Biomarkers of Inflammation in Inflammatory Bowel Disease: How Long before Abandoning Single-Marker Approaches?

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Keywords

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Abstract

Background: Inflammatory bowel disease (IBD) is a chronically relapsing disease with a continuous need for proactive monitoring to decide appropriate treatments and follow-up strategies. To date, gastrointestinal endoscopy with histologic examination of biopsies and contrast-enhanced imaging are mandatory techniques for the diagnosis and the activity assessment of IBD. **Summary:** In recent decades, many research efforts in the IBD field have been placed on finding non-invasive and reliable biomarkers of disease burden that can be easily tested in body fluids without impacting the quality of life of patients. Unfortunately, the ideal biomarker is yet to be discovered and recent studies have investigated the possibility to increase the accuracy of such measurements by combining different markers. In this review, we provide an update about the current knowledge on biomarkers of intestinal inflammation in IBD, focussing on disease diagnosis, correlation with endoscopic findings, and

prediction of relapse. We also summarize composite scores of clinical and laboratory markers that have been recently proposed in various scenarios of disease activity. **Key Messages:** To date, only C-reactive protein and faecal calprotectin can be considered reliable markers of disease activity with demonstrated utility in IBD management. The combination of different parameters has recently shown higher accuracy and might substitute single-marker approaches in the future of research and clinical practice.

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Introduction

Inflammatory bowel disease (IBD) refers to 2 chronic conditions of the digestive tract with a strong impact on the quality of life of the affected individuals, that is, ulcerative colitis (UC) and Crohn's disease (CD). The aetiology and pathogenesis of IBD are still unclear, with the most likely trigger being a dysregulated immune response to the commensal gut flora and external insults in a genetically predisposed individual [1–3].

Fig. 1. Characteristics of the ideal biomarker (adapted from the work by Sands [11]). IBD, inflammatory bowel disease.

Gastrointestinal endoscopy with subsequent histologic evaluation of biopsies is required for the diagnosis and scoring of mucosal inflammation, and cross-sectional imaging provides additional information on the extent of CD in the small bowel, which is technically unreachable by the endoscope [4]. Nevertheless, patients are reluctant to undergo invasive endoscopic follow-ups and both these techniques highly impact the healthcare finances if we consider the need to routinely re-assess a disease with chronic intermittent behaviour. Consequently, an urgent topic of investigation in recent decades has been the study of accurate biomarkers of disease activity to reduce costs and increase patients' compliance.

The Ideal Biomarker

The ability to discriminate between active and inactive disease with non-invasive tools is a diagnostic goal in many medicine fields. In IBD, research has mainly focussed on the identification of reliable biomarkers of intestinal inflammation to guide treatment management and predict disease course.

Approximately 20 years ago, the United States National Institute of Health defined a biomarker as “a characteristic that is objectively measured and evaluated as an indication

of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [5]. The ideal biomarker should satisfy 3 different domains: patient compliance, reliability for the disease, and kinetic stability (Fig. 1). Based on these premises, several issues have been raised in the field of IBD research. First, IBD is a spectrum of 2 different entities (UC and CD) with an unclear “grey area” of unclassified patients and a modification of diagnosis in 5–10% of cases [6, 7]. Second, the overlap between inflammation and fibrosis and the different possible characteristics of the disease (extent, activity, and phenotype and related complications) further complicate the scenario. Therefore, although some markers of inflammation are currently used in clinical practice, the discovery of an ideal biomarker seems unattainable and the combination of different tests is rising as more than an alternative to the classic inflammatory markers.

Biomarkers of Intestinal Inflammation

The natural history of IBD includes flares of acute inflammation and periods of quiescence associated with mucosal healing, which is currently the main therapeutic

Table 1. Biomarkers correlated with intestinal inflammation [79,94,120–148]

Marker	Sample type	Clinical context
α1-Acid glycoprotein	Serum	Correlation with disease activity in IBD [120]
α1-Antitrypsin	Stool	Possible marker of clinical relapse in patients with CD of the terminal ileum [121]
β2-Microglobulin	Serum	Higher values in patients with IBD than in controls and in patients with active disease than in patients in remission [122]
Adenosine deaminase	Serum	Conflicting data with regard to CD activity <clear-delete>[123, 124]</clear-delete>; possible predictor of disease severity in UC [125]
Calprotectin	Stool	See text
CHI3L1	Stool	Correlation with endoscopic score in both UC and CD adult patients [126] and CD paediatric patients [127]
CRP	Serum	See text
DPP-4	Serum	Significantly lower levels were observed in active UC and CD when compared clinically and endoscopically to responders and controls [128]
FGL-2	Intestinal mucosa	Involvement in NF-κB signalling in intestinal epithelial cells and lamina propria dendritic cells, possible future target for therapy [129]
Lactoferrin	Stool	See text
Leukotriene E-4	Urine	Higher levels in patients with active IBD than in patients with inactive IBD and controls [130]
M2PK	Stool	Higher expression in IBD paediatric patients than in healthy controls, with a correlation with disease extent [131, 132]
Methylation of CpG islands	Intestinal mucosa	Higher levels of methylated genes sites and methylating enzymes were found in biopsies of active UC compared to quiescent disease [133, 134]
	Serum	Methylation patterns observed in paediatric CD patients correlate with acute inflammation, and their modification follows disease progression or regression [135]
miRNA	Serum and stool	See text
MPO	Stool	Relation with disease activity in IBD [136] Increased in patients with active or inactive IBD [137]. As accurate as FC in predicting the degree of mucosal damage in patients with IBD [138]
Neopterin	Stool	Higher levels in clinically active CD patients than in patients with inactive CD and controls [139]
	Urine	Higher expression in patients with active IBD than in patients with inactive IBD or IBS [140]
NGAL	Stool	Correlated with ESR and CRP elevation [141]
NGAL-MMP9 complex	Serum	Strong correlation with disease activity in CD, proposed as a surrogate biomarker of MH [142]
PMN-E	Stool	Low levels correlate with MH in UC [79]
Procalcitonin	Serum	Poorly related to disease activity but possible predictor of IBD-related infections [143]
Serum amyloid A	Serum	High levels correlated with lack of MH in CD [144]
Sialic acid	Serum	Higher levels in CD patients than in controls, with an association with disease activity [145]
TFF3	Serum	Increased levels in paediatric CD patients were found, with positive correlation with endoscopic scores [146]
TNC	Intestinal biopsies and serum	Serum levels and intestinal mucosa expression of TNC were reported to be higher in active CD and UC patients and able to predict poor response to infliximab [147]
TPNG	Plasma	Associated with IBD if the levels are compared to those in patients without IBD [94]
Tryptophan and metabolites	Serum	Levels of tryptophan decrease in patients with IBD and are inversely proportional to disease activity and serum CRP (stronger association in CD than in UC). Conversely, levels of its metabolites increase in IBD [148]

CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; CDEIS, Crohn's Disease Endoscopic Index of Severity; CHI3L1, chitinase 3-like-1; CpG, cytosine-phosphate-guanine; CRP, C-reactive protein; DPP-4, dipeptidyl peptidase 4; ESR, erythrocyte sedimentation rate; FGL-2, fibrinogen-like protein 2; IBD, inflammatory bowel disease; M2PK, M2-pyruvate kinase; MH, mucosal healing; MMP9, matrix metalloproteinase-9; MPO, neutrophil myeloperoxidase; NGAL, neutrophil gelatinase B-associated lipocalin; NF-κB, nuclear factor κB; PMN-E, polymorphonuclear elastase; TFF3, trefoil factor 3; TPNG, total plasma N-glycomes; UC, ulcerative colitis.

goal [8]. Unfortunately, gastrointestinal endoscopy with biopsies is the only gold standard to assess deep histologic remission, but it is an invasive and expensive technique. The recent proposal to introduce transmural healing for CD as an additional therapeutic endpoint further complicates IBD assessment [9]. As a consequence, it is crucial to find surrogate biomarkers of clinical, endoscopic, histologic, and transmural activity to increase patient compliance and the cost-effectiveness of IBD management.

The clinical settings in which biomarkers of inflammation could be used are various. In the diagnostic field, a marker of inflammation burden should be able to discriminate IBD from functional disorders, such as irritable bowel syndrome (IBS), otherwise clinically indistinguishable in many cases [10], and assess disease activity. Moreover, the ability to monitor the efficacy of a medical treatment and predict surgical outcomes (risk of colectomy in UC and postsurgical recurrence in CD) is additional unmet needs [11].

In general, stool markers are more likely than blood markers to reflect the degree of intestinal inflammation due to direct contact with the mucosal environment, whereas blood markers often increase for different reasons, and local inflammation does not always correspond to a detectable systemic inflammatory response. Many data on potential biomarkers of intestinal inflammation have accumulated in recent years (Table 1). Nevertheless, serum C-reactive protein (CRP) and faecal calprotectin (FC) are still the most reliable and routinely used markers of disease activity.

C-Reactive Protein

Serum CRP is an acute-phase reactant that is widely accepted as a general inflammatory marker in the IBD field. As it is a non-specific biomarker, CRP can increase in several medical conditions other than IBD, for example, systemic infections and extraintestinal inflammation [12]. On the other hand, approximately 15% of the healthy population [13, 14] and as high as 20–25% of CD patients [12] do not have a CRP response, which is a cause of false-negative results.

CRP has a high sensitivity for discriminating between IBD and IBS, as reported in a meta-analysis of 12 prospective diagnostic cohort studies where CRP levels in the range of normality resulted in a $\leq 1\%$ probability of having IBD [15]. Its levels are often related to the inflammatory burden of UC and CD [12, 16], although a poor correlation is sometimes reported for UC, especially in the setting of mild ulcerative proctitis [16, 17]. Accordingly,

many studies have shown that extensive transmural inflammation in CD presents higher CRP levels than mild-to-moderate mucosal inflammation in UC [18–20]. On the other hand, CRP >45 mg/L combined with a stool frequency between 3 and 8 per day on day 3 (known as the Oxford Criteria) predicts the occurrence of colectomy in 85% of patients with acute severe UC during hospitalization [21], despite recent data have partially reconsidered these results [22].

At the endoscopic level, CRP has been demonstrated to correlate with disease activity, with levels within the normal range in cases of negative ileocolonoscopy [16, 17]. However, in a recent prospective study on 186 patients with UC, Sonoyama et al. [17] reported that serum CRP was not as strongly related to the Mayo Endoscopic Score as FC and no correlation could be observed between CRP and ulcerative proctitis.

With regard to disease monitoring, CRP has been shown to predict clinical relapse and therapy failure in both CD and UC [23–25]. Particularly, a serum CRP of >5 mg/L at week 22 after the initiation of infliximab (IFX) was found to be predictive of loss of response in patients who were in clinical remission at week 14 (HR: 2.5; 95% CI: 1.16–5.26; $p = 0.019$) [25]. In addition, in a large retrospective study involving 1,189 patients with CD, high serum CRP was found to be predictive of a low retention rate of adalimumab treatment over a 4-year period of follow-up [26].

On the other hand, conflicting data have been published regarding the correlation between CRP and CD post-operative recurrence (POR). A randomized controlled trial of 24 patients reported a poor sensitivity (25%) for endoscopic POR at 1 year [27]. Conversely, in a small study ($n = 12$) of IFX withdrawal after 3 years of clinical and endoscopic remission after surgery, CRP levels correlated with POR relapse and subsequent response after resumption of therapy [28]. Moreover, another study reported only a very weak although statistically significant difference between patients with POR and patients with endoscopic remission after a median of 7 months after surgery [29].

Therefore, the settings in which CRP was demonstrated to be mostly reliable are the differential diagnosis between IBS and IBD, the transmural inflammation of CD, the endoscopic activity of extensive and left-sided UC, and the monitoring of disease relapse. Conversely, CRP accuracy in the contexts of ulcerative proctitis and the prediction of POR in CD is rather low and other biomarkers should be preferably considered.

Faecal and Serum Calprotectin

FC is the other fundamental inflammatory biomarker currently used to assess diagnosis and monitor response to treatment in IBD [12, 30]. Calprotectin is predominantly present in neutrophil granules and has an antimicrobial function [31, 32]. Although it is found in various body fluids, its concentration in faeces is 6 times higher than that in blood in healthy individuals [33]. In particular, FC levels increase in patients with active IBD [34] and are correlated with neutrophil margination in the intestinal mucosa [35]. Despite a certain level of intra-individual variability when samples are collected at different timepoints over the course of the same day [36], its use has been widely accepted in clinical practice [4].

In most laboratories, the upper normal limit is set at 50 µg/g, consistent with the results of a meta-analysis of 1,062 patients, in which it was found that a patient with IBS-like symptoms and a FC level of <40 µg/g had a 1% or less chance of having IBD [15]. However, in general gastroenterology, this marker lacks specificity, as it can increase in many other intestinal conditions, such as coeliac disease, colon cancer, diverticulitis, use of anti-inflammatory drugs, and intestinal infections [37]. Furthermore, it is important to know the factors that can influence the test and reduce its sensitivity and specificity [38].

FC has shown a solid correlation with endoscopic activity in IBD. In a recent meta-analysis of 25 eligible studies that examined IBD endoscopic activity in association with FC, the authors reported a pooled sensitivity of 85% and a specificity of 75% in diagnosing active IBD [39]. When UC and CD were considered separately, the same meta-analysis revealed a better performance in UC patients (pooled sensitivity 87.3 vs. 82.4%; specificity 77.1 vs. 72.1%). A possible explanation for these findings lies in the conflicting results of FC accuracy when considering CD patients with isolated ileal disease [40–43]. Accordingly, in a study of 68 UC patients, it has been reported a strong correlation of FC levels with disease extent ($p = 0.006$), Mayo endoscopic score ($p = 0.001$), and Nancy histologic index ($p < 0.001$) [44].

The aforementioned Nancy index and the Geboes score are 2 validated histologic scores of disease activity developed for UC [45, 46]. Defining histologic remission with a Geboes score of <3.1 or a Nancy score of ≤1, a correlation between low levels of FC (<60–100 µg/g) and histologic remission was demonstrated [44, 47]. Moreover, Theede et al. [48] found that levels of FC lower than 40.5 µg/g are able to predict histologic remission with a sensitivity and a negative predictive value of 100%, despite the utilization of a non-validated score. Likewise, elevated FC

values (ranged 72–250 µg/g) showed to predict histologic inflammation in clinically silent patients in more recent studies [49–51]. Recently, a systematic review including 12 studies and 1,168 patients confirmed the correlation between FC and histologic activity, although validated cutoff levels and more prospective studies using validated scores are needed to overcome the heterogeneity of current literature [52].

Regarding disease monitoring, FC has been demonstrated to predict clinical relapse both in UC and in CD [53–57]. Importantly, a tight control of consecutive FC levels, as other inflammatory biomarkers, has shown to be superior to clinical assessment only and should be always recommended [58]. In addition, a recent large prospective study involving 185 clinically quiescent UC patients demonstrated that FC cutoffs of ≥170 µg/g and ≥135 µg/g were able to predict endoscopic and histologic activity, respectively [50].

FC has also a role in predicting the response to treatment. In 2006, Kolho et al. [59] noticed that children with IBD treated with glucocorticoids experienced a decrease in FC levels, but often not below 100 µg/g. In the last few years, many data have accumulated on the capability of FC to predict the response to mesalamine, immunosuppressants, and biological treatments. For instance, dose intensification of mesalamine resulted in significant decrease of FC levels in UC patients in clinical remission despite elevated FC [60]; in the same study, patients with baseline FC ≥200 µg/g showed a higher risk of relapse [60]. In a study involving 88 CD patients on azathioprine, FC levels were found to be strongly linked to thiopurine metabolite concentration: in patients with 6-thioguanine concentrations within the therapeutic range (250–450 pmol/ 8×10^8 red blood cells), FC was significantly lower than in patients with different levels of metabolite concentration [61]. A prospective study involving 53 patients with UC undergoing an induction cycle with infliximab showed that patients with a significant decrease in FC at week 2 were more likely to achieve endoscopic remission at week 10 [62]. In another prospective study, FC <82 µg/g at week 14 predicted clinical remission within 12 months in CD patients treated with anti-TNF agents (sensitivity 93%; specificity 75%) [63]. A post hoc analysis of the GEMINI 1 trial (895 patients) showed that FC levels had higher reductions in patients with UC treated with vedolizumab than placebo; in particular, an absolute FC concentration of ≤150 µg/g was found to be the best predictor of clinical and endoscopic remission at week 6 [64]. Finally, Dulai et al. [65] confirmed the potential role of FC in monitoring clinical and endoscopic response in UC

patients treated with an induction cycle of a biological agent or tofacitinib; the study showed that in patients who had resolution of rectal bleeding and normalization of stool frequency, an FC of ≤ 50 $\mu\text{g/g}$ was able to rule out patients with moderate-to-severe endoscopic activity. Conversely, an FC of ≥ 200 $\mu\text{g/g}$ was able to identify moderate-to-severe endoscopic activity in UC patients with persistent rectal bleeding and increased stool frequency [65].

In the context of monitoring for POR in CD, conflicting results have been published in the last few years. In a cohort of 104 CD patients with previous surgery, Lamb et al. [66] reported a moderate correlation between FC and post-operative Harvey-Bradshaw Index ($r = 0.53$, $p < 0.001$), whereas Yamamoto et al. [67] found no significant correlation between FC and Crohn's Disease Activity Index in a smaller cohort ($r = 0.26$, $p = 0.28$). However, most evidence shows that FC is a more reliable marker than CRP for this purpose, at both clinical and endoscopic levels [29, 68–70].

Furthermore, a meta-analysis of 613 CD patients monitored with FC after resection demonstrated a pooled sensitivity and specificity for POR prediction of 0.82 (95% CI: 0.73–0.89) and 0.61 (95% CI: 0.51–0.71), respectively [71]. As a result, in a survey amongst European gastroenterologists, 90% of respondents reported following CD patients with FC after a first negative colonoscopy [72]. Confidence for the application of this approach is the consequence of data showing the high negative predictive value ($>90\%$) of FC in this setting for levels <100 $\mu\text{g/g}$ [29, 68]. Interestingly, a recent systematic review studied the ability of FC to predict the development of IBD in patients with rheumatologic conditions, including ankylosing spondylitis and spondyloarthritis: endoscopic and histologic inflammation in the intestine was found in up to 80 and 100% of rheumatologic patients with increased FC levels, respectively [73].

Therefore, FC can be considered a reliable surrogate marker of endoscopic activity in IBD (especially in UC), a predictor of clinical and endoscopic relapse in UC, CD, and POR setting, and a useful tool for differentiating between IBS and IBD. On the other hand, the accuracy of detecting intestinal activity in CD patients with isolated ileal disease is significantly lower, as well as its specificity among several intestinal inflammatory conditions and between UC and CD in the context of new IBD diagnoses.

Among biological fluids, calprotectin is also particularly expressed in the blood. Serum calprotectin (SC) has been poorly studied, but the recent literature suggests its

potential role as an inflammatory biomarker in IBD [74]. As an example, in the STORI trial, SC was significantly higher in patients with CD than in controls ($p < 0.0001$) and even higher in clinically active CD than in quiescent disease ($p < 0.0001$), whereas no correlation was reported with endoscopic scores [75]. Therefore, it is likely that SC more accurately indicates a systemic inflammatory status rather than specific intestinal inflammation, resulting in a marginal role in IBD.

Faecal Lactoferrin

Faecal lactoferrin (FL) is another faecal biomarker that has shown results similar to FC [76]. Lactoferrin is an iron-binding 80-kDa glycoprotein that is mainly released by neutrophils in the faeces after margination in the inflamed gut [77].

In a meta-analysis of 10 high-quality studies, FL showed pooled sensitivity and specificity values for assessing UC activity of 0.81 (95% CI: 0.64–0.92) and 0.82 (95% CI: 0.61–0.93), respectively, and of 0.82 (95% CI: 0.73–0.88) and 0.71 (95% CI: 0.63–0.78) for CD, respectively [78]. In a post hoc analysis of a German clinical trial in patients with mild-to-moderate UC, a cutoff value of 11.9 $\mu\text{g/g}$ for FL showed an area under the curve of 0.734 (0.654–0.813, $p < 0.000$), with a 70.2% diagnostic accuracy to detect sustained clinical remission and mucosal healing [79].

In an additional meta-analysis comparing CRP, FC, and FL among symptomatic IBD patients, FC and FL demonstrated higher reliability than CRP both in terms of pooled sensitivity (0.92 for FC, 0.88 for FL, and 0.49 for CRP) and pooled specificity (0.82 for FC, 0.79 for FL, and 0.73 for CRP) [76]. Again, a recent study has confirmed the superiority of FL when compared to CRP in terms of correlation with endoscopic activity [80].

Nevertheless, these presented data do not differ from the results already observed with FC and add no clinical utility when considered separately. A potential future use for FL is the combination of this marker with other serum and faecal measurements to increase the overall accuracy.

MicroRNAs

MicroRNAs (miRNAs) are a class of small, non-coding, negative regulators of gene expression with altered biogenesis due to the inflammatory response [81]. These markers are very attractive because of their stability in the extracellular environment and the ease and specificity at which they can be detected. Data in IBD are starting to accumulate, with identification of several miRNAs in the whole blood [82], serum [83–85], faeces [85, 86], and in-

testinal tissues [83, 87–89] of patients with active IBD that differ from those in patients with quiescent disease and controls. Interestingly, miRNAs have been suggested as potential predictors of response to therapy since levels of let-7d and let-7e were significantly elevated at week 6 of the IFX induction regimen in patients achieving clinical remission [90].

Recently, >800 faecal miRNAs have been investigated in stool samples of patients with both UC and CD and compared to controls [86]. Findings suggest that patients with active CD and UC have distinct faecal miRNA profiles compared to non-IBD patients, particularly higher levels of miRNA-223 and miRNA-1246 [86]. Additional studies of larger cohorts with pooled-data meta-analyses and comparisons with other faecal markers such as FC are necessary to assess the real importance of miRNA in the evaluation of inflammation in IBD.

Glycoproteomics

Protein glycosylation is involved in the pathophysiology of many inflammatory diseases, and a central role has also been postulated in IBD [91–93]. In particular, some expression profiles of total plasma N-glycomes have been recently associated with disease location, severity, need for potent treatment, and resection in a large multicentre study [94]. The ability of this profiling to discriminate non-IBD from IBD cases and active from non-active disease might open a new investigational field for early diagnosis, assessment of activity, and prediction of response.

Others

In addition to CRP, IBD flares are associated with the increase in other positive acute-phase reactants and pro-inflammatory cytokines, such as erythrocyte sedimentation rate, platelet count, ferritin, haptoglobin, tumour necrosis factor α , and coagulation and fibrinolysis factors, among others, and with a decrease in negative acute-phase reactants, such as serum albumin, factor XII, and α 2-macroglobulin [11, 95–97]. However, the vast majority of these serum biomarkers have shown conflicting associations with disease activity and have not demonstrated a clear superiority over CRP, probably because of their longer half-life.

Many other biomarkers have been tested in serum, faeces, and urine but have not entered clinical practice mainly due to unsatisfactory supporting evidence or the high costs of reproducibility. All biomarkers not discussed in the text are summarized in Table 1.

Composite Biomarkers

To date, FC has certainly shown the most robust evidence as a reliable single biomarker of IBD activity, reflecting better than CRP the degree of mucosal inflammation in the intestine [70, 98]. In last European recommendations for diagnostic assessment of IBD [4], an interval of 3 months for FC monitoring in patients with negative calprotectin (“target range”) and of 1 month when above the threshold (“action range”) has been proposed. Although very useful in guiding the management of clear cases, this approach presents 2 issues. First, the cutoff may vary between different IBD clinical contexts and assays provided by the manufacturers [37, 99]. Second, despite the satisfying accuracy, there is still a grey zone (“uncertain range”) that is difficult to be interpreted with FC alone. Therefore, a different and better performing strategy is needed, especially in patients with subclinical disease.

Composite biomarkers are “a combination of 2 or more biomarkers, which are combined by using a stated algorithm or approach to obtain a single interpretive readout” [100]. If we consider the complexity and heterogeneity of IBD, it is clear that a single marker cannot predict the activity of the disease in every single case and that the concept of “precision medicine” should be extended also to this field [101]. Data on composite scores of IBD activity have started to accumulate in the last few years (Table 2), and many more are expected to be published.

Most evidence comes from the combination of biomarkers with clinical symptoms, which is able to improve the predictive value of each item taken alone [102–108]. As an example, the integration of FC with CRP and clinical data (Simple Clinical Colitis Activity Index for UC and Harvey-Bradshaw Score for CD) has been reported to increase the yield of correct classification of IBD patients with “indefinite” FC levels between 100 and 250 μ g/g [103]. Likewise, in a cohort of CD patients treated with anti-TNF, the decrease in FC combined with CRP <2.9 mg/dL and clinical remission at 12 weeks was the best predictor of corticosteroid-free remission at 52 weeks [104].

Another proposed score was the Utrecht Activity Index that combined the frequency of liquid stools in a day with CRP, FC, platelet count, and platelet mean volume with optimal results in terms of endoscopic activity prediction in CD patients [106]. However, in a recent systematic review on published composite biomarkers made of clinical and laboratory parameters, Brand et al. [109] reported that only 2 out of 7 analyzed indexes show lim-

Table 2. Composite biomarkers of IBD disease activity [102–108,110,111,113–117,149]

Composite markers	Clinical context	Ref.
HBI + CRP	Integration of HBI and CRP demonstrated to be superior than FC in predicting CD post-operative recurrence	[102]
Clinical + CRP + FC	Combined use of clinical parameters (i.e., HBI for CD and SCCAI for UC) with CRP and FC was able to correctly classify patients with “indefinite” FC as having active or inactive disease	[103]
CDAI + FC + CRP	Combination of CDAI <150, FC improvement, and CRP <2.9 mg/dL at 12 weeks was the best predictor of corticosteroid-free remission at 52 weeks in a cohort of CD patients treated with anti-TNF	[104]
Symptoms index + FC	An activity index composed of symptoms and semiquantitative FC measurement has shown slight superiority over FC alone in separating active and inactive colonic IBD	[105]
Bowel movements + CRP + FC + PLT (count and mean volume)	An integrated score of [number of liquid stools in a day $\times$ 0.25 + CRP (in mg/L) + PLT count ($\times 10^9$ /L) $\times$ 0.01 + FC (mg/L) $\times$ 0.001 – mean PLT volume (femL) $\times$ 0.2] ranked the best in prediction of disease activity in CD patients	[106]
Clinical + CRP + FC + FL + fPMN-e	A comprehensive activity index including clinical CAI, CRP, and 3 faecal markers (FC, FL, and fPMN-e) has shown to be an accurate pre-test as regards endoscopic activity in UC	[107]
PCDAI + ESR + CRP + FC	A weighted index integrating stool item of PCDAI, levels of ESR, CRP, and FC has demonstrated small but significant superiority over FC in assessing mucosal healing of paediatric CD patients	[108]
Serum amyloid A + IL-6 + IL-8 + eotaxin-1	The combination of these 4 inflammatory markers has reported to be superior to both FC and CRP as predictor of IBD endoscopic activity	[110]
TNF- α + IL-12 + IL-8 + IL-2 + IL-5 + IL1- β + IFN- γ	A panel composed of 7 selected cytokines has shown to predict UC clinical response to anti-TNF with higher accuracy than standard markers	[111]
FC + IL-6 + IFN- γ	The integration of the 3 markers was superior to FC alone for predicting POR at 6 months in operated CD patients	[113]
FC + FIBT	Combination of FC and FIBT resulted in better accuracy than either marker alone for the identification of UC patients with active disease or at risk of recurrence	[114, 116]
FC + EDN	A test combining FC and EDN ranked the highest for IBD detection sensitivity and response to treatment	[115]
FC + IFX-TL	The combination of FC >250 μ g/g and IFX-TL <2 μ g/mL was able to predict loss of response to infliximab better than FC or trough levels alone	[117]
Faecal tests + CRP	Combining FC with CRP or FIBT with CRP was more accurate than FC or FIBT alone for detection of mucosal inflammation in UC patients	[149]

Only studies with clear superiority of the integrated score compared to single markers have been considered. CAI, Colitis Activity Index; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; CRP, C-reactive protein; EDN, eosinophil-derived neurotoxin; ESR, erythrocyte sedimentation rate; FC, faecal calprotectin; FIBT, faecal immunochemical blood test; FL, faecal lactoferrin; fPMN-e, faecal polymorphonuclear neutrophil elastase; HBI, Harvey-Bradshaw Index; IBD, inflammatory bowel disease; IFX-TL, infliximab trough levels; PCDAI, Paediatric CD Activity Index; PLT, platelets; SCCAI, Simple Clinical Colitis Activity Index; TNF, tumour necrosis factor.

Fig. 2. Improvements provided by composite biomarkers. Careful selection of markers and their integration can optimize the diagnostic accuracy of single biomarkers of disease activity and drastically reduce the blind spot resulted from the “grey zone.”

ited benefit over FC or CRP alone in predicting endoscopic activity in CD. A possible explanation for these results is that scores considering patient-reported symptoms might often present subjective biases.

The exclusive integration of objective biomarkers is likely to provide the most reliable results. For instance, an analysis of the serum levels of 10 inflammatory markers involved in IBD pathogenesis reported that the combination of the 4 most predictive biomarkers (serum amyloid A, IL-6, IL-8, and eotaxin-1) was superior to CRP and FC in predicting the presence of endoscopic activity [110]. Similarly, a panel of 7 cytokines (TNF- α , IL-12, IL-8, IL-2, IL-5, IL1- β , and IFN- γ) has been demonstrated a strong predictor of clinical response to anti-TNF induction therapy in UC patients, with a sensitivity of 84.2% and a specificity of 93.3% [111]. Likewise, a recent work by Bertani et al. [112] reported that the combined decrease in IL-6 and IL-8 from baseline to week 6 after introduction of vedolizumab in patients with UC was the most accurate tool to predict mucosal healing at 1-year endoscopic assessment.

In general, the best strategy is probably to maintain FC as the cornerstone of IBD monitoring and increase its diagnostic accuracy by integrating other laboratory parameters, both at serum and faecal levels [113–117]. For example, the comprehensive evaluation of FC with IL-6 and IFN- γ was superior to FC alone in predicting Crohn’s post-operative recurrence at 6 months [113]. In addition,

the combination of FC with the faecal immunochemical blood test has resulted in higher accuracy for the identification of UC patients at risk of relapse than either of the 2 markers alone [114]. Moreover, integrating FC data with trough levels of drugs could predict loss of response to the medication with higher accuracy than single markers, as demonstrated for IFX trough levels <2 $\mu\text{g/mL}$ and FC >250 $\mu\text{g/g}$ [117].

Regarding drug trough levels, their combination with inflammatory biomarkers is certainly a field to expand, as we lack data in this specific topic. An extensive discussion of the isolated relevance of trough levels and therapeutic drug monitoring is beyond the scope of this review.

Conclusion

To date, except for CRP and FC, none of the other presented markers are routinely tested and used in clinical practice. The reasons behind these results are 2-fold: first, most of biomarkers have been tested in small clusters of patients and lack validation in large populations; second, technical issues and expensive procedures result in low practicality and scarce access to the proposed assays.

Evidence has shown that FC is currently the most reliable biomarker of intestinal inflammation, particularly when baseline and consecutive measurements are available [118, 119]. As it is not perfect and its accuracy may

vary among different clinical scenarios and influencing factors, it is fundamental to start introducing new parameters to implement FC precision.

In our clinical practice, we consider FC as the cornerstone of non-invasive intestinal inflammation assessment. As already reported, it is important to have many consecutive evaluations to provide a correct interpretation of the measurement and monitor the situation. For this reason, we always ask a recent FC level at first outpatient visit at our IBD clinic, even in asymptomatic patients in remission. In addition, we recommend patients transitioned from other centres or resident in the surrounding provinces of Tuscany to routinely perform the test in the same place to eliminate the laboratory variable. However, despite our trust in FC, we often seek confirmation of other biological markers, such as CRP and haemoglobin, and of course stool frequency, consistency, and blood presence. With regard to this, we try to explain to each patient all the “red flags” of their disease both at clinical and biomarker level, in order to promptly react and contact us to move up their follow-up visit if needed.

In the end, the purpose for the research in this field should be to eliminate or at least decrease the “grey zone” of each biomarker (Fig. 2), which could either be reached

by improving the sensitivity of detection assays or by integrating together different markers. Therefore, we believe that the validation of non-invasive composite biomarker scores to measure disease activity is an important need for our clinical practice, whose final aim is to reliably manage IBD with less invasive procedures and more empiric decision-making regarding treatment adjustments.

Conflict of Interest Statement

The authors have nothing to disclose.

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Author Contributions

G.D. and T.I. performed the systematic review of the literature and drafted manuscript, tables, and figures. A.G. contributed to the conception and design of the review, provided interpretation of current knowledge, and critically revised the manuscript. All co-authors have approved the final version ahead of submission.

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