Surrogate Fecal Biomarkers in Inflammatory Bowel Disease: Rivals or Complementary Tools of Fecal Calprotectin?

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Background: Current noninvasive methods for assessing intestinal inflammation in inflammatory bowel disease (IBD) remain unsatisfactory. Along with C-reactive protein and erythrocyte sedimentation rate, fecal calprotectin (FC) is the standard test for assessing IBD activity, even though its specificity and accuracy are not optimal and it lacks a validated cutoff. Over the past few decades, several fecal markers released from intestinal inflammatory cells have been investigated in IBD; they are the subject of this systematic review.

Methods: A systematic electronic search of the English literature up to April 2017 was performed using Medline and the Cochrane Library. Only papers written in English that analyzed fecal biomarkers in IBD were included. In vitro studies, animal studies, studies on blood/serum samples, and studies analyzing FC or fecal lactoferrin alone were excluded.

Results: Out of 1023 citations, 125 eligible studies were identified. Data were grouped according to each fecal marker including S100A12, high-mobility group box 1, neopterin, polymorphonuclear neutrophil elastase, fecal hemoglobin, alpha1-antitrypsin, human neutrophil peptides, neutrophil gelatinase-associated lipocalin, chitinase 3-like-1, matrix metalloproteinase 9, lysozyme, M2-pyruvate kinase, myeloperoxidase, fecal eosinophil proteins, human beta-defensin-2, and beta-glucuronidase. Some of these markers showed a high sensitivity and specificity and correlated with disease activity, response to therapy, and mucosal healing. Furthermore, they showed a potential utility in the prediction of clinical relapse.

Conclusions: Several fecal biomarkers have the potential to become useful tools complementing FC in IBD diagnosis and monitoring. However, wide variability in their accuracy in assessment of intestinal inflammation suggests the need for further studies.

Key Words: fecal markers, IBD, Crohn's disease, ulcerative colitis

Noninvasive assessment of inflammatory bowel disease (IBD) still represents a clinical challenge. Symptoms may often be subtle and atypical, leading to a delay in the diagnosis of IBD, especially of Crohn's disease (CD), which might adversely affect the outcome. Traditionally, the diagnosis of IBD is based on endoscopic, histological, and radiological findings. Repeated endoscopy is neither practical nor feasible, being invasive, time consuming, and not always well tolerated or accepted, especially by pediatric patients and by those with clinically inactive disease. A noninvasive, inexpensive, and accurate screening test for objectively measuring gastrointestinal inflammation is therefore needed.

Although the collection of stool samples is less practical than peripheral blood tests, fecal biomarkers can be determined in a single stool sample with an enzyme-linked immunosorbent assay, and they closely reflect the intestinal inflammation status. Along with C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), fecal calprotectin (FC) and fecal lactoferrin (FL) have become part of the current battery of laboratory tests performed during the clinical management of IBD.

A recent meta-analysis by Van Reehnen et al. including both adults (670) and children (371) with suspected IBD showed the overall sensitivity and specificity of FC in differentiating between IBD and functional disorders to be 93% and 96% in adults and 96% and 76% in children, respectively. The same study suggested that FC used as a screening test to identify patients who are candidates for colonoscopy could lead to a reduction in the number of procedures by 67% in adults and 35% in children.[1] Similar results in 3639 adults were also obtained by Mindemark, with the estimated demand for colonoscopies reduced by 50% with a 50-μg/g cutoff and 67% with a 100-μg/g cutoff for a cost avoidance of €1.57 million and €2.13 million, respectively.[2] A systematic review with meta-analysis assessing the diagnostic performance of FL in discriminating IBD from noninflammatory conditions was performed by Wang et al. In this study, the pooled FL sensitivity and specificity were 82% and 95%, respectively, with a better accuracy for the diagnosis of ulcerative colitis (UC; 82% and 100%) than for CD (75% and 100%).[3] We have deliberately chosen not to analyze the roles of FC and FL due to the large number of recent reviews and meta-analyses reported in the literature that have largely highlighted the role of these proteins in current clinical

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practice.[1,3] Therefore, our review is focused on the other fecal biomarkers assessed in IBD including S100A12, high-mobility group box 1 (HMGB1), neopterin, polymorphonuclear neutrophil elastase (PMN-e), fecal hemoglobin (Hb), alpha1-antitrypsin (AAT), human neutrophil peptides (HNPs), neutrophil gelatinase-associated lipocalin (NGAL), chitinase 3-like-1 (CHI3L1), matrix metalloproteinase 9 (MMP9), lysozyme, M2-pyruvate kinase (M2-PK), myeloperoxidase (MPO), fecal eosinophil proteins, human beta-defensin-2 (HBD2), and beta-glucuronidase. A comparison between these fecal markers and both FC and FL was reported, when available.

SEARCH STRATEGY

A systematic electronic search of the English literature up to April 2017 was performed using Medline (EBSCO host) and the Cochrane Library. The search strategy used a combination of Medical Subject (MeSH) headings and key words as follows: "inflammatory bowel disease," "Crohn's disease," "ulcerative colitis," "fecal markers," "fecal biomarkers," "S100A12," "high mobility group box 1," "neopterin, polymorphonuclear neutrophil elastase," "fecal hemoglobin," "alpha1-antitrypsin," "human neutrophil peptides," "neutrophil gelatinase-associated lipocalin," "chitinase 3-like-1," "matrix metalloproteinase 9," "lysozyme,"" M2-pyruvate kinase," "myeloperoxidase," "fecal eosinophil proteins," "human beta-defensin-2," and "beta-glucuronidase." Four authors (M.D.R., F.V., A.C., and G.L.) screened the abstracts and identified relevant articles. Additional studies were identified via a manual review of the reference list of the identified studies and review articles. Any discrepancy was resolved by consensus, referring back to the original article. Out of 1023 citations, 125 eligible studies were identified. Data have been grouped according to each fecal marker. Only papers written in English that analyzed human stool samples were included. In vitro studies, animal studies, studies on blood/serum samples, and studies analyzing FC or FL alone were excluded.

FECAL S100A12

Fecal S100A12 has recently emerged as a promising biomarker in IBD. The S100A12 protein, also known as calgranulin C, extracellular newly identified receptor for advanced glycation end products (EN-RAGE), or cystic fibrosis–associated antigen, is a member of the S100 proteins family, whose name derives from their ability to dissolve in a 100% ammonium sulphate solution.[4] S100A 12 is a small cytoplasmic protein of 10 kDa, with an EF-hand (α helix-loop-α helix), calcium-binding dimeric protein (Table 1). The genes that code for S100 proteins are highly conserved and are mostly clustered in the 1q21 chromosomal region.[5]

The protein is released, like calprotectin, during the activation of granulocytes, via pattern recognition receptors or lysis. However, in contrast to calprotectin, it is less expressed by monocytes and macrophages.[6–9] Within neutrophils, it constitutes 5% of all cytosolic proteins.[10] It shows proinflammatory properties, being a chemoattractant for monocytes[11] and through its capacity to act as a ligand to monocyte Toll-like receptor 4 (TLR4).[12] Being a ligand for RAGE, it induces the production of inflammatory mediators, in particular activating the nuclear factor kappa B (NFKB) signaling pathway, leading to the production of tumour necrosis factor alpha (TNF-α), which promotes the release of S100A12 from neutrophils.[13,14] In addition, S100A12 seems to promote the expression of adhesion molecules on the endothelium, such as vascular adhesion molecule 1, potentially contributing to the invasion of other leucocytes.[15]

S100A12 has been less thoroughly investigated compared with FC. However, it shows similar properties, being stable at room temperature for at least 8 days and being resistant to bacterial endoproteases.

The few studies that have been conducted on S100A12 in the last years have shown controversial results, probably due to the small number of patients in each series (Table 2). In a study by de Jong, stool samples were collected from 23 pediatric IBD patients (22 CD and 1 UC). The protein, using a cutoff of 10 mg/kg, was able to discriminate between IBD patients and healthy controls, showing a sensitivity of 96% and a specificity of 92%.[16] Sidler et al. compared a group of 31 pediatric IBD patients (30 CD and 1 UC) with controls. S100A12 and FC were compared, and both were significantly elevated in IBD patients compared with controls. Using a 10-mg/kg cutoff, S100A12 showed a sensitivity of 97% and a specificity of 97%, while FC, using a cutoff of 50 mg/kg, had 100% sensitivity and 67% specificity. The levels of S100A12 correlated with FC levels in the non-IBD group but not in the IBD group. The 2 fecal markers were correlated in noncontinuous colonic CD, but not in CD pancolitis, suggesting that the 2 proteins might be induced by different factors. No correlations between S100A12 and ESR, CRP, platelet count, and albumin levels were observed, while FC was correlated with CRP.[17]

Similar results were obtained in a different study by Kaiser. S100A12 was significantly higher in IBD patients (32 CD and 27 UC), as well as in patients with bacterial gastroenteritis, but not in irritable bowel syndrome (IBS) or viral gastroenteritis patients (Table 3). The sensitivities of S100A12 in discriminating UC and CD from controls were 91% and 81%, respectively, while the specificity was 100% for both. However, the difference between IBD patients and bacterial gastroenteritis patients was not significant, highlighting the problem of the lack of a disease-related biological marker.[18]

These data are in contrast to those recently reported by Sipponen et al. demonstrating a poor ability of S100A12 to detect ileal CD diagnosed with video capsule endoscopy (VCE) in 84 patients. Using a cutoff of 0.06 μg/g, the sensitivity and specificity in detecting small bowel lesions were 59% and 66%, respectively. However, in this study, neither S100A12 nor FC was not correlated with the Harvey Bradshaw index or the VCE activity score.[19]

S100A12 has also recently been investigated as a potential biomarker of relapse in IBD. Turner et al. showed that S100A12

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TABLE 1. Function and Cellular Source of Fecal Markers

Fecal Marker	Molecular Function	Cellular Source
S100A12	Calcium binding protein	Neutophils, macrophages, monocytes
HMGB1	Nucler nonhistone DNA binding protein	Neutrophils, monocytes, macrophages, dendritic cells, natural killer cells
Neopterin	Metabolite of guanosine triphosfate	T-lymphocytes, monocytes, macrophages
PMN-e	Serine proteinase	Neutrophils
Fecal Hb	Metalloprotein	Red blood cells
AAT	Serine protease inhibitor	Hepatocytes, neutrophils, monocytes, macrophages, enterocytes, Paneth cells
F-HNP	Alpha-defensin	Neutrophils
F-NGAL	Glycoprotein	Neutrophils, epithelial cells, adipocytes
CHI3L1	Chitin hydrolase	Macrophages, neutrophils, condrocytes, sinovial cells
MMP9	Proteinase	Neutrophils, epithelial cells
Lysozyme	Glycoside hydrolase	Neutrophils, macrophages
M2PK	Pyruvate kinase isoenzyme	Leucocytes, cancer cells
MPO	Lysosomal peroxidase enzyme	Neutrophils
ECP	Cytotoxic secretory protein	Eosinophils
EPX	Peroxidase	Eosinophils
HBD2	Cationic antimicrobial peptide	Neutrophils, epithelial cells
β-Glucuronidase	Glycosidase	Mucosal cells, bacteria

AAT = fecal alpha1-antitrypsin; CHI3L1 = fecal chitinase 3-like-1; ECP = eosinophil cationic protein; EPX = eosinophil protein X; F-HNP = fecal human neutrophil peptides; F-NGAL = fecal neutophil gelatinase-associated lipocalin; Fecal Hb = fecal hemoglobin; HBD2 = human beta-defensin-2; HMGB1 = high-mobility group box 1 protein; M2PK = m2-pyruvate kinase; MM9 = fecal matrix metalloproteinase 9; MPO = myeloperoxidase; PMN-e = fecal polymorphonuclear neutrophil-elastase.

has poor accuracy in predicting steroid refractoriness in severe pediatric UC.[20] These data were not confirmed in a recent study by Dabritz et al. that included 147 adults and 34 children with CD (n = 61) or UC (n = 120); fecal S100A12 levels in the relapsing group were significantly higher than those of the nonrelapsing group. The study suggested that an S100A12 level of >0.5 mg/kg is significantly associated with disease relapse within 18 months. At 0.43 mg/kg, the sensitivity and specificity of S100A12 for predicting relapse 8 to 12 weeks earlier were 70% and 83%, respectively. The authors also described a significant and progressive rise in the levels of fecal S100A12 before the relapse. The same study suggests a slightly higher specificity but a lower sensitivity than FC. It is, however, unclear whether persistently high concentrations of fecal S100A12 represent a potential flaw or a determinant of potential disease flare-up.[21]

Data in line with this study have been recently shown by Boschetti et al., who compared both serum and fecal S100A12 vs fecal and serum calprotectin in 32 CD patients undergoing anti-TNFα therapy. The performance of FC measured at 14 weeks was higher (area under the curve [AUC], 0.87) if compared with S100A12 (AUC, 0.70) in discriminating between patients who stayed in remission under maintenance therapy and those who had a loss of response within a year of therapy.[15]

Some studies have evaluated the expression of fecal S100A12 in other clinical conditions. A transient increase has been reported in infants younger than 12 months, but it is unclear whether the higher levels are due to normal expression or subclinical bacterial infection.[22]

Fecal S100A12 is not disease specific, being increased in bacterial gastroenteritis,[18] colorectal cancer and advanced adenomas,[23] and diverticulitis. Less concrete associations with higher levels of S100A12 have also been suggested in immunodeficiency, celiac disease, increased age, obesity, and physical activity. The extent of day-to-day variations in fecal S100A12 is yet to be investigated. A further limitation of the use of fecal S100A12 is the lack of a standardized assay, which may lead to different results among different laboratories. Therefore, it is difficult to assess which of the suggested cutoffs is the most effective in the diagnosis and/or follow-up of IBD.

In conclusion, it is still unclear whether S100A12 has significant advantages over FC. However, in most of the studies comparing both proteins, S100A12 shows a slightly higher specificity.[17, 24] Some studies have also suggested a slightly higher accuracy in the evaluation of small bowel lesions, but there is no agreement among the investigators.[18, 19] Further studies are required to confirm these data.

FECAL HMGB1

HMGB1 is a nuclear nonhistone DNA-binding protein belonging to the HMGB family of 3 nuclear proteins (HMGB1, HMGB2, and HMGB3) expressed in eukaryotic cells (Table 1).[25] It was first isolated by Goodwin et al. in 1973.[26] HMGB1 is a small protein consisting of 215 amino acids with 2 tandem DNA binding-domains (80 amino acids for each domain), called HMG A-box and B-box, respectively, a short flexible linker (24 amino acids), and an acidic C-terminal tail (30 amino acids).[27]

HMGB1 shows different properties and activities. As a nuclear protein, it is involved in the maintenance of nucleosome structure and regulation of gene transcription. Extracellularly, after being released from necrotic cells, it can be a mediator of different inflammatory processes.[25, 28] It is actively secreted by immune cells such as neutrophils, monocytes, macrophages, dendritic cells, and natural killer cells in response to various stimuli.[29–32]

Post-translational modifications of HMGB1 are needed to carry out its activities outside the cell; for example, methylation of Lys42 in neutrophil HMGB1 allows for its passive diffusion to the cytoplasm, while

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TABLE 2. Studies Analyzing Fecal Markers, Their Sensibility, Specificity, and Correlation With Disease Activity in Patients With IBD

Fecal Marker	References	No. Patients	IBD Scores	Marker Cutoff	Sensibility	Specificity	Correlation With	r Value	*P* Value
S100A12	De Jong 2006[16]	22 p CD 1 p UC	PCDAI —	10 mg/kg —	96 —	92 —	Clinical activity Clinical activity	— —	— —
	Kaiser 2007[18]	32 a CD 27 a UC	CDAI CAI	0.8 mg/kg 0.8 mg/kg	81 91	100 100	Clinical activity Clinical activity	0.451 0.44	0.01 0.025
	Sidler 2008[17]	30 p CD 1 p UC	PCDAI —	10 mg/kg —	97 —	97 —	Clinical activity Clinical activity	— —	— —
	Turner 2010[20]	101 p UC	PUCAI	10 μg/g	40	—	Prediction of outcomes/ monitoring response	—	0.11
	Sipponen 2012[19]	14 a CD	HBI	0.06 μg/g	59	66	Small bowel disease activity	—	0.166
HMGB1	Palone 2014[49]	28 a CD 23 a UC	SES CD MES	— —	— —	— —	Endoscopic activity Endoscopic activity	0.763 0.440	0.001 0.05
	Palone 2016[50]	49 p CD 57 a CD 36 p UC 62 a UC	SES CD MES	— —	— —	— —	Endoscopic activity Endoscopic activity	0.75 0.83 0.60 0.81	0.001 0.001 0.001 0.001
Neopterin	Nancey 2013[53]	78 a CD 55 a UC	SES CD Rachmilewitz	200 pmol/g 200 pmol/g	74 74	73 100	Endoscopic activity Endoscopic activity	0.47 0.72	0.001 0.001
	Husain 2013[52]	70 a CD 52 a UC	Capetown; HBI CAI; UC -DAI; EI	87.2–96 ng/g 63–135 ng/g	— —	— —	Clinical activity Clinical/endo activity	— —	0.05 0.05
	Frin 2017[54]	31 a UC	Mayo; MES	150 pmol/g 150 pmol/g	56 63	54 63	Endoscopic activity Endoscopic activity	— —	— —
PMN-e	Adeyemi 1992[57]	20 a CD 16 a UC	CDAI CAI	— —	— —	— —	Clinical activity Clinical activity	0.78 0.9	0.05 0.01
	Andus 1993[59]	70 a CD 24 a UC	SAI Rachmilewitz	— —	— —	— —	Clinical activity Clinical/endo activity	0.0083 0.57	0.485 0.002
	Saitoh 1995[58]	26 a CD 36 a UC	CDAI Mayo; Geboes	0.5 μg/g 0.5 μg/g	— —	— —	Clinical activity Clinical/histo activity	— 0.882	0.05 0.001
	Sugi 1996[100]	34 a CD 41 a UC	CDAI Mayo	0.8 μg/g 0.8 μg/g	— —	— —	Clinical activity Clinical activity	— —	0.01 0.01
	Silberer 2005[60]	21 a CD 18 a UC	Stange Score —	0.124 —	79.5 —	95 —	Endoscopic activity Endoscopic activity	— —	0.0001 —
	Langhorst 2008[61]	43 a CD 42 a UC	SES-CD MES	0.062 μg/mL 0.062 μg/mL	81.8 70.4	70 66.7	Endoscopic activity Endoscopic activity	0.32 0.36	0.05 0.05
	Langhorst 2016[62]	91 a UC	UC-DAI; CAI; EI	0.02 μg/g — —	39.1 — —	86.5 — —	Clinical activity Clinical activity Endoscopic activity	0.29 0.30 0.38	0.000 0.001 0.000
Fecal Hb	Nakarai 2013[64]	152 a+p UC	MES	<100 ng/mL	92	71	Endoscopic activity	0.541	0.0001
	Mooiweer 2014[67]	83 a CD 74 a UC (7 IBD-U)	HBI; MES CAI; MES	1.51 μg/g 1.51 μg/g	74 74	84 84	Clinical activity Endoscopic activity Clinical activity Endoscopic activity	0.22 0.44 0.35 0.72	0.03 0.01 0.01 0.01
	Takashima 2015[65]	92 a UC	MES	<100 ng/mL	95	62	Mucosal healing	—	—
	Inokuchi 2016[66]	71 a CD	SES CD	—	96	48	Mucosal healing	—	—
	Nakarai 2016[69]	194 a UC	MES	≤100 ng/mL	94	76	Mucosal healing	—	0.0001

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TABLE 2. Continued

Fecal Marker	References	No. Patients	IBD Scores	Marker Cutoff	Sensibility	Specificity	Correlation With	r Value	*P* Value
AAT	Meyers 1985[72]	24 a CD	CDAI	20 mg/g	—	—	Clinical activity	0.65	0.001
	Cellier 1994[77]	95 a CD	CDEIS	—	—	—	Endoscopic activity	0.26	0.001
	Moran 1995[76]	7 a CD	Farmer Score	<0.58 mg/g	—	—	Endoscopic activity	0.82	0.001
		21 a UC	—	<0.58 mg/g	—	—	Endoscopic activity	—	—
	Herzog 1996[73]	42 p CD	PCDAI	<1.1 mg/g	—	—	Clinical activity	—	—
	Becker 1999[75]	9 a CD	CDAI	—	—	—	Clinical activity	0.67	0.001
		3 a UC	CAI	—	—	—	Clinical activity	—	0.92
	Biancone 2003[79]	26 a CD	CDAI	—	75	85	Clinical activity	—	0.03
F-HNP	Kanmura 2016[81]	25 a CD	MES	—	—	—	Clinical activity	0.66	—
		45 a UC	—	—	—	—	Clinical activity	—	—
F-NGAL	Nielsen 1999[83]	14 a CD	—	368 ng/mg	—	—	Clinical activity	—	0.02
		33 a UC		442 ng/mg			Clinical activity	—	—
	Thorsvik 2017[84]	30 a CD	HBI;	0.81 mg/kg	94.7	95.7	Clinical activity	—	0.0001
		43 a UC	SES-CD	0.81 mg/kg	94.7	95.7	Endoscopic activity	—	0.001
			Mayo;				Clinical activity	—	0.01
			MES				Endoscopic activity	—	0.05
CHI3L1	Aomatsu 2011[86]	87 a CD	SES CD	13.7 ng/g	81.6	80	Endoscopic activity	0.61	0.01
		94 a UC	Matt's Score	13.7 ng/g	88.2	100	Endoscopic activity	0.73	0.01
	Buisson 2016[87]	54 a CD	CDEIS	15 ng/g	100	63.5	Endoscopic actvity	0.70	0.001
		32 a UC	MES	15 ng/g	81.8	80	Endoscopic actvity	0.44	0.001
MM9	Annahazi 2013[93]	47 a UC	Mayo	<0.22 ng/mL	—	—	Clinical activity	—	0.001
	Farkas 2015[94]	50 a CD	CDAI	0.24 ng/mL	87	87	Clinical activity	0.572	0.129
		54 a UC	SES-CD;	0.24 ng/mL	—	—	Endoscopic activity	0.002	0.450
		(34 with pouch)	Mayo		—	—	Clinical activity	0.287	0.05
			MES		—	—	Endoscopic activity	0.381	0.021
			PDAI		—	—	Clinical activity	0.595	0.009
Lysozyme	Sugi 1996[100]	34 a CD	CDAI	7 µg/g	—	—	Clinical activity	—	0.05
		41 a UC	Mayo	7 µg/g	—	—	Clinical activity	—	0.01
	van der Sluys Veer 1998[102]	112 a CD	CDAI	—	78	—	Clinical activity	0.32	0.001
		46 a UC	AI;	—	78	—	Clinical activity	0.38	0.001
			Truelove Witts	—	94	—	Clinical activity	0.47	0.001
M2PK	Walkowiak 2005[106]	27 a UC with pouch (14 with pouchitis)	PDAI	5.9 UI/g	—	—	Clinical activity (pouchitis)	0.878	0.00001
	Czub 2007[104]	32 p CD	PCDAI	4 UI/g	100	—	Clinical activity	—	—
		75 p UC	Truelove Witts	5 UI/g	94.1	—	Clinical activity	—	—
	Chung-Faye 2007[108]	31 a CD	HBI	3.7 UI/mL	73	74	Clinical activity	—	0.005
		50 a UC	Mayo	3.7 UI/mL	73	74	Clinical activity	—	0.006
	Johnson 2009[112]	46 a UC pouch (19 pouchitis, 27 noninflamed pouch)	OPS;	4 UI/mL	80	70.6	Clinical activity	0.82	0.0001
			PDAI		73.9	71	Clinical activity	0.84	0.0001
			MES;		—	—	Endoscopic activity	0.50	0.0001
			Geboes		—	—	Histological activity	0.68	0.0001
	Turner 2010[20]	101 p UC	PUCAI;	4 UI/g	79	64	Clinical actvity	0.75	0.001
			Lindgren & Seo Scores		—	—	Clinical actvity	0.81	0.001
	Day 2012[105]	17 p UC	PCDAI	3 UI/mL	—	—	Clinical actvity	—	0.0007

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TABLE 2. Continued

Fecal Marker	References	No. Patients	IBD Scores	Marker Cutoff	Sensibility	Specificity	Correlation With	r Value	*P* Value
	Roszak 2015[107]	47 p CD 37 p UC (25 IBD-U)	PCDAI Truelove Witts	0.8 μg/g 0.8 μg/g	— —	— —	Clinical activity Clinical activity	0.820 0.772	0.01 0.01
	Vazquez Moron 2017[113]	71 a CD	CDAI; SES-CD	4.5 ng/g	— —	— —	Clinical activity Endoscopic actvity	— 0.846	0.01 0.001
	Frin 2017[54]	31 a UC	Mayo; MES	50 UI/mL	88 88	80 70	Clinical activity Endoscopic activity	— 0.68	— 0.001
MPO	Sugi 1996[100]	34 a CD 41 a UC	CDAI Mayo	5 μg/g 5 μg/g	— —	— —	Clinical activity Clinical activity	— —	0.01 0.01
	Peterson 2002[116]	7 a CD 11 a UC	— —	3.54 μg/g 3.54 μg/g	— —	— —	Clinical activity Clinical activity	— —	0.0002 —
ECP; EPX	Saitoh 1999[121]	37 a CD 42 a UC	CDAI (EPX) CDAI (ECP) CDAI (EPX +ECP) —	— — — —	— — — —	— — — —	Clinical activity Clinical activity Clinical activity Clinical activity	0.202 0.505 0.594 0.661	0.053 0.001 0.001 0.001
	Peterson 2002[116]	7 a CD 11 a UC	— —	1.69 μg/g 0.57 μg/g	— —	— —	Clinical activity Clinical activity	— —	0.0002 0.0002

a = adult; AAT = fecal alpha1-antitrypsin; AI = activity index for Crohn's disease; CAI = Simple clinical colitis activity index; Capetown; Capetown clinical activity index; CD = Crohn's disease; CDAI = Crohn's disease activity index; CDEIS = Crohn's disease index of severity; CHI3L1 = fecal chitinase 3-like-1; ECP = eosinophil cationic protein; EI = endoscopic index; EPX = eosinophil protein X; Farmer = nonstandard endoscopic scoring system; F-HNP = fecal human neutrophil peptides; F-NGAL = fecal neutophil gelatinase-associated lipocalin; Fecal Hb = fecal hemoglobin; Geboes = Geboes histological score; HBI = Harvey-Bradshaw index; HMGB1 = high mobility group box 1 protein; IBD-U = IBD unclassified; Lindgren and Seo Scores = nonstandard scoring system; M2PK = m2-pyruvate kinase; Matts Score = Matts endoscopic score; Mayo = Mayo scoring system for ulcerative colitis; MES = Mayo endoscopic subscore for ulcerative colitis; MM9 = fecal matrix metalloproteinase 9; MPO = myeloperoxidase; OPS = objective pouchitis score; p = pediatric; PCDAI = pediatric Crohn's disease activity index; PDAI = pouch disease activity index; PMN-e = fecal polymorphonucleate neutrophil-elastase; PUCAI = pediatric ulcerative colitis activity index; Rachmilewitz = Rachmilewitz clinical activity index; SAI = severity activity index; SES-CD = simple endoscopic score for Crohn's disease; Stange = nonstandard endoscopic scoring system; Truelove Witts = Truelove and Witts severity index; UC = ulcerative colitis; UC-DAI = ulcerative colitis disease activity index.

acetylation inhibits its nuclear localization.[29, 33] Direct phosphorylation of HMGB1 after lipopolysaccharide stimulation and interferon-β release allows for its export.[34] HMGB1 contains 3 conserved redox-sensitive cysteines, C23, C45, and C106, whose modifications in the redox state can also affect their activity outside the cell.[35] When all 3 cysteines are in the reduced form, HMGB1 forms a heterocomplex with chemokine (C-X-C) motif ligand 12 (CXCL12), which binds exclusively to CXCR4 to initiate chemotaxis.[36] Oxidation of all 3 cysteines prevents the bond between HMGB1 and CXCR4/TLR4, resulting in the blockage of chemotaxis.[37]

As a proinflammatory cytokine, it appears to play a role in sepsis, atherosclerosis, chronic kidney disease, cancers, IBD, and autoimmune diseases such as systemic lupus erythematosus and rheumatic disease.[38–44]

HMGB1 interacts with different receptors such as TLR2, TLR4, and RAGE.[45] The interaction between HMGB1 and RAGE triggers the mitogen-associated protein kinase-NFKB intracellular pathway with consequent gene activation of pro-inflammatory cytokines such as TNF-α, interleukin-1 (IL-1), IL-6, IL-8, macrophage inflammatory protein 1a (MIP1a), and MIP 2b.[46]

HMGB1 was discovered to be an antigen of perinuclear antineutrophil cytoplasmic antibodies, which are present in 20%–83% of patients with UC.[47]

There are few studies about the role of HMGB1 as an IBD fecal marker. Vitali et al. have assessed the gene/protein expression of HMGB1 in the stools of a pediatric IBD cohort (19 CD and 21 UC) and showed high HMGB1 fecal levels compared with controls ($P < 0.001$).[48] Palone et al. confirmed the previous data in 51 adult patients with IBD (28 CD and 23 UC) and found a significant linear correlation between fecal HMGB1 and mucosal inflammation both in CD (r = 0.763 for simple endoscopic score-CD [SES-CD]) and UC patients (r = 0.440; $P < 0.05$ for Mayo endoscopic subscore [MES]).[49]

Recently, the same authors assessed the role of fecal HMGB1 in detecting gut inflammation and its accuracy in identifying histological features of inflammation in IBD patients and compared it with FC. They analyzed stool samples from 85 children (49 CD and 36 UC) and 119 adults (57 CD and 62 UC) and found not only a significantly increased expression of fecal HMGB1 in both CD and UC patients ($P < 0.001$), but also a strong correlation with the disease severity (r = 0.75 and r = 0.83 with SES-CD in pediatric and adult CD patients, respectively; r = 0.60 and r = 0.81 with MES in pediatric and adult UC patients, respectively; $P < 0.001$) and with FC (r = 0.60 in pediatric and r = 0.72 in adult IBD patients; $P < 0.001$) (Tables 2 and 3).[50] In the same study, the fecal levels of HMBG1 were also high in patients with inactive disease but persistent histological

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TABLE 3. Role of Fecal Markers in IBD

Fecal Marker	IBD vs IBS	IBD vs Non-IBD Colitis	Correlation With Clinical Activity	Correlation With Endoscopic Activity	Correlation With Histological Activity	Response to Therapy	Risk of Relapse	Active vs Inactive Disease	Loss of Response to Therapy	Mucosal Healing
FC	+	-	+	+	+/-	+	+	+	+	+
LF	+	-	+	+	+/-	+	+/-	+	+/-	+
S100A12	+	+/-	+	+	nr	+	+	+	nr	+
HMGB1	+	-	+/-	+	+	nr	+	+	nr	+
Neopterin	+	nr	+	+	nr	-	+	+	nr	+/-
PMN-e	+	nr	+	+	+	+	+	+	nr	+
Fecal Hb	+	nr	+	+	nr	nr	+	+	nr	+
AAT	+	-	+	+	nr	nr	+	+	nr	nr
F-HNP	nr	nr	+	+	nr	nr	nr	+	nr	nr
F-NGAL	+	-	+	+	nr	nr	nr	+	nr	+
CHI3L1	+	nr	-	+	nr	nr	+	+	nr	nr
MMP9	+	nr	+ (uc)	+ (uc)	+ (uc)	nr	nr	nr	nr	+
Lysozyme	+	nr	+	+	nr	nr	nr	-	nr	nr
M2PK	+	-	+	-	+/-	+	nr	+	+	+/-
MPO	+	-	+	-	-	+	nr	nr	+	nr
ECP/EPX	+	-	+	nr	nr	+	+ (epx)	+	nr	nr
HBD2	-	-	nr	nr	nr	nr	nr	nr	nr	nr
β-glucuronidase	+	-	nr	nr	nr	nr	nr	nr	nr	nr

+ = positive results; - = negative results; +/- = contrasting results; AAT = fecal alpha1-antitrypsin; CHI3L1 = fecal chitinase 3-like-1; ECP = eosinophil cationic protein; EPX = eosinophil protein X; F-HNP = fecal human neutrophil peptides; F-NGAL = fecal neutophil gelatinase-associated lipocalin; FC = fecal calprotectin; Fecal Hb = fecal hemoglobin; HBD2 = human beta-defensin-2; HMGB1 = high-mobility group box 1 protein; LF = lactoferrin; M2PK = m2-pyruvate kinase; MM9 = fecal matrix metalloproteinase 9; MPO = myeloperoxidase; nr = not reported; PMN-e = fecal polymorphonuclear neutrophil-elastase; uc = ulcerative colitis.

features of inflammation, and low or absent in patients with inactive disease and complete histological remission. On the other hand, FC was not able to discriminate between patients with or without microscopic inflammation, confirming its limited value in defining the histological activity as reported by Theede et al.[51]

The availability of a noninvasive marker capable of highlighting signs of microscopic inflammation in patients in deep remission should lead to a greater use of HMGB1 in the follow-up and management of the risk of recurrence of IBD in order to obtain longer-lasting disease control. However, more studies evaluating the relationship between fecal HMBG1 and histological findings are needed.

FECAL NEOPTERIN

Neopterin is a pyrazino-[2,3-d]-pyrimidine compound. It is a metabolite of cyclic guanosine monophosphate that is released by activated T lymphocytes and macrophages after induction by γ-interferon (Table 1).[52]

Nancey et al. were the first to analyze the role of neopterin as a fecal marker and evaluate its accuracy for predicting endoscopic disease activity in 133 consecutive IBD patients (78 CD and 55 UC) undergoing a colonoscopy. They measured fecal levels of neopterin and found a positive correlation with clinically and endoscopically active IBD according to the SES-CD and the Rachmilewitz index (r = 0.47 and 0.72, respectively; $P < 0.0001$) compared with patients with inactive disease. Neopterin also showed an accuracy comparable to that of FC in predicting endoscopic activity (74% in CD; 90% vs 88% in UC) by using a cutoff of 200 pmol/g (250 μg/g for FC) (Tables 2 and 3).[53]

Husain et al. studied 122 IBD patients (70 CD and 52 UC) through the quantification of neopterin in feces and found increased levels of neopterin in patients with active and inactive CD (96.0 ng/g and 87.2 ng/g, respectively) and active UC (135.2 ng/g) when compared with the controls (12.0 ng/g).[52]

Frin et al. recently compared the performance of neopterin, FC, FL, and M2PK in predicting the response to therapy in 31 UC patients treated with infliximab (IFX). Neopterin showed weaker performances than the other fecal markers, with an overall accuracy of 56% and 63% and area under the receiver operating characteristics curves (AUROCs) of 0.62 and 0.64 measured at week 2 and week 14 after initiation of IFX, respectively. Neopterin levels were also not significantly correlated with those of FC (r = 0.68; $P > 0.001$).[54]

FECAL PMN-e

PMN-e was identified in 1968 by Janoff and Scherer. It is a leucocyte protein belonging to the family of serine proteases. It is

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a constituent of neutrophil azurophilic granules and is released in response to inflammatory triggers (Table 1).[55] It is overexpressed in the stool and serum samples of patients with IBD and may contribute to tissue destruction in other inflammatory diseases such as lung emphysema and rheumatoid arthritis.[56]

Adeyemi et al. found that fecal levels of PMN-elastase correlated significantly with disease activity in active UC ($P < 0.01$) and active colonic CD ($P < 0.05$).[57] A significant difference between active and inactive disease in 26 CD patients and 36 UC patients was also reported by Saitoh et al. ($P < 0.01$).[58] Similar results were reported by Sugi et al.[59] Andus et al. found high fecal levels of PMN-e both in moderately active CD (70) and UC (24) patients, but a statistically significant correlation with disease activity was only found in the UC group ($r = 0.57$; $P = 0.002$).[59]

Silberer et al. compared PMN-e, FC, FL, lysozyme, and MPO in the feces of 39 patients with IBD (21 CD and 18 UC) and found that PMN-e and FC were not significantly different (AUROC, 0.916 and 0.872, respectively; $P = 0.327$). They also showed differences between the IBD group and the IBS group ($P < 0.0001$) and between the IBD group and healthy people ($P < 0.0001$), and both correlated with the endoscopically assessed severity of inflammation ($P < 0.0001$) (Tables 2 and 3).[60]

In another study, fecal PMN-e was assessed in 85 patients with IBD undergoing colonoscopy, and it showed a similar overall diagnostic accuracy compared with FC and FL (74.1% vs 80.0%). They suggested that fecal PMN-e may be able to differentiate active from inactive IBD ($P < 0.05$).[61]

Recently, Langhorst et al. reported that PMN-e could be used to distinguish between mucosal healing from clinical remission and mild disease in UC patients ($P < 0.001$) and that it was as predictive of flares as FC and FL. In addition, PMN-e was able to categorize mucosal healing by Endoscopic Index (EI; EI = 0 and EI = 1; $P = 0.03$).[62]

FECAL Hb

The fecal immunochemical test (FIT) is a quantitative fecal occult blood test used to screen for colorectal cancer. It can measure the concentration of Hb in feces and, consequently, the amount of blood from the injured intestinal mucosa by using a specific antibody against human Hb with a cutoff of ≤100 ng/mL (Table 1).[63] A significant difference in fecal Hb between active and inactive disease in 26 CD patients and 36 UC patients was reported by Saitoh et al. ($P < 0.01$) (Tables 2 and 3).[58] Nakarai et al. showed that a negative FIT was able to predict mucosal healing in a cohort of 152 UC patients with 92% sensitivity and 71% specificity.[64] In another study, Takashima et al. showed that fecal Hb was significantly correlated with endoscopic activity compared with FC ($r = 0.61$ and 0.58, respectively), and it appeared to be more sensitive than FC for predicting mucosal healing (95% and 82%, respectively).[65] Inokuchi et al. assessed FIT in a cohort of 71 patients with CD and showed a significant correlation of FIT with endoscopic activity ($r = 0.54$) comparable with that of FC ($r = 0.67$), but not in patients with small bowel lesions alone ($r = 0.42$ and 0.78). The sensitivities of FIT and FC for predicting mucosal healing were similar (96% and 87%, respectively), but FIT was less specific (48% and 71%, respectively), especially in patients with disease limited to the terminal ileum (40% and 80%, respectively).[66] Mooiweer et al. showed a similar accuracy of fecal Hb and FC concentrations (1.51-µg/g and 140-mg/kg cutoff values, respectively) in predicting active endoscopic inflammation in 164 IBD patients (83 CD, 74 UC, and 7 IBD-unclassified), with 74% sensitivity, 84% specificity, 72% positive predictive value (PPV), 84% negative predictive value (NPV), and AUROC of 0.81 for FIT; and 86% sensibility, 72% specificity, 64% PPV, 90% NPV, and AUROC of 0.87 for FC ($P = 0.06$).[67]

Kuriyama et al. evaluated the role of fecal Hb in predicting relapse in 78 patients with UC and found that FIT rose 1 or 2 months prior to clinical relapse in about 20%.[68] A recent study by Nakarai et al. assessed the risk of relapse in 194 UC patients in clinical remission using mucosal status and FIT; they found that a negative FIT result (≤100 ng/mL) 1 or more years after the start of remission correlated with mucosal healing (defined as MES = 0) with a sensitivity of 94% and a specificity of 76%.[69]

FECAL AAT

AAT is an acute phase serine protease inhibitor produced mainly by hepatocytes, but also by neutrophils, monocytes-macrophages, enterocytes, and Paneth cells. Its expression can increase in response to acute inflammatory stimuli (Table 1). It can reduce pro-inflammatory cytokine production, inflammatory cell infiltration, and tissue injury. Although one of its most important functions is to protect lung tissue from PMN-e, it can also play roles at extrapulmonary and extrahepatic sites.[70]

The first to evaluate a direct correlation between fecal AAT and disease activity in 25 patients with CD was Karbach in 1983 (Table 3).[71] A significant difference between active and inactive disease in 26 CD patients and 36 UC patients was also reported by Saitoh et al. ($P < 0.01$).[58]

Meyers found a good correlation between fecal AAT and CD activity index (CDAI; $r = 0.65$; $P = 0.01$) (Table 2),[72] while Herzog et al. assessed the role of this fecal marker in differentiating between IBD-related and unrelated diarrhea in 42 pediatric CD patients.[73] Conversely, Parsi et al. found that fecal AAT was not able to distinguish symptomatic patients with and without an inflammatory condition in a cohort of 60 patients with ileal pouch-anal anastomosis.[74] Becker et al. also found a relative correlation between fecal AAT concentration and CDAI score in 9 patients with CD but not in patients with UC (only 3 individuals).[75] Moran et al. identified a high correlation between fecal AAT and endoscopic activity in 28 patients with IBD ($r = 0.82$), while Cellier et al. did not find a significant correlation between fecal AAT and endoscopic activity according to the CD endoscopic index of severity (CDEIS) in 121 patients with CD ($r = 0.26$).[76,77]

Few studies have assessed the role of fecal AAT in predicting relapse in patients with CD. Boirivant et al. showed that 5 of 11 CD patients undergoing terminal ileum resection with a clinical recurrence after 1 year of follow-up had increased levels of fecal AAT at 6 and 12 months after resection, higher than those in patients who

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did not relapse ($P < 0.01$).[78] In a cohort of 26 CD patients in clinical remission, Biancone et al. found that fecal levels of AAT at baseline were higher in patients who flared up within the next 6 months ($P = 0.03$; 75% sensitivity and 85% specificity).[79]

F-HNPs

F-HNPs belong to the group of alpha-defensins, a class of 6 antimicrobial peptides, 4 of which are produced by neutrophils and stored in their granules (Table 1).[80]

Recently, Kanmura et al. assessed the clinical value of F-HNP in assessing disease activity in 45 patients with UC and 25 patients with CD by analysis of F-HNP and FC concentrations in stool samples. They found higher levels of F-HNP in the IBD group than in the controls and a good correlation with the MES (r = 0.66) (Tables 2 and 3). The AUROCs for separation of IBD and non-IBD patients (cutoffs of 32 ng/mL for F-HNP and 240 µg/g for FC) were 0.86 and 0.83, respectively. The sensitivity of F-HNP was 73.7%, the specificity 96.2%, the PPV 93.3% and the NPV 83.3%. For FC, sensitivity, specificity, PPV, and NPV were 89.4%, 73%, 70.8%, and 90.4%, respectively.[81]

FECAL NGAL

NGAL, also known as lipocalin 2, is a glycoprotein expressed in neutrophil granulocytes, adipocytes, and epithelial cells (Table 1). It can act as an iron-sequestering antimicrobial protein, a growth factor, or as a stabilizer of MMP9. It is highly expressed in feces and in the intestinal epithelial cell layer during inflammation.[82]

Nielsen et al. first studied the potential role of fecal NGAL as a disease activity marker in 23 patients with UC and 14 with CD compared with acute infectious enterocolitis patients and healthy controls. They found a significant increase of NGAL in the feces and rectal dialysate from patients with active UC ($P = 0.02$ and $P = 0.003$, respectively) (Table 2).[83]

Recently, Thorsvik et al. assessed fecal NGAL as an IBD marker and correlated it with FC, CRP, and clinical and endoscopic scores (Table 3). They found a significant elevation of fecal NGAL levels in patients with active UC and CD (6.05 and 4.9 mg/kg) compared with the inactive forms (1.3 and 1.5 mg/kg). Comparing active disease with IBS, the AUC was 0.968 (0.93–1.0) for fecal NGAL and 0.985 (0.95–1.0) for calprotectin. The sensitivity and specificity were 94.6% and 90.5%, respectively, for fecal NGAL, while for FC the reported sensitivity and specificity were 97.3% and 100%, respectively.

The 2 markers, using cutoff values of 2.2 mg/kg for fecal NGAL and 150 mg/kg for calprotectin, showed a sensitivity of 86.5% and a specificity of 77.8% for fecal NGAL and 91.9% and 80.6% for FC when distinguishing endoscopically active disease from inactive disease ($P = 0.15$).[84]

FECAL CHI3L1

CHI3L1 is a protein with an unclear enzymatic activity that shows a high affinity to chitin, a polysaccharide similar to cellulose that is absent in mammals (Table 1). It is upregulated in the inflamed colonic mucosa both in vitro and in vivo.[85]

Its potential role as a marker for IBD was studied in a pediatric cohort by Aomatsu et al. They analyzed fecal CHI3L1 and FC and found high fecal CHI3L1 levels in active IBD with a positive correlation with the endoscopic scores (r = 0.73 with UC Matt's Score and r = 0.61 with SES-CD) (Tables 2 and 3). It was also shown that a cutoff value of 13.7 ng/g could predict active disease with a sensitivity of 84.7% and a specificity of 88.9%.[86]

Buisson et al. have recently demonstrated the good accuracy of fecal CHI3L1 in detecting endoscopic activity in 86 IBD adult patients and the positive correlation with CDEIS (r = 0.70; r = 0.78 in ileal CD) and MES (r = 0.44). Furthermore, in CD, levels of CHI3L1 higher than 15 ng/g detected endoscopic ulceration with a sensitivity and specificity of 100% and 63.6%, respectively, while FC levels higher than 250 µg/g showed a sensitivity of 90.5% and a specificity of 59.1%. In UC, levels of fecal CHI3L1 higher than 15 ng/g detected endoscopic activity with a sensitivity and specificity of 81.8% and 80.0% respectively, while levels of FC higher than 250 µg/g showed a sensitivity of 86.4% and a specificity of 80.0%.[87]

FECAL MMP9

MMP9 belongs to a family of enzymes involved in the degradation of the extracellular matrix (Table 1).[88] MMPs are involved in inflammatory processes, and MMP9 is considered the main proteinase implicated in the pathogenesis of IBD, where it recruits and traffics neutrophils and other inflammatory cells into inflamed tissue and through its interplay with angiogenic factors.[89]

Furthermore, some studies have shown an increase of MM9 levels in colonic biopsies, urine, and serum from patients with UC.[90–92] Fecal levels of MMP9 were elevated and significantly correlated with the overall Mayo score, MES, and FC in UC patients.[93] Farkas et al. assessed the diagnostic accuracy of MMP9 compared with FC as a marker of pouchitis, and they found that MMP9 correlated significantly with the severity of pouchitis, with a sensitivity and specificity higher than those of FC.[94] The same group also found a strong association between fecal MMP9 and clinical, endoscopic, and histological activities in patients with UC or pouchitis, but not in patients with CD (87% sensitivity) (Tables 2 and 3).[95]

FECAL LYSOZYME

Lysozymes, also known as muramidase or N-acetylmuramide glycanhydrolase, are a family of enzymes that damage bacterial cell walls through hydrolysis.[96] They are produced by neutrophils and are upregulated in active and inactive UC and in CD, suggesting a potential protective role against pathogenic bacteria that can proliferate in the colon of patients with IBD (Table 1).[97]

An elevation of fecal levels of lysozyme in 41 IBD patients was described by Costongs et al.[98] Similar results were also reported by Gao et al. ($P < 0.001$ for CD and $P < 0.005$ for UC) and Sugi et al. ($P < 0.01$).[99, 100] A significant increase of lysozyme concentration in the feces of IBD patients compared with those of

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IBS and healthy subjects was reported by Hemrika et al.[101] Van der Sluys Veer et al. also found that fecal lysozyme concentration in IBD patients was higher than that in controls, but there was a poor correlation with CDAI and AI in CD and with Truelove and Witts' grading in UC (r = 0.32, r = 0.38, and r = 0.47, respectively) (Tables 2 and 3).[102]

In a comparison with FC, FL, PMN-e, and MMP9, Silberer et al. found that lysozyme levels showed no significant differences between the IBD group and the IBS group ($P = 0.0050$) and a lower ability to identify IBD patients than PMN-e and FC (AUROC, 0.726, 0.916, and 0.872, respectively).[60]

FECAL M2-PK

M2-PK is a protein expressed in proliferating cells such as leucocytes and cancer cells (Table 1).[103] Czub et al. studied the role of M2-PK as a biomarker of IBD and showed an increase of M2-PK in active IBD.[104] Day et al. also found increased fecal levels of M2-PK in a pediatric cohort of 17 patients with active CD compared with controls ($P = 0.0007$).[105] A correlation between fecal M2-PK and active pouchitis was reported by Walkowiak et al. in 18 UC patients ($P < 0.00001$).[106]

The existence of a significant correlation between fecal levels of M2-PK, FC, LF, and clinical activity was demonstrated in 109 children with IBD by Roszak et al., both in CD (M2-PK and FL, $P < 0.01$; FC, $P = 0.005$) and UC patients (M2-PK and FL, $P < 0.01$; FC, $P = 0.004$).[107]

Turner et al. studied a cohort of 101 pediatric patients with severe UC and found that M2-PK could better predict response to corticosteroid treatment than FC, FL, and S100A12 (AUC, 0.75; $P < 0.05$ for M2-PK vs $P < 0.65$ for the other markers). However, M2-PK was inferior to the pediatric ulcerative colitis activity index in predicting outcome (Tables 2 and 3).[20]

Fecal M2-PK has also been shown to differentiate active from inactive IBD ($P < 0.005$ CD; $P = 0.006$ for UC) and IBD from IBS (20–24.3 U/mL vs 0.1 U/mL; 73% sensitivity; 74% specificity) and to have a strong linear correlation with FC levels.[108] However, it is less effective than FC in distinguishing IBD from acute gastroenteritis,[109] IBD from healthy people (AUC difference, –0.10; $P < 0.0001$), and in discriminating patients with mild UC or CD from healthy people (AUC difference, –0.23 and –0.04, respectively).[110]

The accuracy of FC and M2-PK was compared by Jeffery et al., who analyzed their levels in 14 patients with diagnosed organic bowel disease. The sensitivity, specificity, PPV, NPV, positive likelihood ratio (LR+), and negative likelihood ratio (LR-) for the diagnosis of organic bowel disease were 93%, 92%, 62%, 99%, 11.6, and 0.08 for FC and 67%, 88%, 47%, 94%, 5.6, and 0.38 for M2-PK, respectively.[111] An 80% sensitivity and a 70.6% specificity was reported by Johnson et al. in 46 patients with an ileal pouch. The same study found a significant correlation between M2-PK and the endoscopic and histological scores used, as well as with the objective pouchitis score, the pouch disease activity index, and FC (all $P < 0.0001$).[112]

Vazquez Moròn et al. showed that both M2-PK and FC had a high degree of accuracy in predicting endoscopic activity according to the SES-CD in 71 CD patients (AUC, 0.846 and 0.917, respectively; $P < 0.001$).[113]

In the recent study by Frin et al., the ability of M2-PK to predict response to induction therapy at week 2 in 31 UC patients treated with IFX was superior to that of FC and FL (overall accuracy of 84%, 77%, and 77% respectively; AUROC, 0.88, 0.82, and 0.84, respectively). However, it showed worse results than FC and FL in predicting the course of the disease after 1 year under maintenance therapy with IFX (overall accuracy of 82%, 84%, and 93% respectively; AUROC, 0.75, 0.82, and 0.86, respectively). In addition, M2-PK and FL levels showed a good correlation with FC levels (r = 0.85 and r = 0.70, respectively; $P < 0.001$).[54]

FECAL MPO

MPO is a lysosomal protein released by neutrophils during inflammatory processes. Its reaction with hydrogen peroxide or tyrosine forms highly cytotoxic products, which can also contribute to tissue damage in IBD (Table 1).[114] A significant difference between fecal levels of MPO in active disease vs inactive disease in 75 IBD patients was reported by Sugi et al. ($P < 0.01$).[100]

Saiki et al. measured fecal levels of MPO in a cohort of 55 IBD patients (32 CD and 33 UC) and found a statistically significant elevation of MPO in patients with active disease and a significant correlation with the endoscopic score of UC ($P < 0.005$) (Tables 2 and 3).[115] Silberer et al. found the diagnostic accuracy of fecal MPO in detecting patients with IBD to be inferior to FC and PMN-e (AUROC, 0.750, 0.872, and 0.916, respectively).[60]

Peterson et al. also found that fecal MPO concentrations were significantly increased in UC patients, more so than in CD patients and in healthy controls, and suggested that MPO was the best neutrophil marker for studying intestinal inflammation (cutoff, 3.54 µg/g; $P < 0.0002$).[116] High levels of MPO in UC patients with active disease were also reported in the study by Masoodi et al. ($P < 0.001$). However, no significant association between MPO and endoscopic and histological scores was found.[117, 118] Wagner et al. evaluated MPO as a marker of treatment efficacy in 11 patients with CD and 27 patients with UC before treatment and after 4 and 8 weeks of treatment. In order to predict a complete response after 8 weeks of treatment, the authors calculated PPV and NPV, which were 30% (13–53) and 100% (77–100) for FC and 23% (10–42) and 100% (59–100) for MPO, respectively, suggesting that persistently high levels of MPO could predict an incomplete response to treatment.[119]

FECAL EOSINOPHIL PROTEINS

Similar to neutrophils, eosinophils are involved in IBD and contain proteins in their granules, which are released during inflammation (Table 1).[120] Saitoh et al. reported that eosinophil cationic protein (ECP) and eosinophil protein X (EPX) were the highest eosinophil proteins in the feces of IBD patients. They analyzed stool samples from 42 patients with UC and 37 with CD and found the levels of ECP and EPX to be significantly increased in

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active disease ($P < 0.05$) (Tables 2 and 3). They also found significant correlations between EPX and CDAI (r = 0.505; $P < 0.01$) and between ECP and EPX (r = 0.661 in UC; r = 0.594 in CD). The same study also showed significant correlations between the concentrations of fecal eosinophil proteins and other fecal markers such as fecal Hb, AAT, and FL ($P < 0.001$ on a logarithmic scale).[121] Peterson et al. also found markedly increased levels of fecal eosinophil proteins in a smaller cohort of patients (7 CD and 11 UC) and considered EPX to be the best eosinophil marker of inflammation in IBD (1.69 and 0.57 μg/g; $P < 0.0002$).[116] Wagner et al. also showed EPX to be a marker of treatment outcome in IBD, but with an inferior accuracy compared with MPO.[119]

FECAL HBD2

HBD2 is an antimicrobial peptide implicated in the pathogenesis of IBD (Table 1).[122] Kolho et al. assessed the potential role of HBD2 as a marker of IBD by analyzing HBD2 levels in stool samples from 110 pediatric patients with IBD (68 CD, 27 UC, and 15 IBD-U). They found that HBD2 could not classify patients into different groups and did not provide information on disease characteristics (62.8% sensitivity and 51.9% specificity). In the same study, MMP9 was also analyzed; it showed levels significantly higher in active UC ($P = 0.0013$) and a capacity to differentiate between IBD and non-IBD patients comparable with that of FC (AUROC, 0.837 and 0.944, respectively) (Table 3).[123]

FECAL BETA-GLUCURONIDASE

Beta-glucuronidases are enzymes belonging to the glycosidase family. They are released by the lysosomes of colonic cells or bacteria during inflammatory processes (Table 1). Only 1 study analyzed the role of beta-glucuronidase in a cohort of 68 pediatric patients with IBD, and it reported that beta-glucuronidase activity was 2-fold lower in the IBD group than in the healthy group (0.12–81.63 vs 5.82–141.13 mM/mg*h).[124]

DISCUSSION

Although endoscopy with histology remains the gold standard for assessing disease activity and severity of mucosal inflammation in IBD patients, it is limited by its invasiveness, expense, and poor acceptance by asymptomatic patients, as well as posing some risks (eg, bowel perforation). Fecal biomarkers, being noninvasive, inexpensive, and generally more accepted by the patient, can provide a useful tool to monitor disease activity and response to therapy, and indirectly to predict the risk of relapse, especially in pediatric patients.

The search for effective biological markers should therefore be a central issue in the IBD field. The most accurate and widely used fecal biomarker of intestinal inflammation is FC. A meta-analysis by van Rheenen found a pooled sensitivity of 93% and a specificity of 96% in adults and 92% and 76% in children in the diagnosis of IBD. The meta-analysis by Waugh showed a sensitivity of 93% and specificity of 94% in adults and 95%–100% sensitivity and 44%–93% specificity in children. Nevertheless, FC has some limits; FC is not specific to IBD and might be increased in several other gut diseases such as diverticulitis, bacterial gastroenteritis, colorectal carcinoma, nonsteroidal anti-inflammatory enteropathy, graft-vs-host disease, and postradiotherapy patients. FC levels also depend on the age of the patient, as children younger than age 5 years show higher concentrations. In addition, FC may also have considerable day-to-day variation. At the same time, FC does not help to differentiate between CD and UC or describe the localization and extent of intestinal lesions. Moreover, there is no validated FC cutoff to define active disease and clinical remission. Several studies found an FC value higher than 250 μg/g to be indicative of active disease, while levels lower than 100 μg/g are indicative of IBS. Consequently, FC values ranging from 100 to 250 μg/g may be more difficult to interpret. Therefore, FC as a single marker seems insufficient to provide an accurate picture of mucosal inflammation in all IBD patients. We also believe that additional biomarkers should reflect different features of the gut inflammatory process.

A large number of fecal biomarkers have been evaluated for their ability to detect, monitor, and predict inflammation in IBD patients, with good results. Some have demonstrated better performances than FC or FL: S100A12 and F-HNPs have proved to be more specific than FC[17, 24, 81]; HMGB1 has shown the ability to detect histological signs of inflammation[50]; M2-PK and neopterin have been seen to be more effective than FC in predicting response to therapy[20, 54]; PMN-e has demonstrated a greater ability to define mucosal healing,[62] and CHI3L1 has been found to be highly sensitive and specific in locating ileal mucosal damage.[87] However, FC is still the fecal biomarker most extensively assessed in IBD and considered the “gold standard,” and it is widely used in clinical practice. High costs, lack of large-cohort studies and randomized clinical trials, and the availability of meta-analyses of FC and FL only may be some of the reasons that the other fecal markers are not routinely used.

None of the available fecal markers is yet able to satisfy all the characteristics of an ideal fecal biomarker. An ideal fecal biomarker should show high sensitivity and high specificity for IBD. It should discriminate between the different lesion sites and provide information on the activity and severity of the intestinal inflammation. It should be relatively homogeneously distributed in feces, easy to identify, resistant to enzymatic degradation, stable at room temperature, and unaffected by multiple freeze-thaw cycles. It should not show high day-to-day variability or high variability within a single bowel movement. The levels of an ideal fecal biomarker should not significantly vary with age and should not be affected by diet. At the same time, it should be inexpensive and have a short turnaround time for receiving results.

A combination of fecal markers may improve their diagnostic yield, but few studies have focused on this approach. Shroeder et al. found that a combination of PMN-e with FC and FL showed sensitivity, specificity, PPV, and NPV higher than PMN-e alone.[125] Mooiweer et al., instead, found that a combination of fecal Hb and FC did not increase their predictive accuracy when compared with fecal Hb and FC alone.[67]

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Langhorst et al. combined FL, FC, and PMN-e with CRP and CDAI or UC disease activity index in 85 IBD adult patients undergoing diagnostic colonoscopy. This comprehensive index was considered positive when at least 2 fecal markers and either CRP or the clinical index were positive. They found an overall diagnostic accuracy of 95% in UC, while the accuracy in CD was inferior to FC alone (77% and 81.4%, respectively).[62] Interestingly, the combination of FC and M2-PK reported by Jeffery et al. gave a sensitivity of 64%, which was lower than when using M2-PK alone. In fact, the specificity, PPV, NPV, LR+, and LR- of the combination of the 2 markers were 98%, 82%, 95%, 32, and 0.37, respectively.[111] More studies are needed to better define the role of using a combination of fecal markers, which may increase the diagnostic and prognostic value of each of them. However, this approach may lead to higher costs in daily practice.

In the past decade, the microbiota has emerged as a new, important intestinal player in IBD pathogenesis, suggesting that some of its components could be potential new fecal markers. An interesting current field of investigation is the highly complex interaction between the inflamed mucosa and the resident microbiome. Although it is widely accepted that IBD is related to dysbiosis, a direct relationship has not yet been demonstrated. It is well established that the gut microflora can drive immune activation and trigger and maintain chronic inflammation, and that the gut inflammation may affect the various microbiome components (bacteria, yeasts, viruses) and/or their metabolism.[126] Moreover, even though it is unknown whether dynamic changes in the microbiome may contribute to fluctuations in disease activity,[127] the microbiota has been seen to fluctuate in IBD subjects more than in healthy individuals[128]; for instance, it shows a reduced concentration of some bacterial strains (Bacteriodes, Eubacterium, Fecalibacterium) and a greater concentration of others (Actinomices, Bifidobacterium).[129–131] Furthermore, the anti-inflammatory role of single bacterial strains, for example, *Faecalibacterium prausnitzii*, has also been investigated in IBD.[132–134] A pathogenic role has also been suggested for yeasts and viruses.[135, 136]

Microbes are believed to increase susceptibility to IBD also through production of some metabolites.[134]

It has long been known that the stools of patients with severe colitis are characterized by altered concentrations of bacterial metabolites, that is, low butyrate and high lactate and sulphide concentrations[137–139]; however, these changes are not specific, and it remains to be established whether they are a cause or an effect of intestinal inflammation.

More recently, the focus has turned to volatile organic metabolites, an interesting and largely unexplored area of investigation that seems to offer promising prospects.[140]

The interaction between microbes, the host, and the environments is still largely unclear, but it is likely that advances in knowledge and technology will make it possible to identify single microbial strains and/or their specific metabolites as useful biomarkers for IBD diagnosis and monitoring.

Finally, genetic investigations using single nucleotide polymorphisms and proteomic and metabolic studies have shown potential to identify novel candidate serum, tissue, and stool protein biomarkers, yielding promising preliminary results.[141, 142]

In conclusion, several fecal markers have been evaluated as potential noninvasive detectors of inflammation in IBD patients that showed high sensitivity and specificity for differentiating between IBD and functional gut disorders.

In our opinion, some of these fecal noninvasive biomarkers have the potential to become a fundamental mainstay in disease diagnosis and monitoring of IBD in the near future. However, it is necessary to increase the studies and the number of IBD centers and patients involved in trials and to standardize diagnostic kits and single-marker cutoffs in order to better understand their role in the different IBD disease phases and optimally use them in our daily clinical practice.

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