Review paper

Biological markers of disease activity in inflammatory bowel diseases

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Gastroenterology Rev 2023; 18 (2): 141–147
DOI: https://doi.org/10.5114/pg.2023.129412

Key words: inflammatory bowel disease, biomarkers, zonulin, faecal calprotectin, elafin, haptoglobin.

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Abstract

Inflammatory bowel diseases (IBD) are chronic intestinal conditions of multifactorial aetiology including genetic susceptibility, immunological impairment, dysbiosis, and environmental factors. The diagnosis is based on both clinical and endoscopic features, wherein histopathological evaluation remains a gold diagnostic standard. However, fast, reliable, and non-invasive biological markers have been used for years for diagnosis as well as for disease activity monitoring. Currently, commonly used faecal calprotectin is the only biomarker approved and recommended by the European Crohn's and Colitis Organization (ECCO). Nonetheless, other biological markers discriminating between functional and organic bowel conditions have been widely studied. Therefore, the aim of this manuscript was to review new potential biomarkers of inflammation in IBD. The aim of this study was to review currently available biomarkers of intestinal inflammation and increased gut permeability in IBD.

Introduction

Inflammatory bowel diseases (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) are chronic, relapsing intestinal conditions with globally increasing incidence, especially in developed countries [1, 2].

The pathogenesis of IBD, although still unknown, is most probably multifactorial and includes genetics, environmental factors, dysbiosis, and an impaired immune system [3, 4]. The diagnosis is based on both clinical and endoscopic features, and histopathology is the gold diagnostic standard [5]. However, endoscopy has notable disadvantages such as invasiveness, cost, and inconvenience, which limit its use for frequent monitoring of patients with IBD.

Fast, reliable, and non-invasive biological markers of intestinal inflammation and gut permeability have gained a lot of interest in recent years. They help to discriminate between functional and organic conditions, and as well as diagnosis they are used for monitoring disease activity when the diagnosis is already established, e.g. in IBD [6].

Stool biomarkers have higher specificity for intestinal inflammation compared to blood or serum markers. So far, faecal calprotectin (FCP) is the only biomarker approved and recommended by the European Crohn's and Colitis Organization (ECCO).

Nonetheless, new biological markers such as faecal and serum zonulin, elafin, lipocalin, or haptoglobin have already been investigated in IBD [7, 8].

The aim of this review paper was to summarize new potential biological markers of inflammation for IBD and future approaches in their diagnosis and monitoring.

Serum and blood markers

Inflammation in IBD leads to an acute phase response detectable in the serum and blood, characterized by increased concentration of proteins involved in coagulation and fibrinolysis, such as fibrinogen and plasminogen; complement system components; proteinase inhibitors, including α1-antitrypsin, α1-antichymotrypsin; transport proteins such as haptoglobin and ceruloplasmin; and a variety of other proteins such as C-reactive protein (CRP) or ferritin [9]. Inflammatory cascade is mediated by serum proinflammatory cytokines, which are also elevated. These may include tu-

mour necrosis factor-α (TNF-α), interferon-β, transforming growth factor-β, and interleukins (IL)-1β, IL-6, IL-8, IL-12, IL-17, and IL-23 [10].

Cellular components of blood may also indicate inflammation, which is reflected in elevations of white blood cell and platelet counts [10].

The erythrocyte sedimentation rate (ESR) is an indirect measure of inflammation, especially in chronic diseases. The test measures how quickly erythrocytes, or red blood cells, separate from a blood sample. Inflammation or cell damage make the red blood cells clump together, which makes them heavier, so they settle faster. The faster the red blood cells settle and fall, the higher the ESR [11].

Few blood or serum markers of inflammation have been validated in IBD, and CRP and ESR are the most widely available and used.

Serological markers

Various serologic tests have been proposed to differentiate between CD and UC; perinuclear antineutrophil cytoplasmic antibody (pANCA) and anti-*Saccharomyces cerevisiae* antibody (ASCA) are the most notable ones [12, 13]. It has been reported that pANCA is present in 50% to 65% of patients with UC and 10% to 25% of patients with CD, while ASCA is present in 55–65% of patients with CD and 5–20% of patients with UC [14, 15]. In a meta-analysis, combinations of ASCA and pANCA distinguished CD from UC with 40–50% sensitivity and > 90% specificity [16]. However, there are no data on the utility of serological markers to differentiate UC from CC and IBD undifferentiated (IBDU) in children [17]. Serological markers may also be useful to predict a disease behaviour [18]. It has been reported that ASCA is associated with a more aggressive disease course in paediatric CD [19]. Although there is emerging evidence that combining serological markers may increase the accuracy of differentiation between the 2 forms of IBD or their behaviour, the perfect assay or combination of antibodies has not been discovered. They have several limitations, so clinicians must be aware of the evidence on serological markers, interpret them with caution, and always correlate with the clinical presentation.

Calprotectin

Faecal calprotectin, a leukocyte protein of the S100 family belonging to the group of acute phase reactant proteins found in the granulocytes, neutrophil cytosol, in monocytes and activated macrophages, has been used to assess and monitor disease activity, mucosal healing (MH), and disease recurrence in patients with IBD [20]. It has been established that a cut-off point of 50 µg/g discriminates patients with IBD from controls with 79.4% specificity and 91.9% sensitivity and better correlation with clinical activity than C-reactive protein (CRP) [21].

Several factors such as age, diet, use of nonsteroidal anti-inflammatory drugs, exercise, and the presence of blood or mucus in stools may influence the level of FCP [22–25]. Therefore, it is not specific for any disease; it may only help in discrimination between functional and organic conditions.

Tibble *et al.* demonstrated that increased concentration of FCP identified patients with IBD who were at risk of early relapse, which means it is also a good predictor of disease flare [26]. In the study by Costa *et al.* FCP level higher than 150 µg/g in patients in clinical remission was associated with a 2-fold increase in the relapse risk in CD and a 14-fold increase in UC, which may suggest stronger predictive value of clinical relapse in UC than in CD [27].

Ferreiro-Iglesias *et al.* reported a significant decrease in FCP level after 4 weeks of treatment in patients with CD who had complete response to therapy but not in partial or nonresponders [19]. In the study by Kolho *et al.* on children with IBD, a decrease in FCP level and clinical improvement were observed in paediatric patients with active disease treated with steroids, but it rarely dropped to normal values [28]. These outcomes indicate that FCP may serve as a good predictor of the response to therapy, as well.

Many studies have demonstrated good correlation of FCP with endoscopic disease activity in UC and in CD [29, 30]. Additionally, FCP has been shown to predict histopathological remission in children and adults with a cut-off point of 174 µg/g [31, 32].

Zonulin

Zonulin is a 47-kDa human protein that reversibly modulates the intercellular tight junctions crucial for maintaining physiological processes in the intestine [33]. Their impaired function leads to increased permeability in the epithelial layer of the small intestine [34].

Increased serum/plasma zonulin levels have been found in celiac disease, type 1 and type 2 diabetes, in obesity-associated insulin resistance, and in IBD [35–37]. However, both the mechanism of intestinal inflammation development and the correlation between faecal and serum zonulin levels are not clear [38]. There are limited data on the zonulin use in IBD, and the majority of them include adult patients [6, 7, 39].

A Czech study examining 40 patients with IBD for faecal and serum zonulin showed that both of them were elevated in patients with active CD but not in UC. This finding may be explained by the fact that zonulin is considered as the best marker of increased permeability

in the small intestine, and CD can extend to the whole gastrointestinal tract, including the small intestine [40]. Thus, zonulin levels may be higher in CD than in UC, which is restricted to the large intestine (with the exception of rare backwash colitis).

In a study by Caviglia *et al.* that investigated zonulin in patients with IBD and the correlation between its serum and faecal levels, serum concentrations were higher in IBD patients compared to the control group (34.5 (26.5–43.9) ng/ml vs. 8.6 (6.5–12.0) ng/ml, $p < 0.001$), but no correlation was observed between serum and faecal zonulin (rs = 0.15, $p = 0.394$) [8].

On the other hand, Wegh *et al.*, who investigated which markers were most relevant to assess intestinal permeability in patients with UC, demonstrated that serum not faecal zonulin levels were elevated in active disease and had better correlation with other inflammatory markers such as CRP [39].

Our team analysed both serum and faecal zonulin in children with IBD. We demonstrated that faecal zonulin levels were elevated in those patients. Moreover, increased faecal zonulin was associated with CD activity and strongly correlated with FCP [41]. In the study investigating zonulin and I-FABP in our patients and their correlation with FCP, the only observed correlation was between faecal zonulin and FCP, and the strongest one was in CD: CD – $R = 0.73$, UC – $R = 0.67$, All – $R = 0.67$, CG – $R = 0.65$ [42].

Based on the outcomes from the above-mentioned studies, it seems that faecal zonulin may serve as another – along with FCP – biomarker of intestinal inflammation/increased intestinal permeability in IBD, especially in CD, both in adults and children.

Haptoglobin

Haptoglobin (HP) is a haemoglobin-binding protein with immunomodulatory properties.

It has been shown to have a protective effect against experimentally induced colitis – *HP* knockout mice presented with more weight loss and higher macroscopic and histological scores as compared with the wild-type ones [43]. This means that HP may play an important modulatory and protective role in inflammatory colitis in experimental models. Clinical data are contradictory. Maza *et al.* demonstrated that *HP11* was significantly less common in CD, while Papp *et al.* showed its higher frequency in CD [44, 45]. However, in a well-powered study from the Marques group *HP2* was found to be a risk allele for IBD, with a higher frequency in both CD and UC compared with controls [46]. Mouse model studies have demonstrated that Hp knockout mice are more susceptible to experimentally induced colitis than their wild-type littermates, which supports the protective effect of the *HP1* allele in IBD. However, more studies are necessary to draw any firm conclusions on the role of HP in IBD.

Elafin

Elafin is one of the host defence peptides (HDPs) that has antimicrobial and antiprotease properties [47]. Initially, elafin was isolated from psoriatic skin, but it is also synthesized by epithelial cells of the gastrointestinal tract, lungs, or female reproductive system and inflammatory cells, including neutrophils, mast cells, and macrophages [48, 49].

Until now, there have been only a few studies investigating elafin in IBD, and their results are contradictory [50–53].

Wang *et al.* demonstrated that the level of serum elafin was significantly elevated in patients with UC compared with the control group, while in patients with CD elafin was only mildly elevated, which was not statistically significant [52].

In the study by Schmid *et al.* elafin mRNA was expressed predominantly in the colonic biopsies of active UC [53].

On the other hand, Motta *et al.* reported that mucosal expression of elafin was decreased in patients with IBD [54]. Also, in the study by Zhang *et al.* expression of elafin mRNA in peripheral blood leukocytes was significantly decreased in patients with active UC but increased in UC remission. No significant differences in elafin mRNA were observed between patients with CD and controls [51]. Such outcomes – downregulation of elafin in IBD patients – may suggest that protease/antiprotease imbalance may take part in IBD development or may be a consequence of chronic inflammation leading to the destruction of epithelial cells, which are the main source of elafin.

A study from Poland investigating the role of elafin in the pathophysiology of inflammation in paediatric IBD has demonstrated that serum elafin was increased in the active phases of both UC and CD, but only in the remission of UC [55]. Based on the data published so far, elafin appears to be a potential candidate for a biomarker of UC.

Lactoferrin

Lactoferrin is an iron-binding glycoprotein secreted from glandular epithelial cells, and it can be identified in secretions overlying most mucosal surfaces including saliva, tears, vaginal secretions, faeces, synovial fluid, and mammalian breast milk [56]. Lactoferrin also has antibacterial activity [57]. Several reports have demonstrated that lactoferrin might be a primary factor in an acute inflammatory process because it is a major

component of the secondary granules of neutrophils; its levels quickly increase during inflammation [58].

Sherwood reviewed the utility of lactoferrin in differentiating IBD from IBS, and showed that sensitivities for lactoferrin ranged from 78% to 88% and specificities from 85% to 100% [59]. However, there are contradictory data on the utility of lactoferrin in IBD.

Caccaro *et al*. demonstrated that lactoferrin distinguished between organic and functional disease with a sensitivity of 88% and a specificity of 94% [60]. Sugi *et al.* investigated lactoferrin, polymorphonuclear leukocytes (PMN)-elastase, lysozyme, and myeloperoxidase in stool and whole gut lavage fluid from patients with IBD and found that lactoferrin was a superior marker of intestinal inflammation [61]. In a further study, Langhorst *et al.* showed that lactoferrin and calprotectin differentiated active IBD from inactive IBD and IBS in 80% of cases, compared with 74% for PMN-elastase and 64% for CRP [62]. Judd *et al*. compared faecal concentrations of calprotectin, lactoferrin, and S100A12 in children with IBD, and they reported that calprotectin and lactoferrin correlated strongly with each other [63].

On the other hand, Silberer *et al.* reported that among a number of studied biomarkers, only PMN-elastase and calprotectin, but not lactoferrin, were useful to differentiate chronic IBD from IBS [64]. Moreover, other studies indicated that combining lactoferrin and calprotectin does not provide additional benefit [65, 66].

The potential utility of faecal lactoferrin in predicting risk of relapse in IBD has been investigated. Although the study included a small sample size, the results indicate that elevation of faecal lactoferrin may occur prior to clinical flares [67]. In addition, paediatric studies have demonstrated that faecal lactoferrin is a sensitive and specific marker of inflammation in children with IBD; faecal lactoferrin correlated with both clinical disease activity and serum inflammatory markers [59].

Overall, it seems that faecal lactoferrin is a good marker of inflammation. Although faecal lactoferrin may provide similar information to FCP, it has some limitations compared with calprotectin, which limits its utility in IBD.

Table I. Biomarkers used and studied in IBD

Type	Biomarker
Serum/blood	• Coagulation and fibrinolysis proteins: fibrinogen, plasminogen • Complement system components • Proteinase inhibitors: α1-antitrypsin, α1-antichymotrypsin • Transport proteins: haptoglobin, ceruloplasmin • C-reactive protein (CRP) • Erythrocyte sedimentation rate (ESR) • Ferritin • Proinflammatory cytokines: TNF-α, interferon-β, transforming growth factor-β, and interleukin (IL)-1β, IL-6, IL-8, IL-12, IL-17, IL-23 • Morphology: white blood cell, platelet counts
Serological markers	• Perinuclear antineutrophil cytoplasmic antibody (pANCA) • Anti-Saccharomyces cerevisiae antibody (ASCA)
Faecal markers	• Calprotectin • Zonulin
Other	• Elafin • Lipocalin • Haptoglobin • Lactoferrin

Lipocalin

Lipocalin-2 (LCN2), a member of the lipocalin superfamily, also known as 24p3 or neutrophil gelatinase-associated lipocalin (NGAL), is a secreted glycoprotein produced by various cells including neutrophiles, myeloid, and intestinal epithelial cells [68, 69]. LCN2 is strongly induced by pro-inflammatory stimulation, such as IL-1β, IL-22, or Toll-like receptor (TLR) activation, and is secreted into the gut lumen in high concentrations [70, 71]. De Bruyn *et al*. demonstrated that serum LCN2 correlates with endoscopic activity in both CD and UC [72].

Zollner *et al.* compared FCP and faecal LCN2 (FLCN) in a cohort of more than 130 IBD patients and a group of healthy controls, and found comparable ability of both biomarkers in distinguishing between active and non-active disease and between IBD patients and non-IBD controls [73].

Table I summarizes the available biomarkers.

Future approaches

The research investigate more specific, sensitive, and responsive markers of inflammation that may be helpful in IBD, and to do that gene expression arrays, and metabolomic and proteomic platforms are used.

RNA as a biomarker in IBD

Studies of the IBD transcriptome have focused on the pathogenesis of IBD and on differentiation between CD and UC. A small study of the transcriptome of colonocytes from patients with UC was able to differentiate active UC from normal control tissue or UC in remission [74].

Other research used microRNAs (miRNA) – small, short (18–25 nucleotides), noncoding, single-stranded RNA species. The role of miRNA in IBD is not fully un-

derstood, but their ability to interact with messenger RNA and silence expression makes them key regulators of a variety of cellular processes. In one study, RNA was isolated from colonic biopsies of patients with UC and RNA was isolated and run on an miRNA gene expression array. It was shown that increased expression of miR-20b and miR-98 were associated with active UC, while increased expression of miR-125b-1* and let-7e* were associated with UC in remission [75]. Moreover, a study comparing circulating miRNA species in children with CD to healthy controls and children with celiac disease showed that 24 miRNA species were elevated in CD [76]. In addition to good sensitivity and specificity for a diagnosis of CD, these disease-associated miRNA species decreased after 6 months of treatment, which suggests that they may be useful as noninvasive biomarkers of inflammation [62].

Metabolomics

Metabolic profiling holds promise in differentiating IBD from other conditions and CD from UC, as well in potentially measuring inflammation. A variety of methods, such as NMR-spectroscopy, liquid chromatography-mass spectrometry, gas chromatography, or selective ion flow tube mass spectroscopy, have been applied to biospecimens including colonic biopsies, urine, and stool [77, 78]. In one study, volatile organic compounds have been measured in breath, and the results indicated a unique "breathprint" in children with IBD [79].

Proteomics

Protein profiles in serum, plasma, or tissue may also be distinct in IBD. Various techniques have been applied to separate and identify protein species, and they include among others, 2-dimensional gel electrophoresis, liquid chromatography, isobaric tags for relative and absolute quantification (iTRAQ), or tandem mass spectrometry (MS/MS) [80].

Pilot studies of proteomic profiling suggest that these approaches may be useful in identifying serum proteins that differentiate IBD from non-IBD patients, and potentially also response to therapy [81, 82].

Conclusions

Until now, FCP has been the only biomarker approved and recommended by the ECCO; however, many other non-invasive markers have been studied in IBD, and faecal zonulin, elafin, and probably lactoferrin appear to be the most promising ones. The future approaches to distinguish between IBD and other conditions, and to monitor disease activity and response to treatment include RNA, metabolomics, and proteomics.

Conflict of interest

The authors declare no conflict of interest.

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Received: 2.08.2022
Accepted: 2.08.2022