IBD REVIEWS REVIEW ARTICLE

Novel biomarkers in patients with inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) is an idiopathic and chronic inflammatory disorder of gastrointestinal tract which includes Crohn's disease, ulcerative colitis, and indeterminate colitis. These disorders are characterized by cycles of remission and relapse evidenced by frequent fever, abdominal pain, and diarrhea. The differential diagnosis is performed by endoscopic, clinical, and histologic findings. The pharmacological therapy aim is to maintain long time remission and mucosal healing. For monitoring the efficacy of pharmacological therapy, an invasive colonoscopy study and clinical scores of clinical activity evaluation are the major tools employed. The colonoscopy is the better tool for determining the mucosa healing but is uncomfortable and less practical for the patients, and the possibility of perforation is a complication. Clinical activity evaluation does not let evaluate the mucosa healing and employs biochemical and clinical parameters like biochemical biomarkers. Biomarkers are useful in order to reduce invasive procedures such as endoscopy as well as in the prediction of relapses and in the tight monitoring for optimizing medical therapy in IBD patients. (IBD Rev. 2018;4:22-9)

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Key words: *Inflammatory Bowel Disease. Fecal. Serological. Biomarkers. Clinical Outcomes.*

Introduction

Inflammatory bowel disease (IBD) includes three main clinical entities: Crohn's disease (CD), ulcerative colitis (UC), and indeterminate colitis. They are a group of gastrointestinal inflammatory chronic disorders of unknown etiology with repeated cycles of relapse and remission[1,2]. CD and UC differ from each other regarding histological, clinical, and endoscopic features[2,3].

The aim of medical therapy in IBD is maintaining long-term remission and mucosal healing. The disease activity and mucosa healing are evaluated by symptoms, imaging studies, clinical activity, endoscopic indexes, and biochemical parameters[4-6].

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Imagining studies are limited because they do not allow to explore mucosa healing and take tissue samples for evaluating histological inflammation; however, they can provide useful information about structuring and penetrating complications as well as the presence of abscesses and the inflammation of the intestinal wall[1,7].

The gold standard in the evaluation of mucosa healing is based on the ileocolonoscopy procedure, but it may be associated with the risk of intestinal perforation[8]. Biomarkers are non-invasive that aid to different clinical proposes in diagnosis, prognosis, and monitoring IBD evolution[9].

Biomarkers were defined by Definition Working Group as "a characteristic that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process, or pharmacologic response to an intervention[1,10]."

Serological and fecal biomarkers are important in the diagnosis, prognosis, and monitoring of IBD patients[6,10-12].

In this review, we reviewed the clinical utility of several serological and fecal biomarkers as well as new potential markers that can be used in the future as shown in table 1.

Conventional serological biomarkers

C-reactive protein (CRP)

One of the most widely used serological markers for evaluating infection or acute inflammation condition is the CRP. The CRP is produced by hepatocytes with a half-life of 19 h in response to circulating interleukin-6 (IL-6), IL-1β, and tumor necrosis factor (TNF)-α, its levels rise in periods of acute inflammation, and it is not specific for intestinal inflammation because it can increase in infections, autoimmune disorders, or malignancy[1,11,13].

Elevations of CRP are more common in CD than in UC. In discriminating UC from CD, it has been reported an increase in CD but not in all UC cases, even shows a modest correlation with endoscopic disease activity as well as histological inflammation in UC patients, and also correlates with endoscopic activity[10,11,14].

The elevation of CRP was associated with discontinuation of treatment with vedolizumab or loss of response[15]. The role of CRP as a relapse predictor has been confirmed in patients with IBD[10,16].

Erythrocyte sedimentation rate (ESR)

This is not a specific marker of inflammation in IBD, and factors such as age, gender, anemia, polycythemia, or pregnancy can influence the ESR by reducing its accuracy and specificity. Thus, ESR values merit careful interpretation in the evaluation of disease activity in IBD patients[1,10,17].

Some studies have been associated modestly the elevation of ESR with endoscopic activity in UC patients. ESR is less appropriate to detect changes in disease activity; nevertheless, it remains widely used as a biomarker of UC severe activity[10,13].

Other serological markers

They are classified into two categories: autoantibodies and microbial antibodies. There are two main serological antibodies used in IBD patients named antineutrophil cytoplasmic antibodies (ANCAs) and anti-*Saccharomyces cerevisiae* antibody (ASCA)[13,18,19].

ANCAs are antibodies directed against of granules in the neutrophil cytoplasm and are classified according to the staining pattern: cytoplasmic ANCA in which the entire cytoplasm is stained and perinuclear ANCA (p-ANCA)[10,19]. These autoantibodies are used in the differential diagnosis and prognosis in patients with UC, especially p-ANCA, and may be helpful in the differential diagnosis between colonic CD and UC. The positivity of p-ANCA has been associated with response to anti-TNF-α therapy and the development of pouchitis in those UC patients that required proctocolectomy[14,18,20].

Table 1. Conventional and novel fecal and serological biomarkers in IBD

Biomarker	Correlation								
	IBD vs. IBS/ control	CD vs. UC	Active vs. inactive	Clinical activity	Endoscopic activity	Histological activity	Risk of relapse	Mucosal healing	Response to therapy
Serological biomarkers									
CRP		+	+		+	+	+		+
ESR					+				
pANCA		+	+						*
ASCA		*							
NAGL	+		+						
S100A12	+		+						
VICM		+							
C3M		+							
P4NP		+							
BAFF	+		+	+	+	+			
α defensins		+	+		+				
AHSG	+								
Fecal biomarkers									
FC	+		+	+	+	+	+	+	
FL	+	*	+		+	*	*	+	
S100A12	+		+	+	+	+			
FHb	+		+	+	+		+		
M2PK	+		+	+	+	+			+
FMMP9	+		+		+	+			
CHI3L1	+				+				
HNP 1-3	+		+		+				
NAGL	+			+	+				
BAFF	+		+		+				
HMGB1					+				

+: Positive results; *: Contrasting results.
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; pANCA: perinuclear antineutrophil cytoplasmic antibodies; ASCA: anti-*Saccharomyces cerevisiae* antibody; NAGL: neutrophil gelatinase associated lipocalin; VICM: citrullinated vimentin biomarker; C3M: collagen degradation biomarker; P4NP: collagen formation biomarkers; BAFF: B-cell activating factor; AHSG: α2-Heremans-Schmid glycoprotein; FC: fecal calprotectin; FL: fecal lactoferrin; FBh: fecal hemoglobin; M2PK: M2-pyruvate kinase; FMMMP9: fecal matrix metalloprotease-9; CHI3L1: chitinase 3-like 1; HNP1-3: human neutrophil peptides 1-3; HMGB1: high mobility group box 1.

ASCA is an antibody against mannan on the cell wall surface of baker's yeast (S. *cerevisiae*) increased the exposure of yeast antigens to immune cells due to the increase of intestinal permeability. It has been used for the differential diagnosis for CD[14,18-20].

Conventional fecal biomarkers

Fecal biomarkers are non-invasive, stable, simple, easy to perform, rapid, and reproducible. In addition, they are inexpensive and more acceptable as a diagnostic tool in the monitoring of patients with IBD. Fecal biomarkers are also useful in the evaluation of mucosal inflammation such as calprotectin and lactoferrin[21-23].

Calprotectin

Calprotectin is a 36 kDa calcium and zinc-binding protein found in the cytosol of inflammatory cells accounting for 60% of cytosolic protein in neutrophils and is released by the activation of leukocytes, and its name refers to its calcium binding and antimicrobial actions as well as induces apoptosis in normal and cancer cells[24-26].

Depending on the organ affected by inflammation, increased levels of calprotectin can be observed in the plasma, cerebrospinal fluid, synovial fluid, urine, or feces, and it is therefore stable at room temperature for up to 7 days and can be reliably measured in fecal, but in IBD, it is elevated; nevertheless, it is not an exclusive gut inflammation marker[3,23]. In subjects, some factors such as regularly consumption of vegetables and exercise practice decreased fecal calprotectin (FC), and other nutrients such as zinc, vitamin D, fatty acids, and several probiotics can affect FC levels.

Calprotectin is the major non-invasive biomarker used in IBD patients focused in the tight monitoring and also serves as a surrogate marker of neutrophil aggregation in the inflamed intestinal mucosa and as a marker of intestinal inflammation which was also correlated with endoscopic and histological inflammation in adult and children population with IBD. FC is a strong biomarker that helps for distinguishing IBD from other functional gastrointestinal disorders[24,27,28].

In the evaluation of IBD activity, a combination of symptoms and laboratory parameters is employed for their construction[23] as well as endoscopic indexes. FC is a marker for mucosa healing and is also very useful in monitoring, prognosis, and detecting relapse[3,29,30].

Elevated FC is a well-validated marker for risk of relapse in established UC and CD in remission. Elevation of FC concentrations without clinical symptoms can predict clinical relapse in the next 12 months in IBD[17,31].

Lactoferrin

Fecal lactoferrin (FL) is an 80 kDa iron glycoprotein found in neutrophils for this reason and is an important marker in neutrophil infiltration. Similar to FC, FL reflects also neutrophil degranulation and has good sensitivity and specificity in the detection of intestinal inflammation, it shows stability up to 2–5 days in feces[7,21,25].

The clinical utility lactoferrin in the diagnosis of IBD has been investigated in different studies; it has shown high specificity and a modest specificity during the diagnosis of suspected IBD specifically in UC as well as is considered for the differential diagnosis between IBD and irritable bowel disease (IBS)[22,32,33]. LF surrogates for endoscopic monitoring in UC with the strongest association with the endoscopic Mayo clinic score and CD scores and it also correlates with mucosal healing[34-36].

The FL has been evaluated as a marker of relapse in IBD patients. High levels of lactoferrin have been associated with clinical relapse in the subsequent 3 months in the follow-up of IBD patients. In a study, FL with a cutoff value of 140 μg/g was established as an indicator of relapse[37,38].

Novel fecal biomarkers

S100A12

This is a cytoplasmic protein of 10 kDa secreted by activated neutrophils, binding to its

receptor lets the activation of factor nuclear kB that promotes inflammation. Initially, it was reported an increase in different inflammatory disorders including IBD[39]. In 2007, Kaise et al. compared 59 IBD patients (CD = 32, UC = 27) with controls (n = 24) and IBS (n = 24). High levels of S100A12 were found in IBD patients compared to normal controls and IBS (0.006 ± 0.03 mg/kg, p < 0.001and 0.05 ± 0.11 mg/kg p < 0.001, respectively). It has good correlation with the Colitis activity index (ρ = 0.415, p = 0.039) and the inflammation score (r = 0.440, p = 0.025)[40]. To stablish the utility of S100A12 in CD and UC patients compared with IBS, the authors reported a high correlation with Mayo score (r = 0.687, p = 0.001)[41]. Recently, fecal S100A12 is one of the most important biomarkers for the differential diagnosis between IBD and IBS[28].

Hemoglobin (Hb)

In the last year, Högberg *et al*. evaluated that the combination of fecal immunochemical test and Hb is a good biomarker to identify IBD and colorectal cancer with sensitivity and specificity of 100% and 61.7%, respectively[42]. Mooiweer *et al*. evaluated the role of Hb as a endoscopic indicator, in a cohort of 164 IBD patients (CD = 83, UC = 74, unclassified IBD = 7) with 74% sensitivity, 84% specificity, and a cutoff value of 1.51 mg/g that correlates with endoscopic inflammation. A better correlation was identified in UC compared with CD (r = 0.72, p < 0.01 and r = 0.44, p < 0.01; respectively), and in both cases, the Hb had a good correlation with FC[43]. In patients with fecal Hb, concentrations ≤ 100 ng/mL correlated with Mayo score 0 (sensitivity of 0.94 and specificity of 0.76) and was also a marker of sustained endoscopic remission for 12 months[44].

M2-pyruvate kinase (M2PK)

In 2005, Walkowiak et al. evaluated the fecal M2PK in feces of 27 UC patients with ileal pouch-anal anastomosis and it was found a high concentration of M2PK compared with healthy controls (p < 0.0001). The concentration also correlated with Modified Pouch Disease Activity Index (r = 0.878, p = 0.00001)[45]. Jonson et al found that M2PK is a biomarker for distinguishing inflammation vs no inflammation in the pouch (p < 0.0001) and also correlates with pouch disease activity index, endoscopic and histological findings[46].

In another study, Chung-Faye et al. found that M2PK levels were significantly elevated in active compared to inactive CD (p = 0.005 and UC p = 0.006)[47]. Recently, fecal M2PK correlated with the endoscopic Mayo score (r = 0.68, p < 0.001) in active UC patients in patients who received infliximab and a cutoff 50 UI/mL discriminates between responders and non-responders during the induction therapy with a sensitivity 88% and specificity 80%[48].

Matrix metalloprotease-9 (MMP-9)

MMP-9 is an endopeptidase able to degrade cytokines, growth factors, and junction proteins. In a study performed by Annaházi et al., they found lower levels of MMP-9 expression in patients with inactive UC compared with active patients (r = 0.616, p < 0.001) and it was a correlation between MMP-9 and endoscopic Mayo score (r = 0.653; p < 0.001)[49]. Other study found that endoscopic and histologic activity correlated significantly with Mayo and Riley scores (p = 0.021 and p = 0.033, respectively) with a cutoff value of 0.20 ng/mL, with a sensitivity of 96% and specificity of 75% (AUC = 0.806)[50]. Buisson et al. reported that fecal MMP-9 correlated with CD activity index (r = 0.47, p = 0.001) and endoscopic activity evaluated by CD endoscopic index of severity (CDEIS) (r = 0.55, p < 0.001). On the other hand, MMP-9 correlated with clinical SCCAI and Mayo endoscopy sub-score (ρ = 0.50, p = 0.001; ρ = 0.58, p < 0.001; respectively) in UC patients[5].

Chitinase 3-like 1 (CHI3L1)

The CHI3L1 is a glycoprotein with affinity to chitin (polysaccharide found in bacteria, fungi, and others), and it has been observed in chronic inflammatory diseases. A cohort study found a strong correlation between severe CDEIS ($r = 0.70$, $p < 0.001$) and increased levels of CHI3LI > 15 ng/g with a sensitivity of 100% and specificity of 59.1%. They also evaluated patients with UC patients and reported an increased CHI3L1 and correlated with the activity evaluated by Mayo endoscopic subscore ($r = 0.44$, $p < 0.001$) with a cut-off > 15 ng/g, with a sensitivity of 81.1% and specificity 80.0%[5].

Recently, Kanmura et al. studied the fecal human neutrophil peptides 1-3 (HNP 1-3) in 70 patients with IBD and found an increase of HNP 1-3 in active IBD patients compared with those in remission ($p = 0.014$) and was also correlated with Mayo endoscopic subscore ($r = 0.44$, $p = 0.00001$) with a cutoff level of 32 ng/ml for active disease with sensitivity of 73.7% and specificity 96.2%[51].

Neutrophil gelatinase-associated lipocalin 2 (NAGL)

NAGL 2 is a glycoprotein expressed in neutrophilic granulocytes and epithelium of the gastrointestinal tract. There are two studies that evaluated the clinical utility of NGAL. One study found a mild correlation between NAGAL and CDAI ($r = 0.34$, $p = 0.001$) in CD patients; however, a strong correlation was found between NGAL and endoscopic index CDEIS in CD patients with ileal, colonic, and ileocolonic location ($r = 0.70$, $p < 0.001$; $r = 0.92$, $p < 0.001$; and $r = 0.50$, $p < 0.001$, respectively)[5]. The second study found an increased serum and fecal levels of NAGL wich were elevated in active UC and CD patients compared to remission UC and CD ($p < 0.001$ and $p < 0.005$, respectively) with a cutoff value of 2.2 mg/kg in serum (86.5% of sensitivity and 77.8% of specificity) and a cutoff-value of 0.81 mg/kg in feces with a sensitivity of 94.7% and specificity of 95.7%[52].

Novel serological biomarkers

Lipocalin 2 (NGAL)

Lipocalin 2 (NGAL) was evaluated in serum samples with IBD and control patients, and plasma NGAL was elevated in both IBD with a cutoff point of 108 ng/ml to distinguish active IBD from controls (sensitivity of 75.3% and a specificity of 67.9%)[52].

Mortensen et al. evaluated a panel of serum biomarkers named collagen degradation biomarker (C1M, C3M), collagen formation biomarkers (P4NP), and citrullinated vimentin biomarker (VICM) in a cohort of 132 patients with IBD (CD =72, UC = 60) and 32 normal controls. VICM was lower in serum from UC patients compared with CD and normal controls ($p < 0.001$); C3M was higher in UC compared to CD and normal controls ($p = 0.008$), and C1M and P4NP were increased in UC and CD compared with normal controls ($p = 0.029$ and $p = 0.046$, respectively), suggesting a potential clinical use for differentiating between diagnosis of CD and UC[9,53].

B-cell activating factor (BAAF)

BAAF is a cytokine which controls adaptive response and regulates survival and differentiation of antibody production in mature B cells, myeloid cells, and neutrophils. It is an emerging biomarker that was evaluated in serum from CD, UC, and healthy control subjects. The concentration of this biomarker was higher in CD and UC patients compared with healthy controls ($p = 0.005$ and $p = 0.007$, respectively) with a cutoff point of 1414 pg/ml (93% specificity and 53% of sensibility). CD and UC had a higher concentration in patients with active disease ($p = 0.046$). In UC, a positive correlation was found between Mayo score and BAFF levels ($r = 0.810$, $p = 0.015$)[53].

Defensins are molecules presented in neutrophils and epithelial cells, and two classes of defensins are identified: α and β defensin. Cerrillo et al. reported an increased serum expression of α defensins 1-3 and also correlated with endoscopic index in CD patients ($r = 0.934$, $p < 0.01$). It is important to note that a major concentration was found in active CD with ileal location compared to normal controls and remission CD ($p < 0.01$)[54,55].

α2-Heremans-Schmid glycoprotein (AHSG)

Other recently proposed biomarker is AHSG that was evaluated in 96 IBD newly diagnosed patients. The AHSG level in serum was lower in IBD patients compared with IBS and healthy control patients ($p < 0.0001$ for each group), but no significant differences were found between groups[56].

Conclusion

The biomarkers represent a practical, comfortable, and convenient tool for the monitoring and prognosis of IBD. The potential utility of novel fecal and serological markers might help to evaluate the disease activity in IBD patients to decrease the use of invasive tools such as endoscopy to predict the risk of relapse and optimization of medical therapy.

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