




Review Article

Fecal, Blood, and Urinary Biomarkers in Inflammatory Bowel Diseases



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Abstract

The incidence and prevalence of inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease, are rapidly increasing. Currently, colonoscopy is the gold standard for diagnosing and monitoring the course of IBD. However, the recently implemented "treat-to-target" strategy, which involves meticulously pursuing multiple therapeutic objectives, has opened avenues for non-invasive diagnostic and monitoring tools. These tools aim to assess disease activity and predict the likelihood of recurrence. Research studies into serum and fecal biomarkers in IBD have been ongoing for several years. Among the most relevant biomarkers, fecal calprotectin and C-reactive protein have shown the best accuracy, with good-to-optimal correlation with endoscopic, histologic, or transmural activity. Numerous studies have explored the potential advantages of using multi-target tools that combine serum and fecal biomarkers with clinical activity indexes to enhance diagnostic and monitoring effectiveness. Encouraging findings have emerged for fecal lactoferrin, autoantibodies, micro-RNA, gene expression, and many other serological and fecal markers. However, limited evidence has hindered their widespread adoption in routine clinical practice. This review aimed to summarize the available data on the utilization of biomarkers in IBD.

Introduction

The burden of inflammatory bowel diseases (IBD), primarily Crohn's disease (CD) and ulcerative colitis (UC), is spreading worldwide.¹ Recent incidence rates are steadily rising, reaching up to 17.8 cases per 100,000 person-years for CD and even higher for UC (up to 28.4 per 100,000 person-years).² Over the past 20 years, there has been a shift in the incidence and prevalence of IBD, with the onset occurring in older populations and varying geographically.³ In highly developed countries such as those in North America, age-standardized prevalence rates are significantly higher than in less developed regions.⁴ Currently, endoscopic assessment through ileo-colonoscopy remains the most viable and guideline-advised tool for assessing disease activity and monitoring treatment response in IBD patients.⁵ Monitoring new disease flares and pre-

dicting disease course and long-term outcomes are crucial in IBD management.⁶ Symptom-based tools, such as the partial Mayo score or the Harvey Bradshaw index (HBI),^{7,8} have not shown optimal correlation with disease activity and endoscopic remission, presenting low negative and positive predictive values.⁹ In the first STRIDE consensus by the International Organization for the Study of IBD, short-term endoscopic response and long-term endoscopic remission were identified as the primary therapeutic targets of IBD work-up.¹⁰ Nevertheless, several cross-sectional imaging techniques, such as magnetic resonance enterography and intestinal ultrasound, have become essential for diagnosing and monitoring IBD patients.¹¹ These imaging modalities offer the advantage of equal accuracy with less invasiveness, enabling comprehensive evaluation of disease status and therapeutic response.¹² While cross-sectional imaging and endoscopy serve as primary methods for patient monitoring, their inconsistent availability often leads to prolonged waiting lists, requiring patients to visit hospitals for these investigations. Thus, relying on serum, fecal, and urinary biomarkers of disease activity is crucial for implementing close monitoring of IBD, with some biomarkers potentially being analyzed directly from home.¹³ A biomarker is defined as a measurable compound or substance that can be objectively identified and quantitatively evaluated in a biological sample, such as blood, urine, tissue, or feces.¹⁴ Recently, the novel "treat-to-target" ap-

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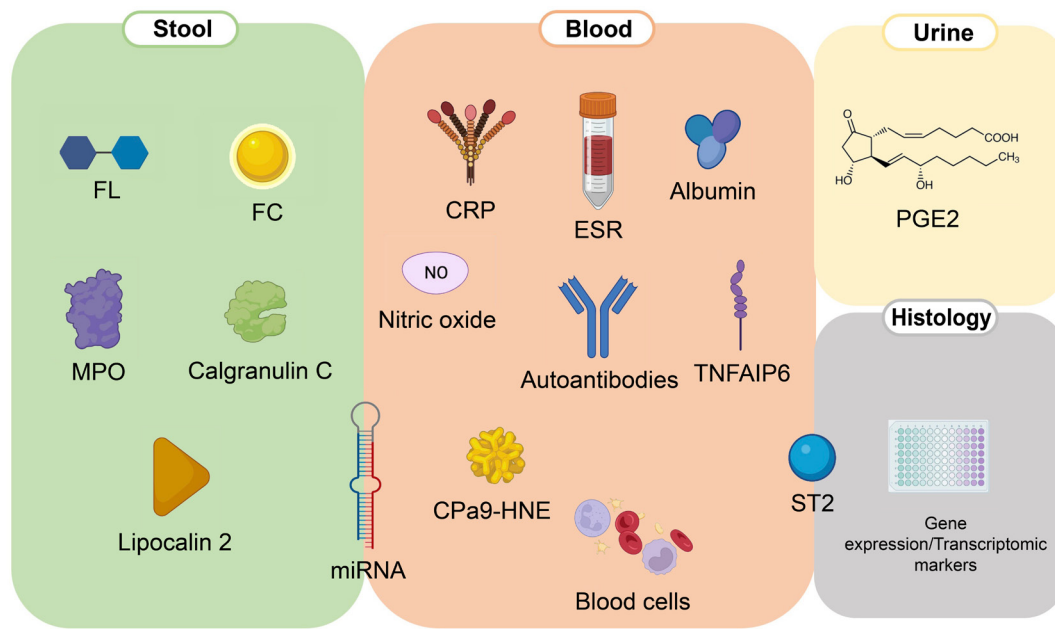


Fig. 1. Summary of fecal, urinary, blood, and histologic biomarkers. FC, Calprotectin; FL, Lactoferrin; MPO, Myeloperoxidase; CRP, C-reactive-protein; ESR, Erythrocyte sedimentation rate; ST2, Suppression of tumorigenicity 2, TNFAIP6, TNF- α -induced protein 6.

proach in managing IBD patients has heightened the significance of these biomarkers, as it fully depends on the periodic monitoring of markers of disease activity, leading to treatment optimization or therapy changes.⁶ The CALM trial was the first to demonstrate that promptly escalating treatment, guided by both clinical symptoms and biomarkers, yields superior clinical and endoscopic outcomes in individuals with early CD compared to decisions based on clinical symptoms alone.¹⁵ The STRIDE-II consensus initiative confirmed that, in IBD patients, clinical response and remission, endoscopic healing, and normalization of C-reactive protein (CRP)/erythrocyte sedimentation rate and fecal calprotectin (FC) represent the most crucial targets.¹⁶ Other serological, fecal, urine, and tissue transcriptomic markers have been tested and are still being studied to assess disease activity or predict response to therapies in IBD (Fig. 1). This review aimed to provide an overview of available biomarkers in IBD, focusing on their role in diagnosis, disease activity evaluation, prediction of response to therapy, endoscopic and histological healing, and disease recurrence.

Review criteria

PubMed, Embase, and Scopus databases were screened up to November 30, 2023, to identify studies reporting the accuracy, sensitivity, specificity, and overall feasibility of different biomarkers in IBD. The following search terms were used: ‘biomarkers’, ‘fecal calprotectin’, ‘calprotectin’, ‘polymerase chain reaction (PCR)’, ‘C-reactive protein’, ‘protein C’, ‘erythrocyte sedimentation rate’, ‘anti-neutrophil cytoplasmic antibodies’, ‘auto-antibodies’, ‘non-invasive markers’, combined with ‘Crohn’, ‘Crohn’s disease’, ‘terminal ileitis’, ‘IBD’, ‘post-surgical CD’, ‘peri-anal’, ‘peri-anal disease’, ‘peri-anal CD’, ‘UC’, ‘ulcerative colitis’, ‘colitis’, ‘segmental colitis’. Only articles published in English were considered. Four authors (SB, FD, FF, and AZ) independently reviewed titles and abstracts to identify eligible studies. The full texts of the selected articles were examined for inclusion, and relevant references in their lists were

hand-searched to identify studies missed by the electronic search. Abstracts and articles were included based on their relevance.

Fecal biomarkers

FC

FC is a cytosolic calcium and zinc-binding protein composed of two monomeric subunits, S100A8 and S100A9, expressed by neutrophils, dendritic cells, monocytes, macrophages, and squamous cells.¹⁷ FC is a stable protein, capable of persisting in a fecal sample at room temperature for one week¹⁸ due to its calcium-binding capacity, which confers resistance to proteolysis. The concentration of FC in feces is about six times higher than in plasma and urine samples.¹⁹ FC is a hallmark of neutrophil activation during various inflammatory processes in the gut, and its presence in feces is recognized as a marker of gastrointestinal organic disorders,²⁰ such as IBD, non-steroidal anti-inflammatory drug enteropathy,²¹ and even colorectal cancer.²² The quantification of FC in stools is determined by different types of assays, mainly based on the use of mono or polyclonal antibodies that bind specific epitopes of the FC heterodimer via various techniques (such as immunofluorescence or chemiluminescence).²³ The concentration of FC in the stool may vary depending on patient-related characteristics and intrinsic biases encountered during the two phases of FC determination: the pre-analytical phase (stool storage and FC extraction) and the analytical phase during which the test and thresholds are decided.²⁴ To address these issues, an international consensus recently standardized practical tips to homogenize FC assessment: the preference for quantitative FC determination and the consistent use of the same quantification technique whenever possible.²⁵

Fecal calprotectin and diagnosis

FC is the most studied biomarker in the field of IBD. FC was first quantified in IBD patients by Roseth and colleagues in 1992.²⁶ In a

subsequent study, the same authors reported higher levels of FC in patients with low-to-mild UC compared to healthy controls (HC).²⁷ Several pooled analyses have comprehensively aggregated FC accuracy data, distinguishing between IBD and functional patients. Von Roon and colleagues,²⁸ pooling data from 5,983 patients, reported a sensitivity of 0.95 (95% CI 0.93–0.97) and a specificity of 0.91 (95% CI 0.86–0.91) in discriminating IBD from HC. Menees *et al.* identified an optimal cut-off value for FC around 50 µg/L, which could exclude organic inflammation from IBS patients with fair accuracy.²⁹ A more recent meta-analysis, which retrieved data from 2,145 patients subgrouped into organic versus functional gastrointestinal disorders (of which 1,059 had IBD), reported a pooled FC sensitivity and specificity of 88% (95% CI, 80–93%) and 72% (95% CI, 59–82%) in differentiating IBD from IBS.²⁹

Fecal calprotectin and endoscopy

Non-invasive measurement of FC concentration accurately predicts endoscopic disease activity. Petryszyn *et al.* demonstrated that colonoscopy could be avoided in 66.7% of cases by incorporating FC into the diagnostic work-up for IBD.³⁰ Mosli *et al.* pooled data from 2,499 patients, equally divided between UC and CD, reporting an overall sensitivity of 0.88 (95% CI 0.84–0.90) and specificity of 0.73 (95% CI 0.66–0.79) for predicting endoscopic activity in IBD patients.³¹ The authors identified a relatively low cut-off threshold for endoscopic activity assessment at 50 µg/g, demonstrating a high negative predictive value of FC determination with a pre-test probability of IBD of 25%.³¹ A recent study by Rokkas and colleagues set optimal cut-off values for FC at 50 µg/g for best sensitivity and 100 µg/g for best specificity in detecting IBD activity,³² while other studies found higher concentrations in patients in remission.³³ Schoepfer *et al.* extensively evaluated the role of FC in describing disease activity, reporting higher FC levels (396 ± 351 versus 18.1 ± 5 µg/g) in active versus non-active UC patients.³⁴ The correlation between FC and the Mayo endoscopic score was strong (Spearman score 0.834).³⁴ The authors identified an overall accuracy of 89% for FC in detecting endoscopic activity in UC.³⁴ Similarly, Walsh and colleagues defined thresholds of 71 µg/g, 91 µg/g, and 67.7 µg/g of FC, correlating well with endoscopic, histologic, and combined activity in 39 UC patients.³⁵ The area under the curve (AUC) of FC for combined activity prediction was above 0.90.³⁵ In CD, the correlation between FC levels and endoscopic activity was stronger when the disease was located in the colon rather than the ileum.³⁶ The most recent large-cohort (273 CD patients) analysis on the clinical prediction value of FC in CD, provided by Li *et al.*, reported optimal sensitivity and specificity in predicting endoscopic remission (sensitivity 68.02% and specificity 85.53%; AUC 0.83 with a cutoff value of 80.84 µg/g).³⁷ Brand and colleagues conducted an intricate analysis, extrapolating 27 prediction models for assessing endoscopic activity in CD patients.³⁸ Specifically, the researchers calculated that by setting a cut-off value of < 100 µg/g for FC, up to 72.5% of ileo-colonoscopies could be avoided, while potentially overlooking active CD in about 19.8% of cases as per the application of this predictive model.³⁸ Schoepfer and colleagues also analyzed FC in CD, evidencing similar results, with a slightly lower correlation between FC and the SES-CD score (Spearman 0.75) but comparable accuracy of 87% with an FC cut-off of 70 µg/g.³⁹ In both studies, FC performed better than clinical activity scores and blood component evaluations, such as blood leukocyte count.^{34,39}

Fecal calprotectin and histology

FC has also been evaluated as a potential predictor of histologic

activity in UC patients by Guardiola *et al.* More elevated levels of FC were found in histologically active UC patients (278 mg/g) compared to histologically inactive ones (68 mg/g; $p < 0.002$).⁴⁰ In early retrospective evaluations of new rapid tests, FC has been found to correlate closely with both endoscopic and histologic activity for CD⁴¹ and UC.⁴²

Fecal calprotectin and disease monitoring

Considering the severity of the disease, specifically for UC, FC values were also evaluated in acute-severe UC (ASUC). An FC > 800 µg/g at admission independently predicted the need for medical rescue therapy (OR 2.61, 95% CI, 1.12–6.12) and surgery within three months (OR 2.88, 95% CI, 1.01–8.17).⁴³

FC is also a useful tool in disease monitoring, correlating with endoscopic and clinical remission,⁴⁴ or predicting disease relapses during stable remission.⁴⁰ Considering UC, a post hoc analysis of the GEMINI 1 study showed that an FC < 150 µg/g was associated with clinical and endoscopic remission (AUC range 0.70–0.77). Endoscopic evaluation should be indicated for patients with FC levels ≥250 µg/g, but not for those with FC values < 100 µg/g.⁴⁵ An FC value of 130 µg/g predicted remission, while an FC > 300 µg/g was associated with relapse.⁴⁶ These data are confirmed by the i-Support Therapy-Access to Rapid Treatment approach for patient-centered therapy in mild-to-moderate UC, which emphasizes the importance of regularly conducting FC measurements to monitor patients and promptly detect relapses to initiate suitable treatment.⁴⁷ Exploring the latest studies on the use of FC to assess therapeutic response, Bertani and colleagues found a statistically significant correlation between FC levels and the prediction of mucosal healing (Mayo endoscopic score ≤1) in UC, with a sensitivity of 75% and specificity of 88.9%. This correlation was observed 8 weeks after the initiation of anti-TNF or Vedolizumab therapy.⁴⁸ Similar prior studies have corroborated these findings.^{49,50} In UC, a recent standardization on the use of biomarkers to monitor inflammation during remission of treatment response has been achieved.⁵¹ The American Gastroenterology Association developed a clinical practice guideline, advising an FC cut-off of >150 µg/g as optimal for specificity and sensitivity in predicting endoscopic activity in patients with moderate to severe symptoms of UC.⁵¹ Considering CD, Haisma *et al.* found that early pursuit (< 12 weeks) of FC normalization was associated with lower disease relapse and better long-term outcomes.⁵² For patients with persistently high levels of FC, the probability of experiencing a relapse could potentially reach 83%.⁵³ This data was confirmed in subsequent studies, which reported that high FC values predicted relapse after anti-TNF therapy discontinuation, in both CD and UC.^{46,54} Guidi *et al.* assessed FC values before and one year after induction and maintenance therapy with anti-TNF α in CD patients, finding a mean decrease of FC from 308 µg/g to 106 µg/g ($p < 0.0001$) for the sustained clinically responsive group, with no significant reduction in those who did not improve clinically (sensitivity of 83% and specificity of 74% for post-induction FC).⁵⁵ A recent study by Magro and colleagues, which collected prospective data from 289 CD patients treated with infliximab (IFX) for two years, analyzed the progression of the disease through two composite outcomes. The first composite outcome integrated clinical factors such as initial occurrence of surgery or hospitalization related to IBD, or the emergence of new fistulas, abscesses, or strictures, and drug-related factors such as initial prescription of either at least one course of oral corticosteroids or more than 10 mg of prednisolone per day, or the initiation of de novo azathio-

prine or methotrexate, or a swap/switch to biological therapy (to adalimumab, golimumab, vedolizumab, or ustekinumab), or an increase in azathioprine dose unrelated to weight fluctuation, or an escalation in IFX dose or a reduction in dosing intervals. The second composite outcome considered adjustments in IFX dose and/or frequency.⁵⁶ The authors demonstrated that FC > 500.0 µg/g was associated with complications of CD and the need for corticosteroid treatment during biological therapy (43.1% in composite outcome 1 and 26.9% in composite outcome 2; OR 3.069).⁵⁶ Mild elevations (250.1–500.0 µg/g) were relevant when observed in at least two consecutive visits (33.3 % in composite outcome 1 and 15.2 % in composite outcome 2; $p < 0.001$ and $p = 0.04$ respectively). These results were confirmed by Cao *et al.*, who demonstrated that high FC levels (>238 µg/g) during IFX maintenance treatment in CD patients predicted endoscopic activity within a one-year follow-up ($p < 0.001$).⁵⁷

Considering the relevance of FC in clinical practice, easy-to-use and feasible kits for rapid assessment of FC in stools, such as “CalproSmart,” “QuantoCal,” and “IBDoc,” have been developed and can be performed directly at home.⁵⁸ Sjoukje-Marije Haisma *et al.* compared these three home tests and found that within a low calprotectin range (≤ 500 µg/g), IBDoc, QuantOnCal, and CalproSmart demonstrated agreement rates of 87%, 82%, and 76%, respectively, with their corresponding ELISA. In the elevated range (>500 µg/g), the agreement stood at 37%, 19%, and 37%, respectively. The imprecision in the high range is of lesser concern because any calprotectin result exceeding 500 µg/g is interpreted as indicative of active disease, regardless of the specific concentration. Moreover, the CalproSmart and QuantOnCal smartphone applications experienced significantly higher rates of reading errors compared to the IBDoc application, with rates of 5.8% and 4.8% versus 1.9% ($p = 0.002$ and $p = 0.012$), respectively.⁵⁹

Fecal calprotectin in the post-operative recurrence (POR)

Fecal biomarkers have demonstrated accuracy in predicting POR in CD patients.⁶⁰ Initially, FC < 272 µg/g revealed a strong correlation with endoscopic remission following surgery in CD patients (area under the receiver operating characteristic curve [AUROC] 0.93). The correlation between FC and the Crohn’s disease index of severity was stronger ($r = 0.722$; $p < 0.001$) compared to CRP ($r = 0.362$; $p < 0.001$) and leukocyte count ($r = 0.327$; $p = 0.003$).⁴¹ Additionally, an analysis by Yamamoto and colleagues demonstrated a positive correlation between FC and endoscopic recurrence in CD patients who underwent ileocolonic resection ($p = 0.0001$).⁶¹ Conversely, blood markers such as CRP and leukocyte count did not perform as well. In the POCER trial, a subgroup analysis revealed higher FC values in patients who experienced a recurrence (275 µg/g vs. 72 µg/g, $p < 0.001$).⁶² This data was confirmed in a real-world cohort of patients with CD and endoscopic recurrence after ileocolic resection.⁶³ Specifically, with an FC threshold of 50 µg/g, sensitivity was 90% and negative predictive value was 93%, while specificity and positive predictive value were 48% and 38%, respectively.⁶³ Recently, it was identified that a combination of FC > 50 µg/g and bowel wall thickness measured by standard intestinal ultrasound had a very high positive predictive value (94%), with an OR of 8.58 ($p < 0.001$) in predicting post-operative recurrence in adult CD patients.⁶⁴

In conclusion, the STRIDE-II consensus has revisited and refined key messages from the initial consensus, emphasizing the importance of biochemical non-invasive evaluation as an early to intermediate objective. This approach aims to enhance and expe-

dite the follow-up of IBD, not only in terms of treatment response but also in specific scenarios such as POR.¹⁶

Fecal Lactoferrin (FL)

FL is an 80 kilodaltons (kDa) monomeric glycoprotein that exerts the main function of binding iron in its Fe³⁺ form and transporting it in the blood (with the capacity to also bind other ions like magnesium and zinc).⁶⁵ FL plays a key role in innate immunity processes, being released by several immune cells, primarily neutrophils (from secondary granules).⁶⁶ FL exerts both anti-bacterial and antifungal activities thanks to its Fe³⁺ binding capacity.⁶⁷ Even anti-parasitic effects (mostly against *Entamoeba histolytica* due to lipid membrane binding and disruption⁶⁸) and antiviral effects (towards non-enveloped RNA viruses by viral entry inhibition⁶⁹) of FL have been described. Overall, FL plays an anti-inflammatory role by reducing the production of pro-inflammatory cytokines,⁶⁶ promoting apoptosis of uncontrolled damaged cells, and blocking the cellular cycle of malignant cells, thus exhibiting potential anticarcinogenic activity.⁷⁰

FL has been proven as a useful fecal biomarker during various inflammatory conditions,⁷¹ with recent increasing interest in IBD pathogenesis and diagnosis.⁶⁶ Menees *et al.*, evaluating the available literature on FL, were unable to conclusively determine its discriminative power of FL for distinguishing IBD from HC or IBS patients.²⁹ In the context of IBD, the highest predictive likelihood for disease using FL was 20.4% at a concentration of 1,810 µg/g. A level of 10 µg/g was associated with a 2% probability of IBD. Among all individual markers, FL exhibited the highest predictability for IBS at 74% with a concentration of 2,960 µg/g.²⁹ Zhou and colleagues analyzed data from 1,012 patients, revealing a pooled sensitivity of 0.78 (95% CI 0.75, 0.82), specificity of 0.94 (95% CI 0.91, 0.96), combining for an AUC of 0.94 (95% CI: 0.90, 0.98), and OR of 52.65 (95% CI: 25.69, 107.91) in discriminating IBS from IBD using FL.⁷² Similar results were found in subsequent reports.⁷³

FL has shown a correlation with endoscopic and histologic activity in colonic CD,⁷⁴ even in pediatric patients.⁷⁵ The most recent pooled analysis of 10 available studies identified good sensitivity and specificity values for FL in detecting activity. The combined sensitivity and specificity values for evaluating UC activity were 0.81 (95% CI 0.64–0.92) and 0.82 (95% CI 0.61–0.93), respectively. Additionally, the pooled sensitivity and specificity values for assessing CD activity were 0.82 (95% CI 0.73–0.88) and 0.71 (95% CI 0.63–0.78), respectively. Notably, the diagnostic performance of the FL assay in UC patients appeared to be superior to that in CD patients.⁷⁶ In some studies, FL levels correlated with endoscopic disease activity. A meta-analysis of patients with CD reported combined sensitivity and specificity values for FL in detecting endoscopic activity at 75% and 80%, respectively.⁷⁷

FL has also gained recognition as a fecal biomarker in monitoring IBD.⁶⁶ It has shown a strong correlation, particularly following anti-TNF therapy in CD.⁷⁸ A cutoff of 10 mg/g correlated with endoscopic response defined as a Crohn’s disease index of severity (≤ 3) (Spearman’s r 0.773, $p < 0.001$).⁷⁸ In a recent post-hoc analysis of UNIFI and PURSUIT trials, FL has been targeted as a potential marker of worse long-term outcomes following anti-TNF α or anti-IL12-23 therapy in UC.⁷⁹ FL levels above 84.5 µg/mL predicted a low likelihood of clinical (OR 0.43; $p < 0.001$), endoscopic (OR [95% CI]: 0.40 [0.29, 0.56]; $p < 0.001$), and histological (OR [95% CI]: 0.27 [0.14, 0.53]; $p < 0.001$) remission.⁷⁹ Despite this, the use of FL is not widespread.

Moreover, FL’s role in predicting response (assessed with HBI

and partial Mayo score) to biological agents during induction in both UC and CD has been demonstrated.⁸⁰ Baseline FL values were higher in the non-responder group compared to responders ($2,221 \pm 1,910$ vs. $773 \pm 1,054$ $\mu\text{g/mL}$, $p = 0.02$). Similarly, the initial FL drop was less on average in non-responders, although the difference was not statistically significant (62 ± 36 vs. $83 \pm 16\%$, $p = 0.09$).⁸⁰

Serum biomarkers

CRP

CRP is an acute-phase inflammatory protein, which can increase by 1000-fold during an acute response in various settings.⁸¹ CRP is a pentameric protein with five non-covalently linked subunits of 206 amino acids and a molecular weight of 23 kDa.⁸² CRP has numerous functions within the human immune system. CRP is produced by the liver in response to increased levels of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, or TNF α .⁸³ IL-6 has been identified as the primary and most significant stimulator of CRP production by hepatocytes during inflammatory states.⁸⁴ CRP rises within the first few hours following the initial causative stress (such as tissue injury or infection), typically reaching an initial concentration of around 0.8 mg/L.⁸⁵ Moreover, CRP shows a late-onset curve in serum, especially during acute bacterial infections, compared to other infectious biomarkers like white blood cells.⁸⁶ The main role of CRP in immune system activation and defense is to promote the activation of the complement cascade.⁸⁷ Additionally, it plays a crucial role in cell-mediated immunity by binding to the Fc ϵ R1 receptor of immunoglobulin-G (IgG) during antigen presentation.⁸⁸

CRP has been studied as a tool to aid in the differential diagnosis between IBD and IBS since the mid-1980s.⁸⁹ In a landmark population-based study by Fagan and colleagues, mean CRP values were found to be elevated in both CD and UC patients. Specifically, CRP levels were consistently higher in patients with moderate to severe disease compared to those with mild to moderate disease. Furthermore, CRP values were higher in CD compared to UC at the same disease activity level (4, 0–65 mg/L in mild CD vs 0, 0–15 mg/L in mild UC; 15, 1–100 mg/L in moderate CD vs 3, 0–29 mg/L in moderate UC; 85, 15–183 mg/L in severe CD vs 12, 2–33 mg/L in severe UC).⁹⁰ The work by Menees and colleagues evaluated the accuracy of CRP in differentiating IBS, IBD, and HC from four available studies using a Bayesian predictive algorithm, demonstrating a 90% likelihood probability of suffering from IBD for a threshold CRP level above 2.7 mg/L.²⁹ Despite some limitations in CRP accuracy,⁹¹ its usefulness as a biomarker in IBD remains relevant.⁹² Particularly when combined with other biomarkers, CRP can significantly improve diagnostic performance.⁹³

CRP is traditionally recognized as one of the principal serum biomarkers of disease activity in IBD.⁹⁴ Schoepfer *et al.* extensively evaluated the role of CRP in describing disease activity, reporting higher levels (16 ± 13 versus 3 ± 2 mg/L) in active UC versus non-active patients, with 69% accuracy in detecting endoscopic activity.³⁴

The role of CRP is significant in ASUC. Particularly, the CRP/albumin ratio (CAR) is a better predictor of response to steroid therapy in ASUC than CRP or albumin values alone ($\text{AUC} = 0.75$; $p < 0.001$). The optimal CAR to predict response to steroids on day 3 is 0.85 (sensitivity 70%, specificity 76%).⁹⁵ Moreover, following IFX rescue therapy for ASUC, CAR emerged as a straightforward biomarker, demonstrating robust predictive capabilities for

the likelihood of colectomy. A day 3 CAR cutoff of 0.47 had 79% sensitivity, 80% specificity, and 94% negative predictive value to predict colectomy.⁹⁶

CRP showed lower accuracy values compared to FC in detecting endoscopic activity. Specifically, in an intermediate pre-test scenario, an 11.5% rate of false positives for elevated CRP levels has been determined.⁴⁸

CRP is also a useful marker in disease monitoring, allowing therapy adjustment according to the treat-to-target strategy.¹⁰ In patients with CD treated with IFX, an increase in CRP levels >10.0 mg/L in at least one visit was associated with a higher need for oral corticosteroid treatment during biological therapy [(44.3% in composite outcome 1 and 25.7% in composite outcome 2; OR 3.187); composite outcome 1 and 2 are described in the section 3.1.4].⁵⁶ Mild elevations (3.1–10.0 mg/L) were only considered relevant when observed in at least two visits, whether consecutive or not (35.2% in composite outcome 1 and 18% in composite outcome 2; $p < 0.001$ and $p = 0.007$, respectively). These results were confirmed by Cao *et al.*, who demonstrated that high CRP levels (>3.00 mg/L) during IFX maintenance treatment in CD patients predicted moderate endoscopic activity outcomes within a one-year follow-up ($p = 0.012$).⁵⁷

Despite its utility as a non-invasive biomarker for IBD, it is important to note that 20–25% of IBD patients do not produce CRP during a flare.⁹⁷ Additionally, CRP demonstrates a weaker correlation with disease activity in UC patients compared to those with CD.⁹⁸

The accuracy in diagnosis, disease activity, and disease monitoring of FC, FL, and CRP is shown in Table 1.

Autoantibodies in IBD assessment

There has been a suggestion regarding the potential involvement of antibodies in the diagnostic differential process of IBD.⁹⁹ Antibodies against neutrophil cytoplasmic antigens (ANCA) are currently serum biomarkers useful in the diagnostic assessment of several inflammatory diseases.¹⁰⁰ ANCA can be divided into two sub-groups: cytoplasmic ANCA and pre-nuclear ANCA (pANCA), depending on the location within the cell where the antigens bound by these autoantibodies are detected by ELISA staining.¹⁰¹ pANCA are directed against myeloperoxidase, while cytoplasmic ANCA target proteinase 3.¹⁰²

These autoantibodies were first evaluated in IBD by Rump and colleagues in 1990. They found elevated titers of p-ANCA in UC patients with active disease, while no increase was reported in the CD cohort.¹⁰³ Ruehmele and colleagues found a specificity of 100% for both IgA and IgG pANCA levels for UC diagnosis versus non-IBD controls, while lower levels of this marker were reported in CD patients.¹⁰⁴ Other authors evaluated high levels of pANCA in CD patients with UC-like colitis characteristics, suggesting a potential clinical subgroup distinction using this biomarker.¹⁰⁵ In contrast, in CD, an increased titer of anti-Saccharomyces cerevisiae (ASCA) antibodies has been reported for years.¹⁰⁶ ASCA are specific autoantibodies known to directly bind the mannose residue on the cell surface mannan of Saccharomyces Cerevisiae. They were described long ago in coeliac disease.¹⁰⁷ Other microbial species, namely Candida albicans, have also been shown to be immunogenic for ASCA production in CD.¹⁰⁸ Peeters and colleagues in 2001 investigated the accuracy of ASCA and pANCA in the differential diagnosis of IBD, achieving considerable levels of specificity (up to 92%) but very low sensitivity (around 60%), even when combined, indicating limited diagnostic feasibility in IBD.⁹⁹

Other antibodies associated with the diagnosis of IBD include

Table 1. Summary of accuracy of principal biomarkers in diagnosis, disease activity, and disease monitoring of IBD

	Diagnosis	Disease activity	Disease monitoring
FC	IBD from HC: se: 0.95 (95% CI 0.93–0.97); sp: 0.91 (95% CI 0.86–0.91). IBD from IBS: se: 88% (95% CI, 80–93%); sp: 72% (95% CI, 59–82%).	Predicting endoscopic activity in IBD: se: 0.88 (95% CI 0.84–0.90); sp: 0.73 (95% CI 0.66–0.79). UC: correlation between FC and MES (Spearman score 0.834): accuracy of 89% in predicting endoscopic activity; AUC of 0.90. CD: correlation between FC and the SES-CD score (Spearman 0.75); accuracy of 87% in predicting endoscopic activity.	IBD: repeated FC measurements above the study's cutoff level had a 53% to 83% probability of developing disease relapse. UC: AUC range 0.70–0.77 for FC reduction >90%, FC ≤ 150 µg/g that indicates clinical and endoscopic remission; se: 75% and sp: 88.9% in predict mucosal healing after 8 weeks of treatment. CD: se: 83% and sp: 74% for FC post-induction treatment.
FL	IBD from IBS: se: 0.78 (95% CI 0.75, 0.82), sp: 0.94 (95% CI 0.91, 0.96).	UC: se: 0.81 (95% CI, 0.64–0.92); sp: 0.82 (95% CI, 0.61–0.93); CD: se: 0.82 (95% CI, 0.73–0.88) sp: 0.71 (95% CI, 0.63–0.78).	FL > 84.5 µg/mL predicted a low likelihood of clinical (OR 0.43) endoscopic (OR 0.40) and histological (OR 0.27) remission.
CRP	Differentiating IBS, IBD and HC: CRP > 2.7 mg/L probability of 90% to suffer from IBD.	UC: 69% accuracy in detecting endoscopic activity.	

CD, Crohn's Disease; CRP, C-Reactive Protein; FC, Fecal Calprotectin; FL, Fecal Lactoferrin; HC, healthy controls; IBD, Inflammatory Bowel Diseases; IBS, Irritable Bowel Syndrome; Se, Sensitivity; Sp, Specificity; UC, Ulcerative colitis; MES, Mayo Endoscopic score; SES-CD, Simple Endoscopic Score for Crohn's disease.

anti-outer-membrane porin C and anti-CBir1 antibodies.¹⁰⁹ Their detection is associated with the diagnosis of IBD.^{110,111}

A recent study reported a correlation between primary non-response to anti-TNF therapy in UC patients and high levels of pANCA antibodies ($p > 0.0002$), suggesting a role for autoantibodies in predicting treatment response in IBD.¹¹² The previously mentioned low sensitivity of autoantibodies as biomarkers of inflammation has severely limited their use as diagnostic non-invasive tools, reducing research interest in them.⁹⁹ Nonetheless, individually assessing the feasibility of all these potential biomarkers could be debated. It is crucial to consider the utilization of a panel of multiple biomarkers when aiming to enhance the accuracy of disease prediction in IBD patients.¹¹³ A recent validation of this approach is represented by the study of Plevy and colleagues, which explored the diagnostic performance of a multi-panel test based on inflammatory markers (such as CRP) in combination with several serological markers (especially ASCA, ANCA, outer-membrane porin C, CBir1). This study demonstrated a substantial increase of the AUC in discriminating IBD from non-IBD patients (from 0.80 to 0.87; 95% CI, -0.4 to 0.4; $p < 0.001$) and between CD and UC (from 0.78 to 0.93; 95% CI, -0.4 to 0.4; $p < 0.001$).¹¹⁴

Erythrocyte sedimentation rate (ESR)

The ESR is a serological biomarker of inflammation, reflecting the degree of blood serum aggregation influenced by increased production of acute inflammatory proteins and metabolites.¹¹⁵ ESR values are affected by hematocrit and red blood cell aggregation.¹¹⁶ The most comprehensive examination of ESR as a diagnostic marker for IBD stems from the aforementioned study by Menees *et al*. In this study, no ESR levels demonstrated statistically significant prediction of IBD, indicating that ESR did not exhibit notable accuracy compared to FC and CRP.²⁹ Even ESR has been evaluated in active IBD cohorts, showing no significant differences between the two disease phenotypes, UC versus CD.¹¹⁷ However, there was concordance with CRP and FC in predicting endoscopic and clinical activity.¹¹⁸

Data available from more recent meta-analyses in the literature on the feasibility of the principal biomarkers assessed in the diagnosis of IBD, both in adult and pediatric settings, are summarized in Table 2.^{27,29–31,76}

Urine markers

Urine biomarkers have shown limited utility in assessing IBD activity; however, certain studies have explored urine as a potential source of non-invasive biochemical indicators for disease activity.¹¹⁹ Prostaglandins are synthesized through the action of A2 phospholipase, which catalyzes the phosphorylation of arachidonic acid. This process is triggered by various inflammatory triggers.¹²⁰ Prostaglandins serve numerous functions, particularly in mucosal inflammation and the recruitment of inflammatory cells.¹²¹ PGE2 is excreted in urine in the form of several processed metabolites, primarily Tetranor-prostaglandin E metabolite. Arai and colleagues evaluated urinary concentrations of Tetranor-prostaglandin E metabolite, demonstrating good agreement with FC in describing active inflammation in UC ($p < 0.01$), and also predicting histological and endoscopic remission.¹²² In pediatric patients, urinary PGE2 showed a positive correlation with both endoscopic and clinical activity indexes in UC ($r = 0.594$ and $r = 0.462$, respectively).¹²³ Recently, the role of PGE2 in predicting disease relapse has been investigated, with a reported predictive value of 25.2 µg/g and an AUC of 0.721 (95% confidence interval: 0.556–0.886).¹²⁴ Patients with PGE2 values ≥ 25.2 µg/g experienced a significantly shorter relapse-free period (log-rank test: $p < 0.001$).¹²⁴ The feasibility of PGE2 and its metabolites in urine warrants further research efforts. Future studies are needed to explore this potential avenue.

Unveiling novel IBD indicators: Exploring promising markers on the horizon

Several biomarkers have been evaluated since the end of the 1990s to assess the presence of CD or UC.¹⁸ Other biomarkers with promising results are currently under investigation but still need to be incorporated into clinical practice.

Myeloperoxidase

Myeloperoxidase (MPO) is a heme-containing enzyme primarily found in azurophil granules of neutrophils and in monocyte lysosomes.¹²⁵ MPO is a crucial component of the cytoplasmic antimicrobial compartment in phagocytic cells. MPO produces oxygen reactants, particularly hypochlorous acid.¹²⁶ One of the first

Table 2. Studies focusing on diagnostic accuracy of biomarkers in IBD diagnosis in adults and children

Author and date	Study design	Cohort N.	Patients type	Disease	Assessed biomarker	Accuracy
Von Roon <i>et al.</i> ²⁷	Metanalysis	5,983 (CD = 663, UC = 361, IBD = 186)	Adults	IBD vs HC	FC	Se 0.95 (95% CI 0.93–0.97); Sp 0.91 (95% CI 0.86–0.91)
Menees <i>et al.</i> ²⁹	Metanalysis	2,145 (IBD = 1,059)	Adults	IBD vs IBS vs HC	FC, FL, ESR, CRP	Maximal predictive value of IBD for FC: 78.7% at 1,000 µg/g; IBS exclusion for FC < 40 µg/g; Maximal predictive value of IBD for CRP: 90% at 2.7 mg/dl; FL and ESR no predictive values
Mosli <i>et al.</i> ³¹	Metanalysis	2,499 (UC = 1,069, CD = 1,033)	Adults	IBD	FC, FL, CRP	CRP: Se 0.49 (95% CI 0.34–0.64); Sp 0.92 (95% CI 0.72–0.96); FC: Se 0.88 (95% CI 0.84–0.90); Sp 0.73 (95% CI 0.66–0.79); FL: Se 0.82 (95% CI 0.73–0.88); Sp 0.79 (95% CI 0.62–0.89)
Petryszyn <i>et al.</i> ³⁰	Metanalysis	5,032 (IBD = 620)	Adults and children	IBD vs HC	FC	Se: 0.88 (95% CI, 0.827–0.921); Sp: 0.79 (95% CI, 0.693–0.875)
Dai <i>et al.</i> ⁷⁶	Metanalysis	936 (IBD = 773)	Adults	IBD	FL	UC: Se 0.81 95% CI, 0.64–0.92; Sp 0.82 95% CI, 0.61–0.93; CD: Se 0.82 95% CI, 0.73–0.88; Sp 0.71 95% CI, 0.63–0.78

CD, Crohn's Disease; CRP, C-Reactive Protein; ESR, Erythrocyte Sedimentation Rate; FC, Fecal Calprotectin; FL, Fecal Lactoferrin; IBD, Inflammatory Bowel Diseases; IBS, Irritative Bowel Syndrome; Se, Sensitivity; Sp, Specificity; UC, Ulcerative colitis.

influential studies on fecal MPO and IBD was conducted in 1998 by Saiki T. The study enrolled a total of 33 UC patients, 32 CD patients, and 15 HC, demonstrating significantly elevated levels of MPO concentration in stools among IBD patients compared to the control group ($p < 0.001$).¹²⁷ Regarding disease activity, a paired analysis highlighted reduced levels of MPO in stools of patients with UC in remission compared to those with active disease ($p < 0.001$).¹²⁷ Anezaki and colleagues found concordant levels of MPO and IL-8 (measured with ELISA testing) in the stools of active UC patients versus inactive ones.¹²⁸ Regarding disease monitoring, Sangfelt *et al.* evaluated the decrease in MPO levels after seven days of corticosteroid treatment in patients with distal and rectal UC.¹²⁹ However, there is currently insufficient evidence to support the use of MPO as a biomarker, and it is not recommended for clinical practice.

Calgranulin C

Calgranulin C, a calcium-binding protein related to the S100 superfamily, has been found to increase in stools during inflammatory flares.¹³⁰ Calgranulin C is expressed exclusively by neutrophils and has the ability to target the RAGE protein, inducing NF-κB and MAP-kinase pathways, leading to increased production of pro-inflammatory cytokines.¹³¹ In 2003, Foell and colleagues reported a statistically significant increase in calgranulin C levels in both CD and UC compared to HC using sandwich ELISA testing ($p < 0.0001$).¹³² A recent study aimed to analyze the concentration of calgranulin and FC in IBD pediatric patients.¹³³ The authors found a direct positive correlation between FC and calgranulin C levels, as well as significant consistency in calgranulin levels assessed during active disease versus remission ($p = 0.02$).¹³³ However, despite these findings, the use of calgranulin C as a biomarker is not authorized in clinical practice.

Lipocalin 2

Another potential marker of inflammation in IBD patients is Lipocalin 2 (L2). L2 is an adipo-cytokine, a member of the lipocalin family, small proteins capable of binding hydrophobic mole-

cules on cell surfaces and forming large aggregates.¹³⁴ L2 has been studied in various diseases, including diabetes mellitus,¹³⁵ and in IBD as well.¹³⁶ In a study by Thorsvik *et al.*, the L2 concentration levels in the stools of 73 IBD patients were compared to HC and IBS patients, with significantly higher values of this marker in IBD compared to the other groups (difference of 0.3 mg/kg in UC and CD vs HC, $p < 0.001$ for both; difference of 0.4 mg/kg in UC and CD vs IBS, $p < 0.001$ and $p = 0.004$, respectively).¹³⁷ Another study by Magro and colleagues identified a cutoff level of L2 in stool samples (approximately 12 µg/g) to predict endoscopic and histologic remission in UC.¹³⁸ L2 is relevant in gut inflammation also due to its interaction with another fecal biomarker: fecal matrix metalloproteinase-9 (MMP-9), a member of zinc-dependent endopeptidases known for degrading extracellular matrix, crucial in cell-extracellular environment interactions.¹³⁹ Moreover, L2 can physiologically form complexes with neutrophil gelatinase-associated lipocalin, intricate structures of 198 amino acids with anti-inflammatory functions, equipped with terminal domains capable of binding and stabilizing several proteins such as MMP9 itself.¹⁴⁰ Recently, Buisson and colleagues evaluated the accuracy of MMP9 along with L2 in CD patients, finding good reliability of MMP9 (sensitivity up to 90%) and slightly lower for L2 (sensitivity up to 87.5%) in detecting endoscopic and clinical activity.¹⁴¹

Micro-RNA (miRNA)

A novel trend in the non-invasive evaluation of IBD is represented by miRNA assessment in fecal samples.¹⁴² miRNAs consist of a group of small non-coding RNAs with variable expression in various diseases, from neurological disorders to cancer.¹⁴³ miRNAs can be assessed in serum as well as in all biological specimens, including stools, using various molecular techniques, primarily PCR and Next Generation Sequencing.^{143,144} miRNA 21 is one of the most important miRNAs found to be increased in IBD compared to non-IBD controls.¹⁴⁵ miRNA 21 and miRNA 223 were evaluated in the stools of IBD patients, with higher levels observed in UC compared to CD (miRNA 223 was 5.7-fold higher for CD and 10.2-fold higher for UC compared to controls).¹⁴⁶

Cytokines and interleukins

Serum pro-inflammatory cytokines and interleukins have been extensively characterized in IBD.¹⁴⁷ TNF α has been found to be elevated in the colonic and ileal mucosa of patients suffering from IBD.¹⁴⁸ Despite discordant results in previous studies on serological samples,¹⁴⁹ Komatsu and colleagues used PCR methods and found a statistically significant increase in serum concentrations of TNF α in IBD patients (both CD and UC) compared to HC ($p < 0.0001$).¹⁵⁰ These results suggest a potential use of TNF α serum levels as a biomarker for assessing IBD activity. Serum concentrations of IL-10 in IBD patients have also been evaluated by Kucharzik and colleagues, who reported higher concentrations of serum IL-10 in active CD and UC patients compared with HC ($p < 0.001$).¹⁵¹ Mitsuyama and colleagues conducted an analysis of three inflammatory biomarkers—IL-6, CRP, and IL-10—in patients with UC, CD, and HC. The authors found increased levels of serum IL-6 and CRP in active UC and CD compared to HC ($p < 0.0001$), while higher serum concentrations of IL-10 were observed only in active UC compared to HC ($p = 0.0086$).¹⁵² The role of serum interleukins in assessing and monitoring IBD requires further evaluation through larger observational studies.

Nitric oxide

Additionally, nitric oxide (NO) is a recognized mediator of several processes related to local and systemic inflammation.¹⁵³ NO is synthesized through the oxidation of the amino acid L-arginine by a family of enzymes known as NO synthases.¹⁵³ There exist three isoforms of NO synthase: neuronal, expressed in the brain and peripheral nervous system; endothelial, found in endothelial cells; and inducible NO synthase, which activates in response to microbial products such as IL-1 or TNF- α .¹⁵⁴ Inducible NO synthase activity has been demonstrated in active UC.¹⁵⁵ The potential role of NO as a mediator in the inflammatory processes of IBD has garnered significant interest. Various manifestations of IBD have been found to correlate with NO, including vasodilation and increased vascular permeability, directly or indirectly.¹⁵³ Avdagić *et al.* conducted a study exploring the potential of serum NO as a biomarker for diagnosing UC and CD.¹⁵⁶ The results indicated statistically significant differences in serum NO levels among UC patients, CD patients, and HC. The median NO concentrations in UC patients, CD patients, and HC were 15.3 μ M, 14.5 μ M, and 13.3 μ M, respectively. Using a cutoff of 17.4 μ M, both the sensitivity and specificity of NO in distinguishing between active and inactive UC patients were 100%. With a cutoff of 14 μ M, the sensitivity and specificity of NO in distinguishing between active and inactive CD patients were 88% and 69%, respectively. These findings suggest that serum NO could potentially serve as a biomarker for IBD.

Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and albumin-to-globulin ratio

There has been increasing attention on blood cell count-based ratios such as the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), and their potential relation to various immune-mediated inflammatory disorders.¹⁵⁷ A recent study by Soufli *et al.* assessed serum concentrations of NO, NLR, and PLR in complicated CD patients compared to HC, finding statistically significant differences in these markers between the two groups ($p < 0.001$) before and after corticosteroid or anti-TNF therapy. Cut-off values for disease prediction were identified (NLR > 2.43 ; PLR > 156.4 , respectively).¹⁵⁸ Additionally, the albumin-to-globulin ratio (AGR) may emerge as a promising tool to aid clinicians in differentiating IBD and evaluating disease activity.¹⁵⁹ AGR is

notably reduced in IBD patients compared to those without IBD. In individuals with UC, there is an inverse correlation between serum AGR and the Mayo score ($r = -0.413$, $p < 0.001$), whereas in patients with CD, serum AGR shows an association with HBI ($r = -0.471$, $p < 0.001$).¹⁵⁹

Suppression of tumorigenicity 2 (ST2) and TNF- α -induced protein 6 (TNFAIP6)

ST2 and TNFAIP6 are two novel serological biomarkers recently studied in patients with IBD. ST2 is an interleukin belonging to the IL-1 superfamily.¹⁶⁰ It has been reported that ST2 is positively correlated with endoscopic and clinical activity in UC and CD compared to HC.¹⁶¹ Furthermore, serum levels of ST2 were found to be lower in individuals with UC who responded to treatment (conventional therapies such as 5-ASA derivatives, corticosteroids, or immunomodulators) compared to non-responders ($p < 0.05$).¹⁶² Additionally, among patients who responded to treatment, the level of intestinal ST2 remained consistent within the cellular infiltrate of the lamina propria during the six-month follow-up. In contrast, patients experiencing reactivation showed an elevation in total ST2 within the inflamed mucosa, though it remained confined to the cellular infiltrate.¹⁶² Similarly, elevated TNFAIP6 levels (a 35 kDa glycoprotein with terminal halves sharing homologous regions with terminal peptides of other immune proteins such as C1r/C1s or EGF)¹⁶³ have been detected during episodes of acute inflammation.¹⁶⁴ Its potential for diagnosing and monitoring IBD appears promising, but further clarification is certainly warranted.¹⁶⁵

Autoantibodies against malondialdehyde-acetaldehyde adduct

A promising serological marker for diagnosing IBD is autoantibodies against the malondialdehyde-acetaldehyde adduct (MAA). IBD is associated with immune responses that involve oxidative stress, where elevated levels of malondialdehyde contribute to the formation of a highly stable and immunogenic MAA.¹⁶⁶ Specifically, Duryee *et al.* demonstrated that IgG anti-MAA antibody levels could accurately identify UC with a sensitivity of 75%, specificity of 71%, and an AUC of 0.81.¹⁶⁶

Calprotectin neo-epitope (CPa9-HNE)

Another serological marker that correlates with disease activity in IBD is CPa9-HNE.¹⁶⁷ Serological levels of this marker are higher in both CD and UC patients compared to HC ($p < 0.0001$ for both). CPa9-HNE shows a significant association with the SES-CD ($r = 0.61$, $p < 0.0001$) and the full Mayo score ($r = 0.52$, $p = 0.0013$). It can effectively differentiate between CD and UC patients in terms of endoscopic remission and moderate/severe disease activity (CD: AUC = 0.82, UC: AUC = 0.87). Moreover, other two serological markers of disease activity in IBD are dipeptidyl peptidase activity circulating and proteins of extracellular matrix remodeling such as biomarkers of type III collagen degradation and formation, type IV collagen degradation and formation, and type V collagen formation.^{168,169}

Anti-integrin α v β 6 antibodies

Integrin α v β 6 functions as a receptor for extracellular matrix proteins, notably fibronectin, and its expression is limited to epithelial cells. It plays a significant role in maintaining epithelial barrier functions.¹⁷⁰ Recent research, particularly in the Japanese population, has demonstrated elevated circulating levels of IgG against colonic epithelial integrin α v β 6 in adult patients with UC compared to those with CD and HC.¹⁷¹ These antibody levels were found to correlate with the severity of the disease.¹⁷¹ Subsequent

studies in small Swedish and Italian cohorts confirmed these findings.^{172,173} Additionally, anti-integrin $\alpha\beta6$ autoantibodies appear to precede the clinical diagnosis of UC and are associated with adverse UC-related outcomes.¹⁷¹

Transcriptomic markers

Considering the heterogeneity and varied therapeutic outcomes among IBD patients who exhibit similar clinical, endoscopic, and histologic activity, a more personalized approach is necessary.¹⁷⁴ Perez *et al.*, in a meta-analysis, examined transcriptomic profiles of 1,047 samples from five cohorts to distinguish UC, colonic CD, ileal CD, and pouchitis in comparison to normal colonic and ileal mucosa. They subsequently conducted a meta-analysis focusing on distinct transcriptomic signatures associated with ileal and colonic manifestations of these diseases.¹⁷⁵ They identified specific markers indicating inflammation in the ileum (FOLH1, CA2) and colon (REG3A), and demonstrated that as the disease progresses, specific cells in the ileum begin expressing markers typically associated with the colon. Immunohistochemistry validated the specificity of these markers for ileal or colonic diseases. These findings highlighted that colonic CD resembles UC more than ileal CD, which shares similarities with pouchitis. Transcriptomic analysis, in addition to aiding diagnosis, may also predict endoscopic and histologic healing in IBD patients. Biopsies from 111 UC patients treated with ritlecitinib (an oral JAK3/TEC inhibitor) were analyzed by Hassan-Zahraee *et al.* Ten genes (CXCL1, FCAR, CKAP4, SPINK4, CXCL17, OSM, CD4, CXCL9, IL17A, and GZMB) exhibited significant alterations from baseline in responders compared to non-responders at week 8, particularly in terms of endoscopic improvement or histological remission. Additionally, these genes showed a marked increase at baseline between colon biopsies with inflammation and those without inflammation.¹⁷⁶ New transcriptomic biomarkers can predict therapeutic response, as Abreu *et al.* identified immune cell phenotypic and gene expression patterns associated with vedolizumab response.¹⁷⁷ In this study, Treg cells, especially from the ileum, showed the most transcriptional differences at baseline in responders vs non-responders to vedolizumab, irrespective of CD or UC diagnosis ($p < 0.05$). This evidence supports molecular disease stratification over reliance solely on clinical criteria, opening possibilities for designing drugs that target diseases more specifically.

Discussion

The assessment of IBD activity is crucial for disease monitoring and evaluating treatment responses.¹⁰ The pursuit of achieving disease remission should be guided by a “treat-to-target” strategy, involving regular evaluations of disease activity.¹⁷⁸ This approach aims to streamline treatment adjustments and enhance long-term outcomes.⁶ Serum and fecal biomarkers are the fastest and easiest tools to monitor the course of IBD.¹⁸ FC remains the most accurate marker of gut inflammation, demonstrating exceptional accuracy, sensitivity, and specificity in assessing disease activity, treatment efficacy, and predicting relapse.²⁰ Despite its recognition as an inflammation marker, FL still faces challenges such as low specificity and a lack of significant validation studies, preventing its widespread adoption in routine clinical practice.⁹¹ Regarding serum markers, CRP, ESR, and blood cell counts are routinely used.⁹² Enhancing diagnostic accuracy through multi-target panels of biomarkers has demonstrated improved sensitivity and specificity. Novel biochemical targets such as fecal MPO, serum pro-inflammatory cytokines, interleukins, serum or fecal miRNAs, Calgranulin C, Lipocalin 2, NO, CPa9-HNE, transcriptomic

markers, and urinary components (especially PGE2) are gaining attention in IBD research. However, further studies are needed to integrate them into clinical practice.¹⁷⁹ Expanding the repertoire of biomarkers could serve as a pivotal role in implementing the treat-to-target strategy for monitoring IBD.¹⁸⁰ While the potential of blood, fecal, and urinary biomarkers in IBD is promising, challenges remain. Achieving standardization in terms of assays, defining appropriate cut-off values, and establishing uniform interpretation criteria are crucial for their smooth integration into routine clinical practice. Longitudinal studies are also essential to confirm their efficacy in predicting disease flares, treatment response, and long-term outcomes. The evolving landscape of blood, fecal, and urinary biomarkers in IBD presents an exciting opportunity to revolutionize diagnostic and monitoring methodologies for these complex diseases. The incorporation of non-invasive biomarkers into clinical practice offers the potential for early detection, evaluation of disease activity, and tailored treatment approaches. As ongoing research unveils the complexity of IBD, integrating biomarkers into patient care becomes increasingly feasible. Through collaborative efforts and rigorous research, we can pave the way towards more effective management and improved quality of life for individuals navigating the challenges of IBD.

Conclusion

Assessing IBD activity is crucial for understanding treatment responses and long-term outcomes. A “treat-to-target” strategy, supported by regular evaluations, guides remission targets. Biomarkers serve as non-invasive tools for diagnosing and monitoring IBD, yet further studies are required to fully integrate them and predict relapses and treatment outcomes. The evolving landscape of biomarkers presents an opportunity to improve IBD management and promote personalized medicine.

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Author contributions

Conceiving the article (MA, FF), writing the article and creating

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Data sharing statement

No new data were generated or analyzed in support of this research.

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