fecal calprotectin and is superior to CRP.<sup>29</sup> Like fecal calprotectin, fecal lactoferrin may be influenced by the extent and location of the inflamed mucosa. However, there is limited data regarding its prognostic value.<sup>25</sup>

### Novel biomarkers

#### Mucosal addressin cell adhesion molecule-1 (MAdCAM-1)

MAdCAM-1 is expressed by endothelial cells and stimulates intestinal inflammation by binding adhesion molecules on immune cells. Elevated MAdCAM-1 expression in tissue correlates with endoscopic and histologic evidence of inflammation. Higher levels are also noted in patients with a Mayo Endoscopic Score of one who subsequently relapse.<sup>53</sup> These associations make it a promising biomarker for monitoring disease and stratifying relapse risk.

Vedolizumab, a biologic used to treat UC and CD, blocks the interaction of MAdCAM-1 with its integrin receptor to reduce inflammation. Vedolizumab is considered a “slow-acting” biologic

due to its relatively delayed onset of action. Therefore, identifying biomarkers that could predict response to vedolizumab would be particularly helpful in avoiding long periods of ineffective treatment. MAdCAM-1, particularly its adhesion to CD4<sup>+</sup> T cells in the peripheral blood of IBD patients, correlates with subsequent clinical response to vedolizumab therapy in small studies.<sup>54</sup> On the other hand, if intestinal endothelial cells do not express MAdCAM-1, there will likely be no clinical response to vedolizumab.<sup>55</sup>

The OPERA study evaluated a monoclonal antibody directed against MAdCAM-1 as a potential treatment option for moderate-to-severe CD but did not achieve a greater treatment effect than placebo.<sup>56</sup> Some suggest this finding may be related to dose effect or drug delivery methods, as vedolizumab, which utilizes a similar pathway, has proven effective in treating CD patients.<sup>57</sup>

#### Oncostatin M (OSM)

Oncostatin M belongs to the IL-6 cytokine family and is involved in liver repair, cardiac tissue remodeling, osteoclastogenesis, and hematopoiesis. However, excessive OSM production can contribute to skin and lung inflammation, atherosclerosis, and various cancers.<sup>58</sup> Both OSM and its receptor, OSMR, consistently show elevated levels in both the blood and inflamed mucosa of IBD patients.<sup>9,59</sup> A single nucleotide polymorphism on chromosome 5 in the human OSM locus is strongly associated with the risk of IBD development.<sup>58,60,61</sup> Therefore, serum OSM testing could be a promising diagnostic biomarker for identifying IBD patients, especially those with a first-degree relative.<sup>9</sup>

Hematopoietically derived OSM appears to promote inflammatory responses by enhancing the production of chemokines, cytokines, and adhesion factors by intestinal stromal cells. Overexpression of OSM in intestinal mucosa is consistently associated with an increased risk of resistance to anti-tumor necrosis factor (TNF) therapy.<sup>58</sup> Since up to 40% of patients do not respond to anti-TNF agents, identifying alternative therapeutic targets could reduce corticosteroid usage.<sup>60,61</sup> Because mucosal OSM correlates closely with histopathological disease severity, it raises the question of whether OSM signal is truly predictive for lack of response to anti-TNF agents specifically or is simply a marker of a more refractory and difficult-to-treat disease. The routine use of OSM in predicting clinical response is currently limited by the fact that the mucosal signal of OSM could not be reliably translated into either whole blood or serologic OSM biomarker levels.<sup>59</sup>

In tissues with increased extracellular matrix protein deposition, such as those observed in chronic inflammation and fibrosis, OSM's effects may be amplified due to its increased stability in such environments.<sup>58</sup> Up to 15% of CD patients will develop fibrostenotic disease with strictures within a decade after initial diagnosis.<sup>62</sup> Despite the widespread knowledge of this common phenomenon, there are no available therapeutic agents targeting intestinal fibrosis. Data in mouse models suggest that OSM exerts significant fibrogenic activity. However, its potential as a target for stricturing CD has not been investigated and no data have yet proven that neutralizing OSM can reverse fibrosis.<sup>60,63</sup> Interestingly, OSM levels are also elevated in the colonic mucosa of patients with UC despite UC not being as strongly associated with fibrosis.<sup>58</sup>

OSM has been identified as a potential mediator of nociception and is associated with common comorbidities of IBD, such as psoriasis and arthritis. Thus, OSM blockage could be beneficial for IBD patients, not only in reducing gut inflammation but also in alleviating various comorbid conditions.<sup>58</sup> Recent studies suggest that OSM may sensitize sensory afferents in IBD patients, lead-



ing to increased colonic afferent discharge. These findings suggest that OSM may contribute to the severity of abdominal pain in IBD and could be a potential target for managing chronic pain in IBD patients.<sup>64</sup>

OSM thresholds have not been established and may differ between UC and CD. This should be investigated in future studies.<sup>61</sup> Additionally, OSM may not be predictive of disease response in pediatric patients.<sup>65</sup> As a newly discovered biomarker, the potential value of OSM has garnered significant attention, but its potential uses and reliability in IBD require further evaluation.<sup>9</sup>

### Nucleotide-binding oligomerization domain protein 2 (NOD2)

First identified in 1996 on chromosome 16, NOD2 is expressed by many leukocytes as well as Paneth cells, fibroblasts, and epithelial cells. NOD2 acts as a positive regulator of immune defense, partly by regulating autophagy.<sup>67</sup> NOD2 is one of several susceptibility loci recognized in relation to IBD risk, but it is associated with CD risk alone.<sup>29</sup> It has the highest expression in terminal ileal Paneth cells, supporting its role in the development of ileal disease.<sup>67</sup> One of the crucial pathogenic mechanisms of NOD2 may involve impaired bacterial clearance. This leads to increased bacterial invasion into the mucosa, activating inflammatory pathways that contribute to the deeper, often transmural, inflammation seen in ileal CD.<sup>66,67</sup> The intestinal microbiome may play a key role as a trigger for the inflammation seen in NOD2-related CD. Knockout NOD2 [−/−] mice did not develop spontaneous colitis in sterile conditions but only developed inflammation when introduced to bacteria.<sup>68</sup>

Interestingly, the most common NOD2 mutations occur in Caucasians, and NOD2 mutations associated with CD are not observed in Asian or sub-Saharan African populations.<sup>66</sup> Therefore, sequencing for NOD2 variants could have important impacts for Caucasians as it could correlate with CD risk, but it is controversial for other ethnicities.<sup>29</sup> Between 30–50% of CD patients in the Western hemisphere carry disease-causing mutations in at least one NOD2 allele. Patients with double-dose mutations typically experience disease onset at a younger age than those with no mutation.<sup>69</sup>

However, it is worth noting that normal, healthy individuals may have NOD2 mutations on both chromosomes with no evidence of active disease.<sup>69</sup> Smoking has been proposed as a possible modulator of NOD2 mRNA expression and function, suggesting that epigenetic modification of NOD2 may confer an increased risk of developing CD through gene-environment interaction.<sup>70</sup> NOD2 variants have been associated with a familial CD with a predisposition to stricturing disease.<sup>29</sup> However, some studies suggest that NOD2 may not be directly associated with stricturing itself after accounting for disease location in the ileum.<sup>71</sup> Establishing a timely diagnosis of NOD2-associated disease could allow for more targeted treatment with either new or existing therapies to prevent irreversible fibrosis or the need for surgery. However, the utility of this strategy remains hypothetical as there have been no studies to date investigating this specific use of NOD2 as a treatment decision tool.<sup>67</sup>

### Anti-integrin $\alpha\beta6$

Integrins are cell surface glycoprotein receptor heterodimers composed of  $\alpha$  and  $\beta$  subunits. They are involved in cell signaling, proliferation, adhesion, and migration.<sup>72</sup> Integrin  $\alpha\beta6$  appears to be exclusive to epithelial cells and functions to maintain the epithelial barrier. It also attenuates the innate immune system's surveillance of the GI tract through its interaction with the extracellular matrix.<sup>36,73</sup> Loss of epithelial barrier integrity could be an

early feature of UC pathogenesis, making the appearance of anti- $\alpha\beta6$  autoantibodies a potential preclinical biomarker of disease.<sup>73</sup> While previous studies have noted reduced  $\alpha\beta6$  expression in the mucosa of CD patients,<sup>74</sup> the majority of studies have focused on the correlation with UC.

Anti- $\alpha\beta6$  autoantibodies were significantly higher among individuals who developed UC compared with controls up to 10 years before diagnosis in PREDICTS. The increasing prevalence of anti- $\alpha\beta6$  autoantibodies is superior to that of pANCA in diagnosing and predicting disease outcomes.<sup>73</sup> The presence of anti-integrin  $\alpha\beta6$  autoantibodies showed a sensitivity of 92% and a specificity of 94.8% for diagnosing UC in adult patients compared to healthy controls. Ten years before diagnosis, the anti- $\alpha\beta6$  autoantibody seropositivity was 12.2%, increasing to 54% at UC diagnosis, compared to 2.7% seropositivity across multiple time points in healthy controls. Those with recently diagnosed UC and elevated anti- $\alpha\beta6$  autoantibodies were at an increased risk of adverse outcomes, including hospitalization, disease extension, colectomy, systemic steroid use, and/or escalation to biologic therapy.<sup>73</sup> These findings have been supported in multiple studies in various populations, including Japan, the United States, Sweden, and pediatric populations as well.<sup>36,73</sup>

### Epigenetics

#### MicroRNAs (miRNAs)

MiRNAs are short, non-coding RNAs that negatively regulate gene expression at the post-transcriptional level.<sup>75</sup> The imbalance of miRNAs could explain the pathophysiologic processes of multiple diseases, such as arrhythmias, schizophrenia, cancer, and immune-related diseases. An ever-expanding number of serological miRNAs appear to be upregulated or downregulated in IBD.<sup>29</sup> Deciphering this variability could serve as a non-invasive measure of disease activity. Recent studies have shown that miRNAs mediate inflammatory responses and intestinal barrier function in the pathogenesis of IBD as well as playing an important role in endoplasmic reticulum stress and interactions with gut microbiota.<sup>9,76,77</sup>

There are multiple miRNA sequences that tend to be overexpressed in patients with IBD compared to healthy controls, and some may eventually be useful in distinguishing between CD and UC for those with unspecified IBD.<sup>9,78</sup> Comprehensive microarray profiling and quantitative PCR have been used to determine the different miRNA profiles in CD, UC, and non-IBD subjects.<sup>79</sup> Several miRNAs that show promise in the identification and treatment of IBD include:

- miRNA-192: It appears to be downregulated in the colonic mucosa of patients with active UC. It is an important inhibitory mediator of the expression of a pro-inflammatory chemokine, macrophage inflammatory peptide 2a;<sup>79,80</sup>
  - miRNA-223: It has been associated with increased inflammation of the colonic mucosa in IBD patients. It targets claudin-8, a crucial protein of the tight junctions in the intestinal mucosa, through the IL-23 pathway and impairs intestinal barrier function.<sup>81</sup> Increased levels in circulation correlate closely with disease activity in CD and UC;
  - miRNA-16: Serum expression of miRNA-16 correlates with CD localized to the small bowel as well as stenosis and penetrating forms of the disease. The activity also appears to correspond with the Crohn's Disease Activity Index. Increased levels can also be found in extensive UC. However, miRNA-16 levels did not correlate with any treatment given in CD or UC.<sup>82</sup>
- The most extensively studied miRNAs with respect to the

pathogenesis of intestinal fibrosis are the miRNA-200 family, which may induce the epithelial-to-mesenchymal transition,<sup>83</sup> and the miRNA-29 family, whose downregulation has been associated with pulmonary, cardiac, and hepatic fibrosis as well as stricturing phenotypes.<sup>84</sup>

Several research studies have shown that certain miRNAs determine the extent of glucocorticoid response in multiple diseases, including hematologic neoplasms and airway hyperresponsiveness. Further research could establish the specific roles of miRNAs in predicting glucocorticoid resistance in IBD and determine whether miRNAs could be adopted as biomarkers and/or therapeutic targets in these patients.

The utilization of miRNAs as therapeutic targets would necessitate the identification of all miRNA targets and those that are consistently dysregulated. The uptake of miRNAs beyond the target organ presents a challenging obstacle to initiating miRNA as a therapeutic intervention in diseases like IBD. Additionally, the lack of consistency between experimental processes and improper controls for normal miRNA levels present significant barriers to the utilization of miRNAs as disease biomarkers.<sup>79</sup> Any given miRNA can regulate multiple genes, consequently, targeting a single miRNA could affect several different disease processes. Because of this, therapeutic use of miRNAs is limited due to the potential for off-target effects as well as the possibility of undesirable on-target effects.<sup>85</sup> Many studies have investigated gut/colonic expression of miRNAs in IBD, but few have examined serum miRNAs, which will determine if they will actually be useful biomarkers in clinical practice.<sup>9</sup> To increase the feasibility of miRNA-based therapeutics, the field needs to address miRNA-regulated genes and gene networks, efficient miRNA delivery, and develop animal models that mimic critical aspects of IBD to enable testing the physiological role of miRNA and the impact of miRNA-targeted interventions.<sup>85</sup>

## DNA methylation

The study of epigenetics aims to define heritable changes in phenotype that affect gene expression and cannot be explained by changes in the fundamental DNA sequence.<sup>86</sup> Various forms of epigenetic modifications include DNA methylation, non-coding RNAs, histone modification, and the positioning of nucleosomes, each of which is influenced by the interplay between the environment and the genome. DNA methylation is a chemical modification of DNA involving the covalent bonding of a methyl group to cytosine, which primarily occurs at cytosine-phosphate-guanine (CpG) dinucleotides. Regions with a relatively high concentration of CpG dinucleotide clusters are named CpG islands. These islands lead to decreased transcriptional activity in that region. Compared to genetic biomarkers, DNA methylation incorporates the influence of age as well as cumulative environmental experiences such as smoking and diet. Furthermore, DNA biomarkers remain stable in the bloodstream, body tissues, and stool, making them advantageous for detection and preservation.<sup>87</sup>

The importance of lifestyle in disease susceptibility is supported by the rising incidence of CD in newly industrialized countries in Africa, Asia, and South America.<sup>86,88</sup> Epigenetic modifications shaped by environmental factors may help to explain the increasing incidence of IBD. The dynamic and reversible nature of epigenetic gene modifications gives them potential as novel therapeutic targets.<sup>88</sup> The methylation of genes changes their transcriptional activity, and in the context of IBD, these gene alterations could impact disease risk and progression. Varied methylation status also appears to correlate with endoscopic disease severity. However,

there are limited studies comparing DNA methylation signatures in peripheral blood compared to mucosal biopsies in active and inactive disease states.<sup>87</sup>

Distinct methylation patterns were identified in genome analysis of treatment-naïve UC patients, which identified hypermethylation of genes involved in homeostasis and defense, and hypomethylation of genes for cytokines and chemokines involved in the immune response.<sup>89</sup> Jousra *et al.*<sup>15</sup> recently reported on three validated panels of highly stable epigenetic biomarkers that could be used to predict clinical and endoscopic response in CD patients treated with adalimumab, vedolizumab, or ustekinumab. They identified distinct CpG loci that, in combination, accurately predicted clinical and endoscopic responses. Notably, for these CpG loci, methylation levels remained stable during both induction and maintenance of treatment, regardless of inflammatory status and therapeutic intervention.<sup>15</sup>

Different mucosal methylation changes of several genes in IBD patients have been used to distinguish between CD and UC as well as differentiate from healthy controls. However, there are limitations due to differences in methylation profiles in different cell types and sites, as well as technical limitations and high cost.<sup>90</sup> Single-cell profiling could circumvent the problem of cellular contamination by cell types with differing DNA methylation. Recent studies have begun to develop cutting-edge methodologies demonstrating the achievability of performing genome-wide epigenetic profiling on a single-cell level.<sup>86,91</sup>

## Combined biomarkers

### Panels

Given the variability of disease presentation in IBD, several prior studies have suggested that utilizing a panel of multiple biomarkers for disease assessment could be more useful than applying each biomarker individually. These “composite biomarkers” can consist of multiple values that may then be incorporated into an algorithm to interpret data based on various aspects of this complex and heterogeneous disease.<sup>92</sup>

Plevy *et al.*<sup>93</sup> developed a tool composed of 8 serological markers, 4 genetic markers, and 5 inflammatory markers, all previously described in association with IBD, to accurately identify IBD patients and differentiate between CD and UC. This panel is currently in use in clinical practice and has proven useful for establishing a diagnosis when it is otherwise unclear.<sup>93</sup>

Integrating multiple biomarkers into clinical decision-making is especially useful in circumstances where the biomarker may be in a “gray” or “indeterminate” zone. For example, FC levels between 100–250 µg/g may be difficult to interpret in isolation. However, adding CRP and clinical scoring indices (Simple Clinical Colitis Activity index for UC and Harvey-Bradshaw score for CD) could aid in the correct classification and treatment of IBD patients, as well as predict the clinical course following remission.<sup>92</sup>

Scores that incorporate patient-reported symptoms are prone to subjective biases. Thus, the incorporation of objective data can mitigate bias and produce more reliable results. An example of this is seen in the Utrecht Activity Index, which combines the patient-reported frequency of liquid stools with CRP, FC, platelet count, and platelet mean volume. This index shows promise in predicting endoscopic activity in CD patients, with a cutoff score of 3.0 demonstrating a sensitivity of 80% and a specificity of 92% in predicting active disease (defined as a Crohn’s Disease Endoscopic Severe Score of 3 or more).<sup>94</sup>

Biomarker panels were created to aid in predicting clinical re-

sponse and mucosal healing to limit delays in effective treatment and undue harm from ineffective treatments. Obratzov *et al.*<sup>95</sup> created a panel of 7 cytokines (TNF- $\alpha$ , IL-12, IL-8, IL-2, IL-5, IL-1- $\beta$ , and IFN- $\gamma$ ), which individually have limited predictive value but, in combination, were able to correctly classify nearly 90% of UC patients as responders or non-responders to anti-TNF therapy.<sup>95</sup> Likewise, Bertani *et al.* observed that a significant decrease in IL-6 and IL-8 from baseline to 6 weeks after starting UC patients on vedolizumab could predict mucosal healing and clinical remission.<sup>96</sup>

The Endoscopic Healing Index is another panel composed of 13 biomarkers known to be involved in the proinflammatory cascade of CD. It was comparable to FC and superior to CRP in predicting endoscopic inflammation, aiming to reduce the need for repeating endoscopy or collecting stool samples to assess responses to treatment. A score of 20 points could rule out the presence of large ulcers with a sensitivity of 93%. Conversely, a score of 50 points could rule in the presence of large ulcers with a specificity of 87%. However, there is variability in results for those with isolated ileal disease, necessitating further research to guide adaptation of the index for these patients.<sup>4</sup>

### Ratios

Current research has expanded to incorporate ratios based on commonly acquired labs, specifically the complete blood count with differential, which is nearly universally monitored at a relatively low cost compared to other biomarkers.<sup>97</sup> The inflammation of IBD causes characteristic changes in circulating white blood cells, resulting in increased recruitment of neutrophils and monocytes to sites of inflammation, as well as thrombocytosis, lymphocyte dysfunction, and reduced responsiveness. This results in increased circulating neutrophils, monocytes, and platelets, and a decrease in lymphocytes at both the peripheral and mucosal level.<sup>5</sup>

#### The neutrophil-to-lymphocyte ratio (NLR)

NLR has been studied for its potential utility in multiple diseases, in addition to IBD.<sup>5,98</sup> Higher NLR values are observed in CD (range of 2.13–2.85) and UC (range of 2.26–4.70), which can reliably differentiate from healthy controls (range of 1.65–1.7).<sup>98</sup> A related meta-analysis showed that NLR was elevated during active disease in UC and CD patients compared to those in remission.<sup>99</sup> Furthermore, various studies documented a decreased NLR over time following initiation of treatment with infliximab, which could be used to predict loss of response to infliximab in UC and CD patients.<sup>98</sup>

#### Lymphocyte-to-monocyte ratio (LMR)

The LMR, conversely to NLR, has been shown to decrease in UC and CD patients compared to healthy controls. Although variable cutoffs have been reported, in one study, UC patients with clinically active disease had LMR levels around 2.1, while the level in quiescent disease was around 2.9, and healthy controls typically had levels around 3.5.<sup>97</sup> An LMR of 2.88 or less could indicate active UC with both a sensitivity and specificity of 90%.<sup>5</sup>

#### Platelet-to-lymphocyte ratio (PLR)

The PLR also shows promise in monitoring response to therapy and predicting long-term treatment response for patients with UC and CD, although the research on this ratio is less robust. PLR values, like NLR, were higher for UC patients presenting with mucosal ulceration at baseline endoscopy. Patients with an elevation of NLR and PLR at baseline,  $\geq 2$  and  $\geq 183$ , respectively, were

unlikely to achieve mucosal healing after 54 weeks of anti-TNF therapy.<sup>100</sup> Continued research in this area with larger sample sizes would be needed to strengthen these findings.

#### CRP-to-lymphocyte ratio (CLR)/CRP-to-albumin ratio (CAR)

Con *et al.*<sup>101</sup> used a similar approach, utilizing ratios of commonly collected biomarkers to predict the risk of colectomy for patients with acute severe UC following infliximab salvage therapy. They specifically focused on the CLR and CAR. Of the two, CLR appeared to offer superior risk stratification. A value  $\geq 6.0$  mg/10<sup>9</sup> obtained on day 3 following infliximab salvage therapy achieved a sensitivity and specificity of 84% and a negative predictive value of 96% for predicting colectomy within 1 year.<sup>101</sup> A recent abstract has even suggested that the CAR correlates well with endoscopic disease activity in patients with UC and could serve as a cost-effective and practical biomarker.<sup>102</sup>

#### Additional cutting-edge diagnostics

Alongside advancements in biomarker research, the area of endoscopic diagnosis has also expanded to include the development of confocal laser endomicroscopy and endoscopic visualization of biofilms. Confocal laser endomicroscopy was introduced in 2004 with the idea of creating “optical biopsies” to obtain targeted biopsies with higher diagnostic yield.<sup>103,104</sup> It requires the integration of a miniature confocal microscope into a conventional colonoscope, along with the addition of a topical or systemic contrast agent, typically acriflavine or fluorescein, respectively. This technique can be used to provide real-time microscopic analysis of inflammatory activity and rapid differentiation between neoplastic and non-neoplastic lesions, which is particularly valuable for dysplasia surveillance in CD and UC.<sup>104</sup>

Confocal laser endomicroscopy has also proven beneficial in assessing biofilms in the digestive tract. Biofilms appear as yellow-green adherent layers lining the intestinal wall and are commonly dismissed as stool remnants on routine colonoscopies. However, previous studies have confirmed these adherent structures as biofilms containing abundant bacteria protected by an exopolysaccharide matrix.<sup>105,106</sup> Biofilms were more prevalent in patients with irritable bowel syndrome (57%), UC (34%), and CD (22%) compared to healthy controls (6%). In UC patients, biofilms were associated with increased disease extent, histologic inflammation, and elevated fecal calprotectin.<sup>106</sup> This connection could provide new opportunities for diagnostic and treatment approaches with ongoing research focusing on biofilm eradication, particularly in the prosthetics industry as well as bioactive compounds, such as Punica Granatum derived from pomegranates, which could reduce biofilm formation in IBD.<sup>107,108</sup>

### Conclusion

There is no single gold-standard test for diagnosing IBD, much less differentiating between Crohn’s disease and ulcerative colitis. Therefore, diagnosis is commonly made through multiple modalities with varying availability, cost, and complexity. Biomarkers for inflammatory bowel disease have long been sought as potential non-invasive indicators of gastrointestinal tract inflammation. They can help distinguish between IBD and functional gut symptoms, aiding in disease monitoring for prompt diagnosis while avoiding unnecessary invasive examinations and harmful treatment delays.

In addition to older markers such as CRP, ESR, and fecal biomarkers, recent studies have identified genes and epigenetic modi-



fications to recognize at-risk populations. Further evaluations are required to elucidate the best test(s) for assessing mucosal healing and predicting the risk of future relapse. The concept of disease interception is emerging as a treatment paradigm aimed at early detection through the identification of at-risk individuals in a pre-disease state when pathologic molecular changes can be detected but the patient has not yet developed symptoms. Individualized interventions can then commence aimed at preventing irreversible damage from delayed or ineffective therapy.

Despite significant progress, no single biomarker can be used in isolation for the diagnosis of IBD. However, many show promise when incorporated into matrices of predictive tools for diagnosis, management, and prediction of disease severity and relapse. Nonetheless, while advanced genetic testing improves our understanding of IBD and individual variability, it's not yet widely used in clinical practice.

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