SYSTEMATIC REVIEWS

Are faecal markers good indicators of mucosal healing in inflammatory bowel disease?

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Abstract

AIM: To review the published literature concerning the accuracy of faecal inflammatory markers for identifying mucosal healing.

METHODS: Bibliographical searches were performed in MEDLINE electronic database up to February 2015, using the following terms: "inflammatory bowel disease", "Crohn´s disease", "ulcerative colitis", "faecal markers", "calprotectin", "lactoferrin", "S100A12", "endoscop*", "mucosal healing", "remission". In addition, relevant references from these studies were also included. Data were extracted from the published papers including odds ratios with 95%CI, *P* values and correlation coefficients. Data were grouped together according to each faecal marker, Crohn's disease or ulcerative colitis, and paediatric compared with adult study populations. Studies included in this review assessed mucosal inflammation by endoscopic and/or histological means and compared these findings to faecal marker concentrations in inflammatory bowel diseases (IBD) patient cohorts. Articles had to be published between 1990 and February 2015 and written in English. Papers excluded from the review were those where the faecal biomarker concentration was compared between patients with IBD and controls or other disease groups, those where serum biomarkers were used, those with a heterogeneous study population and those only assessing post-operative disease.

RESULTS: The available studies show that faecal markers, such as calprotectin and lactoferrin, are promising non-invasive indicators of mucosal healing. However, due to wide variability in study design, especially with regard to the definition of mucosal healing and evaluation of marker cut offs, the available data do not yet indicate the optimal roles of these markers. Thirty-six studies published between 1990 and 2014 were included. Studies comprised variable numbers of study participants, considered CD (15-164 participants) or UC (12-152 participants) separately or as a combined group (11-252 participants). Eight reports included paediatric patients. Several indices were used to document mucosal inflammation, encompassing eleven

endoscopic and eight histologic grading systems. The majority of the available reports focused on faecal calprotectin (33 studies), whilst others assessed faecal lactoferrin (13 studies) and one study assessed S100A12. Across all of the biomarkers, there is a wide range of correlation describing the association between faecal markers and endoscopic disease activity (*r* values ranging from 0.32 to 0.87, *P* values ranging from < 0.0001 to 0.7815). Correlation coefficients are described in almost all studies and are used more commonly than outcome measures such as sensitivity, specificity, PPV and/or NPV. Overall, the studies that have evaluated faecal calprotectin and/or faecal lactoferrin and their relationship with endoscopic disease activity show inconsistent results.

CONCLUSION: Future studies should report the results of faecal inflammatory markers in the context of mucosal healing with clear validated cut offs.

Key words: Crohn's disease; Ulcerative colitis; Mucosal healing; Faecal calprotectin; Inflammatory bowel disease; Faecal lactoferrin

Core tip: With regard to mucosal inflammation and response to therapy in Crohn's disease and ulcerative colitis patients, mucosal healing may be a more reliable target for treatment than clinical and biochemical assessment. The available studies in this review show that faecal biomarkers are promising non-invasive indicators of mucosal healing and they could be an appropriate surrogate to endoscopy (the gold standard) in inflammatory bowel diseases patients. However, due to a wide variability in the use of clinical indices and marker cut offs, it's difficult to compare their performances. Moreover, a clear definition of mucosal healing is needed.

INTRODUCTION

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic diseases characterised by inflammatory changes in the gastrointestinal tract, which can present at any age and are defined according to disease location, extent and histological findings[1]. IBD features chronic inflammatory changes, with a relapsing/remitting course. Symptoms of active disease typically include abdominal pain, diarrhoea, haematochezia and nutritional compromise. Although predominantly involving the gastrointestinal tract, extra-intestinal manifestations such as skin lesions, joint changes and hepatobiliary disease, may be seen in both CD and UC.

Historically, the treatment goal of IBD has been symptom palliation with clinical remission or response used as the primary outcomes in clinical trials and for registration by regulatory bodies such as the FDA. Clinical disease indices such as the Simple Clinical Colitis Activity Index (SCCAI) are examples of indices used in this way, while composite indices such as the CD activity index (CDAI) use a combination of clinical and laboratory data[2,3]. While this approach has many merits, emerging data suggest that other goals may be associated with an improved prognosis.

Mucosal healing (MH) is associated with improved outcomes in clinical trials and has been suggested as the gold standard for remission as it is a direct measure of inflammation of the target organ[4]. In clinical trials of biological drugs, MH has been associated with a lower risk of hospitalisation and colectomy[5], improved symptom control and reduced corticosteroid use[6], and a reduced risk of clinical and surgical relapse following ileocolic resection in patients with CD[7]. Despite much discussion concerning MH as a treatment goal, there is not yet a clear consensus on its definition[8]. In addition to MH, the concept of deep remission (encompassing both clinical remission and mucosal healing) has been developed although is yet to be formally tested in clinical trials[9].

Recently a working group of the International Organisation for the study of Inflammatory Bowel Diseases (IOIBD) published a detailed description of potential targets for the management of IBD. The process leading to a "treat to target" approach in IBD has mirrored that seen in other diseases where tight disease control has led to improved patient outcomes[10]. For both CD and UC, a key target identified is mucosal healing, in addition to the absence of clinical symptoms. While biochemical markers of inflammation in blood (*e.g.*, C-reactive protein (CRP)) and stool (*e.g.*, faecal calprotectin (FC)) were thought to be adjuvant targets, it was concluded that insufficient data exist for them to be used as treatment targets in their own right. At present, the assessment of MH requires ileocolonoscopy[11]. While ileocolonoscopy is the gold standard in assessing the severity and extent of mucosal inflammation and healing in individuals with IBD[12], it is invasive, expensive and, therefore, not appropriate for repeated regular assessment of disease activity.

Faecal tests of inflammation have significant promise as non-invasive biomarkers that may reflect intestinal inflammation. These proteins can be measured easily in a single stool sample and efficiently quantified by enzyme-linked immunosorbent assay (ELISA). Furthermore, a number of these proteins can be measured using point of care devices

facilitating rapid clinical decision-making based on the current inflammatory burden[13]. Recent studies have considered the potential of those non-invasive markers as ways to assist in the diagnosis of IBD and as indicators of the response to therapy[14]. However, for faecal biomarkers to have a key role in the management of IBD in the treat to target era, it is essential that there are robust, accurate and validated data to support specific cut-off values to aid clinical decision making.

This review aims to examine studies that assess mucosal healing by non-invasive faecal tests. The role of several faecal markers will be discussed with comparison to endoscopic assessment.

MATERIALS AND METHODS

Bibliographical searches were performed in MEDLINE electronic database up to February 2015, using the following terms: "inflammatory bowel disease", "Crohn's disease", "ulcerative colitis", "faecal markers", "calprotectin", "lactoferrin", "S100A12", "endoscop*", "mucosal healing", "remission". In addition, relevant references from these studies were also included.

Studies included in this review assessed mucosal inflammation by endoscopic and/or histological means and compared these findings to faecal marker concentrations in IBD patient cohorts. Articles had to be published between 1990 and February 2014 and written in English. Papers excluded from the review were those where the faecal biomarker concentration was compared between patients with IBD and controls or other disease groups (*e.g.*, irritable bowel syndrome), those where serum biomarkers were used, those with a heterogeneous study population and those only assessing post-operative disease. No specific funding was obtained for this study.

RESULTS

Research design
Thirty-six studies published between 1990 and 2014 were included[15-49] Summaries of the studies are shown in Tables 1-6. Studies comprised variable numbers of study participants, considered CD (15 to 164 participants)[15-26,28,38,41-43,45,46,50,51] or UC (12 to 152 participants)[16,22,23,26,28,35-37,39,42,45-48,50,52] separately or as a combined group (11 to 252 participants)[22,23,28-34,40,42,44,50]. Eight reports included paediatric patients[16,31,34,35,38,43,44,50].

Several indices were used to document mucosal inflammation, encompassing eleven endoscopic and eight histologic grading systems. Some of these systems have been validated (*e.g.,* CDEIS and SES-CD), whilst others utilised grading systems that have not been widely validated. The majority of the available reports focused on faecal calprotectin (33 studies)[15-37,42-44,50], whilst others assessed faecal lactoferrin (13 studies)[17-20,22-24,26,28,30,32,38] and one

study assessed S100A12[42]. In addition, studies utilised different faecal biomarker concentration cut offs for the assessment of markers and scoring systems.

Across all of the biomarkers, there is a wide range of correlation describing the association between faecal markers and endoscopic disease activity (*r*-values ranging from 0.32 to 0.87, *P*-values ranging from < 0.0001 to 0.7815). Correlation coefficients are described in almost all studies and are used more commonly than outcome measures such as sensitivity, specificity, PPV and/or NPV. Overall, the studies that have evaluated faecal calprotectin (FC) and/or faecal lactoferrin (FL) and their relationship with endoscopic disease activity show inconsistent results (Tables 1 and 2). Fewer studies have studied the correlation between FC and FL with histologic severity (Tables 4 and 5).

Faecal calprotectin and endoscopic severity
Of the 28 studies investigating the ability of FC to determine endoscopic disease activity in patients with IBD (Table 1), 17 specifically included patients with CD. Two reports demonstrate high sensitivity and specificity[15,21]. However, the number of patients in these studies was relatively low (*n* = 64 and 122, respectively). In a cohort of 64 CD patients, Schoepfer and colleagues used a FC cut off concentration of 70 μg/g to demonstrate a sensitivity and specificity of 89% and 72% for the identification of MH, respectively[21]. On the other hand, in a cohort of 122 CD patients, af Björkesten *et al*[15] found a sensitivity of 84% and a specificity of 74% with a FC cut off of 94 μg/g. While these values are comparable, the studies used different SES-CD scores to reflect endoscopic remission (SES-CD ≤ 3 and ≤ 2 respectively). Reanalysis using a SES-CD score of 0 (absence of ulcers) in Björkesten's study reduced the ability of FC to detect remission. In both studies only FC was capable of discriminating between various degrees of disease activity in contrast to other indicators such as CRP and the CDAI.

Where calculated, PPV and NPV are variable between the studies of FC in CD patients. Much of this variability appears to be secondary to differences in the cut off concentrations for the faecal biomarkers and the cut off endoscopic scores used to define MH.

Thirteen studies described the use of FC concentration and its correlation with mucosal inflammation and healing in UC patients (Table 1). For all of these studies there was a statistically significant association between FC concentration and mucosal inflammation. However, only seven of these studies reported sensitivity, specificity, PPV and NPV for their studies with respect to a specific FC cut off concentration[16,23,27,45-48]. The largest study, which included 115 patients with UC, demonstrated a sensitivity of 93%, specificity of 71%, PPV of 91% and NPV of 81% using a FC cut off of 50 μg/g[27]. Re-evaluation of this data using a higher cut off of

Table 1 Studies investigating the correlation between faecal calprotectin concentrations and endoscopic activity in subjects with inflammatory bowel diseases

Ref.	Number of participants		Population	Endoscopic index used	Endoscopic index cut off	Faecal calprotectin cut off (μg/g)	Outcome measures				Correlation	
	CD	UC					Sensitivity	Specificity	PPV	NPV	*r* value	*P* value
Crohn's disease studies												
Falvey *et al*[46]	59		Adults	SES-CD	≤ 3	125	71%	71%	85%	50%	0.55%	< 0.0001
						200	60%	79%	88%	45%		
Lobatón *et al*[51]	85		Adults	CDEIS	< 3	274 ELISA	77%	97%	75%	98%	0.784%	< 0.001
					< 3	272 QPOC	79%	97%	76%	98%	0.722%	< 0.001
					0	262 ELISA	75%	76%				
					0	200 QPOC	75%	77%				
Nancey *et al*[45]	78		Adults	SES-CD	≤ 2	250	71%	78%	79%	71%	0.53%	< 0.0001
						100	88%	38%	62%	73%		
D'Haens *et al*[47]	87		Adults	CDEIS	≤ 3	< 250	94.1%	62.2%	48.5%	96.6%	0.419%	< 0.001
				SES-CD	0	< 250	51.6%	82.6%	89.2%	38%	0.49%	< 0.001
af Björkesten *et al*[15]	64		Adults	SES-CD	≤ 2	< 100	81%	74%	-	-	0.56%	< 0.001
						< 94	84%	74%	-	-		
				SES-CD	0	< 94	82%	78%	-	-		
Aomatsu *et al*[16]	18		Paediatrics	SES-CD	0	100	94.7%	50%	87.8%	71.4%	0.76%	< 0.01
						150	94.7%	50%	87.8%	71.4%		
Sipponen *et al*[17]	19		Adults	SES-CD	≤ 2	< 100	-	80%	-	-	-	-
Schoepfer *et al*[21]	122		Adults	SES-CD	≤ 3	< 50	89%	58%	89%	61%	0.75%	< 0.01
						< 70	89%	72%	88%	76%		
Langhorst *et al*[23]	43		Adults	SES-CD		> 6	100%	30%	82.5%	100%	0.35%	< 0.05
						> 48	81.8%	80%	93.1%	57.1%		
Schoepfer *et al*[22]	36		Adults	SES-CD	≤ 19	50	-	-	-	-	-	< 0.0001
Sipponen *et al*[18]	61		Adults	SES-CD (total)	≤ 3	< 100	-	-	-	-	0.662%	< 0.001
				SES-CD (colon)	≤ 3	< 100	-	-	-	-	0.642%	< 0.001
				SES-CD (ileal)	≤ 3	< 100	-	-	-	-	0.317%	> 0.05
Sipponen *et al*[19]	15		Adults	CDEIS	≤ 2	< 200	87%	100%	100%	70%	0.831%	< 0.001
Sipponen *et al*[20]	77		Adults	CDEIS	≤ 2	< 50	91%	44%	76%	73%	0.729%	< 0.001
						< 100	81%	69%	84%	66%		
						< 200	70%	92%	94%	61%		
Jones *et al*[24]	164		Adults	SES-CD	≤ 6	≤ 50	-	-	-	-	0.45%	< 0.05
Denis *et al*[25]	28		Adults	CDEIS	≤ 5	< 50	-	-	-	-	-	0.57
Schoepfer *et al*[26]	24		Adults	SES-CD	≤ 19	< 50	-	-	-	-	-	0.0001
D'Incà *et al*[28]	31		Adults	SES-CD		> 80	-	-	-	-	0.48%	0.008
Mixed inflammatory bowel disease population studies												
Molander *et al*[29]	183	69	Mixed	SES-CD; Mayo	≤ 2; ≤ 1	< 100	-	-	-	72%	-	< 0.0001
Vieira *et al*[30]	38	40	Adults	CDEIS; Mayo	≤ 2; ≤ 2	> 200.01	88.6%	97.1%	97.5%	86.8%	-	0
Schoepfer *et al*[22]	36	28	Adults	SES-CD; Rachmilewitz	≤ 19; ≤ 4	50	-	-	-	-	-	< 0.0001
Canani *et al*[50]	26	32	Paediatrics	Saverymuttu	≤ 1	143	-	-	-	-	0.46%	≤ 0.05
Fagerberg *et al*[31]	27	10	Paediatrics	Saverymuttu		< 85.7	-	-	-	-	0.65%	< 0.001
Silberer *et al*[32]	21	18	Adults	Stange		18.6	61.5%	95%	-	-	-	< 0.0001
Røseth *et al*[33]	17	28	Adults	Farup		< 50	0%	100%	-	97.8%	-	-
Bunn *et al*[34]	2	9	Paediatrics	Saverymuttu			-	-	-	-	0.65%	< 0.05
Ulcerative colitis studies												
Falvey *et al*[46]		38	Adults	Baron	0	125	74%	80%	85%	67%	0.55%	< 0.0001
						200	58%	95%	95%	59%		
Nancey *et al*[45]		55	Adults	Rachmilewitz	≤ 2	250	91%	87%	87%	91%	0.75%	< 0.0001
						100	100%	53%	85%	100%		
Kristensen *et al*[48]		62	Adults	Mayo	0	61 Cal	84.1%	83.3%	92.5%	68.2%		< 0.001
					0	96 BM	90.9%	83.3%	93%	78.9%		< 0.001
					≤ 1	110 Cal	80%	66.6%	69.2%	78%		
					≤ 1	259 BM	83.3%	71.9%	73.5%	82.1%		
D'Haens *et al*[47]		39	Adults	Mayo	0	< 250	71%	100%	100%	47.1%	0.56%	< 0.001
Komraus *et al*[35]		16	Paediatrics	Rachmilewitz		< 50	-	-	-	-	0.52%	0.0391
Aomatsu *et al*[16]		17	Paediatrics	Matts	≤ 6	100	94.1%	50%	88.9%	66.7%	0.84%	< 0.01
						150	91.2%	87.5%	96.9%	70%		
Schoepfer *et al*[27]		115	Adults	Rachmilewitz	< 4	< 50	93%	71%	91%	81%	0.83%	< 0.001
						< 100	86%	88%	96%	65%		
Langhorst *et al*[23]		42	Adults	Mayo		> 6	100%	6.7%	6.6%	100%	0.49%	< 0.001
						> 48	81.5%	72.3%	84.6%	68.8%		

Ref.	N	Pop	Index	Cut	Val							P
Schoepfer et al[22]	28	Adults	Rachmilewitz	≤ 4	50	-	-	-	-	-	-	0.0025
Schoepfer et al[26]	12	Adults	Rachmilewitz	≤ 1	< 50	-	-	-	-	-	-	0.0335
D'Incà et al[28]	46	Adults	Mayo		> 80	-	-	-	-	-	0.511%	0.001
Hanai et al[36]	31	Adults	Matts	≤ 1		-	-	-	-	-	0.81%	< 0.001
Røseth et al[37]	62	Adults	Sandborn	≤ 1	< 10	-	34%	-	-	-	0.57%	< 0.0001
					< 20	-	62%	-	-			

CD: Crohn's disease; UC: Ulcerative colitis; PPV: Positive predictive value; NPV: Negative predictive value; SES-CD: Simple endoscopic score for Crohn's disease[54]; CDEIS: Crohn's disease endoscopic index of severity[55]; ELISA: Enzyme linked immunosorbant assay; QPOC: Quantitative point of care test; Mayo: Mayo endoscopic sub-scoring of ulcerative colitis[56]; Rachmilewitz: Rachmilewitz endoscopic score[57]; Saverymuttu: Non-standard endoscopic scoring system[58]; Stange: Non-standard endoscopic scoring system[59,60]; Farup: Non-standard endoscopic scoring system[61]; Baron: Baron score; Matts: Matts score[62]; Sandborn: Non-standard endoscopic scoring system[63]; Cal: Calpro ELISA: Calpro Calprotectin ELISA, Calpro AS, Norway; BM: BM ELISA, EK-CAL, Buhlmann Laboratories AG, Switzerland; Farmer: Non-standard endoscopic scoring system[64]; Faecal Hb: Faecal haemoglobin; PMN-e: Polymorhonuclear elastase; Hb-Hp: Haemoglobin; Haptoglobin complex; D'Haens: Non-standard histologic scoring system[65]; Fazio: Non-standard histologic scoring system[66]; Floren: Non-standard histologic scoring system[67].

Table 2 Studies investigating the correlation between faecal lactoferrin concentrations and endoscopic activity in subjects with inflammatory bowel diseases

Ref.	Number of participants		Population	Endoscopic index used	Endoscopic index cut off	Faecal lactoferrin cut off (μg/mL)	Outcome measures				Correlation	
	CD	UC					Sensitivity	Specificity	PPV	NPV	r value	P value
Crohn's disease studies												
Sipponen et al[17]	19		Adults	SES-CD	≤ 2	< 7.25	-	80%	-	-	-	-
Pfefferkorn et al[38]	54		Paediatrics	Unique score		≥ 7.25	100%	43%	70%	100%	-	< 0.001
						≥ 60	84%	74%	81%	77%		
Sipponen et al[18]	61		Adults	SES-CD (total)	≤ 3	< 7.25	-	-	-	-	0.705%	< 0.001
				SES-CD (colon)	≤ 3	< 7.25	-	-	-	-	0.627%	< 0.001
				SES-CD (ileal)	≤ 3	< 7.25	-	-	-	-	0.18%	> 0.05
Sipponen et al[19]	15		Adults	CDEIS	≤ 2	< 10	77%	100%	100%	58%	0.865%	< 0.001
Sipponen et al[20]	77		Adults	CDEIS	≤ 2	< 10	66%	92%	94%	59%	0.773%	< 0.001
						< 7.25	71%	83%	89%	60%		
Jones et al[24]	164		Adults	SES-CD	≤ 6	< 7.25	-	-	-	-	0.48%	< 0.05
Langhorst et al[23]	43		Adults	SES-CD		> 7.25	81.8%	60%	87.1%	50%	0.42%	< 0.01
						> 7.05	81.8%	60%	87.1%	50%		
Schoepfer et al[22]	36		Adults	SES-CD	≤ 19	7	-	-	-	-	-	< 0.0001
Schoepfer et al[26]	24		Adults	SES-CD	≤ 19	< 7	-	-	-	-	-	0.0008
D'Incà et al[28]	31		Adults	SES-CD			-	-	-	-	0.192%	0.545
Mixed inflammatory bowel disease population studies												
Vieira et al[30]	38	40	Adults	CDEIS; Mayo	≤ 2; ≤ 2	4-8	93.2%	76.5%	83.7%	89.7%	-	0
Schoepfer et al[22]	36	28	Adults	SES-CD; Rachmilewitz	≤ 19; ≤ 4	7	-	-	-	-	-	< 0.0001
Silberer et al[32]	21	18	Adults	Stange		6.64	33.3%	95%	-	-	-	0.0059
Ulcerative colitis studies												
Langhorst et al[23]		42	Adults	Mayo		> 7.25	88.9%	66.7%	82.8%	76.9%	0.56%	< 0.001
						> 7.05	92.6%	66.7%	83.3%	83.3%		
Schoepfer et al[22]		28	Adults	Rachmilewitz	≤ 4	7	-	-	-	-	-	0.078
Schoepfer et al[26]		12	Adults	Rachmilewitz	≤ grade 1	< 7	-	-	-	-	-	0.7815
D'Incà et al[28]		46	Adults	Mayo			-	-	-	-	0.354%	0.023

CD: Crohn's disease; UC: Ulcerative colitis; PPV: Positive predictive value; NPV: Negative predictive value; SES-CD: Simple endoscopic score for Crohn's disease[54]; CDEIS: Crohn's disease endoscopic index of severity[55]; Mayo: Mayo endoscopic sub-scoring of ulcerative colitis[56]; Rachmilewitz: Rachmilewitz endoscopic score[57]; Stange: Non-standard endoscopic scoring system[59,60].

100ug/g resulted in values of 86%, 88%, 96% and 65%, respectively. The correlation coefficient of $r = 0.83$ for UC was higher than found in CD patients ($r = 0.75$). Again, FC was the only marker that was able to discriminate inactive from mild, moderate and highly active disease. A further study evaluating patients with UC using the Mayo Endoscopic Subscore and FC with a cut off of 48 μg/g, determined a sensitivity of 81.5% and specificity of 72.3%[23]. In contrast, an earlier study from the same region reported specificity of only 34% (for FC with cut off of 10 μg/g), or 62% using a cut off of 20 μg/g[37]. Four more recent studies have been more thorough in describing the association between FC concentration and endoscopic remission[45-48], although in relatively modest numbers of patients (38-62 patients only). Kristensen et al[48] analysed both Mayo 0 and Mayo 0 and 1 combined for two different commercial FC assays. Not surprisingly, specificity and PPV were greater when using the Mayo 0 score with both FC assays. On the other hand, in a

Table 3 Studies investigating the correlation between other faecal marker concentrations and endoscopic activity in subjects with inflammatory bowel diseases

Ref.	Number of participants		Population	Endoscopic index used	Endoscopic index cut off	Faecal marker measured	Faecal marker cut off	Outcome measures				Correlation	
	CD	UC						Sensitivity	Specificity	PPV	NPV	r value	P value
Nancey et al[45]	78		Adults	SES-CD	≤ 2	Neopterin	200 pmol/g	74	73	73	74	0.47	< 0.001
							150 pmol/g	80	65	68	78		
		55	Adults	Rachmilewitz	≤ 2	Neopterin	200 pmol/g	74	100	100	73	0.72	< 0.001
							150 pmol/g	84	100	100	78		
Nakarai et al[39]		152	Mixed	Mayo	0	Faecal Hb	< 100 ng/mL	92	71	37	97	0.5409	< 0.0001
						Faecal Hb	< 60 ng/mL	94	74	40	98		
				Mayo	≤ 1	Faecal Hb	< 100 ng/mL	60	87	85	64		
						Faecal Hb	< 60 ng/mL	58	90	88	64		
Langhorst et al[23]	43		Adults	SES-CD		PMN-e	< 0.062 μg/mL	81.8	70	90	54.8	0.32	< 0.05
		42	Adults	Mayo		PMN-e	< 0.062 μg/mL	70.4	66.7	79.2	55.6	0.36	< 0.05
Silberer et al[32]	21	18	Adults	Stange		PMN-e	0.124	79.5	95	-	-	-	< 0.0001
						Lysozyme	1.3	47.5	95	-	-	-	< 0.0001
						α1-AT	158	20	95	-	-	-	-
						Faecal Hb	1.8	61.5	95	-	-	-	-
						Hb-Hp	0.8	64.1	95	-	-	-	-
Moran et al[40]	7	21	Mixed	Farmer		α1-AT	≤ 0.58 mg/g	-	-	-	-	0.83	0.001
Cellier et al[41]	95		Adults	CDEIS		α1-AT		-	-	-	-	0.26	0.001

CD: Crohn's disease; UC: Ulcerative colitis; PPV: Positive predictive value; NPV: Negative predictive value; SES-CD: Simple endoscopic score for Crohn's disease[54]; CDEIS: Crohn's disease endoscopic index of severity[55]; Mayo: Mayo endoscopic sub-scoring of ulcerative colitis[56]; Rachmilewitz: Rachmilewitz endoscopic score[57]; Stange: Non-standard endoscopic scoring system[59,60]; Farmer: Non-standard endoscopic scoring system[64]; Faecal Hb: Faecal haemoglobin; PMN-e: Polymorhonuclear elastase; Hb-Hp: Haemoglobin and Haptoglobin complex.

study of 39 adults with UC, D'Haens et al[47] used Mayo 0 and a FC cut off of < 250 μg/g leading to 100% specificity and NPV, but just 50% PPV. Therefore, this would suggest that while a FC concentration of greater than 250 μg/g is highly predictive of the presence of mucosal inflammation in UC, this concentration is no better than flipping a coin for determining whether a patient has mucosal healing.

Faecal calprotectin and histological assessment
FC has been compared with histological activity in only 11 studies (Table 4). A study of 61 CD patients showed a significant correlation between FC concentration (r = 0.563, P < 0.01) and colonic or ileocolonic disease, but not with ileal disease[18]. This is consistent with an earlier study published by the same group[19], which demonstrated a significant association between FC concentration and pretreatment colonic disease (r = 0.522, P = 0.046) although only 15 patients were included.

For UC, the patient groups are small in all of the studies and there are mixed results regarding the correlation between FC concentration and histological appearance. Furthermore, few studies report a FC cut off concentration that optimally reflects MH.

Paediatric studies evaluating faecal calprotectin
Only one of eight paediatric studies documented sensitivity, specificity, PPV and NPV in addition to correlation coefficients[16]. Although the sensitivity was high in both CD (94.7%) and UC patients (94.1%), when utilising a cut off of 100 μg/g, the specificity was only 50%. Using a cut off of 150 μg/g, the specificity

for active UC increased to 87.5%. Furthermore, Aomatsu et al[16] demonstrated that FC correlates closely with the SES-CD and Matt's grading (r = 0.760 and 0.838, respectively) - the strongest correlations identified amongst these studies.

Fagerberg et al[43] studied a paediatric group with predominantly colonic CD. Experienced gastrointestinal histopathologists divided the patients into two groups (inflamed and non-inflamed), based upon conventional criteria for IBD. Using a FC cut off of 50 μg/g resulted in sensitivity 95%, specificity 93%, PPV 95% and NPV 93%. In 2007 Fagerberg et al[31] evaluated a mixed group of children with CD and UC. Using a cut off of 85.7 μg/g for FC, the authors demonstrated a sensitivity 93%, specificity 82%, PPV 93% and NPV 82% for the identification of mucosal healing.

It is difficult to directly compare paediatric and adult studies of faecal biomarkers due to the heterogeneity of the study designs, particularly with respect to the use of different endoscopic indices and the definition of MH.

Faecal lactoferrin and endoscopic severity
Ten of twelve studies focusing on FL included just patients with CD (Table 2). For example, Langhorst et al[20] used the SES-CD to demonstrate a sensitivity of 81.8% and specificity of 60% for FL (r = 0.35, P < 0.05)[23]. In another study using the same cut off concentration (< 7.25 μg/g), a sensitivity of 71% and a specificity 83% were demonstrated with a PPV of 89% and NPV of 60%. A PPV of 100% has been shown in a further report including just 15 patients: however the NPV in this series was only 58%[19].

In a group of patients with UC, Langhorst et al[28]

Table 4 Studies investigating the correlation between FC concentrations and histologic activity in subjects with inflammatory bowel diseases

Ref.	Number of participants CD	UC	Population	Histology index used	Histology index cut off	Faecal calprotectin cut off (μg/g)	Sensitivity	Specificity	PPV	NPV	r value	P value
Crohn's disease studies												
Sipponen et al[18]	61		Adult	D'Haens (ileocolonic)		< 100	-	-	-	-	0.563	< 0.01
				D'Haens (ileal)		< 100	-	-	-	-	0.311	> 0.05
Sipponen et al[19]	15		Adult	D'Haens (pretreatment colonic)		< 200	-	-	-	-	0.522	0.046
				D'Haens (posttreatment colonic)		< 200	-	-	-	-	-	> 0.05
				D'Haens (ileal)		< 200	-	-	-	-	-	> 0.05
Canani et al[50]	26		Paediatric	Saverymuttu	≤ 1	143	-	-	-	-	0.681	< 0.0001
Kaiser et al[42]	32		Adult	Unique score	0	< 50	-	-	-	-	0.412	< 0.05
D'Incà et al[28]	31		Adult	Fazio		> 80	81%	80%	95%	-	0.117	0.545
Fagerberg et al[43]	22		Paediatric	Unique score		< 50	95%	93%	95%	93%	-	< 0.00001
Mixed inflammatory bowel disease population studies												
Vieira et al[30]	38	40	Adult	Unique score		> 200	77%	100%	100%	68%	-	0
Canani et al[50]	26	32	Paediatric	Saverymuttu	≤ 1	143	94%	64%	81%	87%	0.655	< 0.05
D'Incà et al[28]	31	46	Adult	Fazio; Floren (SES-CD; Mayo)	> 80	79	74%	92%	-	-	-	
Fagerberg et al[31]	27	10	Paediatric	Saverymuttu	≤ 2	< 50	93%	73%	90%	80%	0.75	< 0.001
						< 85.7	93%	82%	93%	82%		
				Saverymuttu			-	-	-	-	0.79	< 0.001
Kolho et al[44]	9	16	Paediatric	Farup		50	-	-	69%	100%	-	-
						100	-	-	72%	96%	-	-
Bunn et al[53]	2	9	Paediatric	Saverymuttu	≤ 6	6.3	100%	80%	-	-	0.74	< 0.01
Ulcerative colitis studies												
Canani et al[50]		32	Paediatric	Saverymuttu	≤ 1	143	-	-	-	-	0.661	< 0.0001
D'Incà et al[28]		46	Adult	Floren		> 80	78%	70%	90%	-	0.323	0.042
Kaiser et al[42]		27	Adult	Unique score		< 50	-	-	-	-	0.311	0.14
Røseth et al[37]		62	Adult	Farup	≤ 1	< 10	-	50%	-	-	0.70	< 0.0001
						< 20	-	81%	-	-		

CD: Crohn's disease; UC: Ulcerative colitis; PPV: Positive predictive value; NPV: Negative predictive value; SES-CD: Simple endoscopic score for Crohn's disease[54]; Mayo: Mayo endoscopic sub-scoring of ulcerative colitis[56]; Saverymuttu: Non-standard endoscopic scoring system[58]; Farup: Non-standard endoscopic scoring system[61]; D'Haens: Non-standard histologic scoring system[65]; Fazio: Non-standard histologic scoring system[66]; Floren: Non-standard histologic scoring system[67].

used the Mayo Endoscopic Subscore (without a given cut off concentration for FL), leading to specificity and sensitivity of 92.6% and 66.7%, respectively, with a correlation of $r = 0.56$, $P < 0.001$[23]. A different cohort using the same index showed a lower correlation coefficient for FL ($r = 0.354$, $P = 0.023$)[28].

Faecal lactoferrin and histological assessment
Four studies have evaluated correlations between FL and histologic severity[18,19,28,30]. Sipponen et al[18] described a significant correlation between FL and colonic or ileocolonic CD (r=0.543), but not for ileal disease ($r = 0.291$)[18]. Subsequently, the same authors divided patients into subgroups of pretreatment colonic, post-treatment colonic and ileal disease[19]. This report did not find a significant correlation between FL and mucosal histology. An additional study performed by D'Incà et al[28] with only 15 participants demonstrated moderate sensitivity (77%), specificity (80%) and PPV (95%), which was comparable to the performance of FC in the same

group of patients.
Only one study has measured FL and histologic severity in patients with UC. A sensitivity of 75%, a specificity of 60%, a PPV of 87% and a significant correlation ($r = 0.544$) was ascertained in this report[28].

Paediatric studies evaluating faecal lactoferrin
Only one study has assessed FL in children with CD[38]. Using an unvalidated endoscopic grading system, the patients were divided into active and inactive groups. A cut off of 7.25 μg/g demonstrated a sensitivity of 100% and a specificity of 43%, whereas a cut off of 60 μg/g resulted in a lower sensitivity (84%) but higher specificity (74%). Again, it is hard to compare these outcomes to the adult studies evaluating FL due to marked variability in study design.

Other faecal markers
Although the majority of studies included in this review have evaluated FC and FL, other faecal markers have also been assessed including α1-antitrypsin,

Table 5 Studies investigating the correlation between faecal lactoferrin concentrations and histologic activity in subjects with inflammatory bowel diseases

| Ref. | Number of participants | | Population | Histology index used | Histology index cut off | Faecal lactoferrin cut off ($\mu g/mL$) | Outcome measures | | | | Correlation | |
	CD	UC					Sensitivity	Specificity	PPV	NPV	*r* value	*P* value
Crohn's disease studies												
Sipponen *et al*[18]	61		Adults	D'Haens (ileocolonic)	< 7.25		-	-	-	-	0.543	< 0.01
				D'Haens (ileal)	< 7.25		-	-	-	-	0.291	> 0.05
Sipponen *et al*[19]	15		Adult	D'Haens (pretreatment colonic)	< 10		-	-	-	-	0.482	0.069
				D'Haens (posttreatment colonic)	< 10		-	-	-	-	-	> 0.05
				D'Haens (ileal)	< 10		-	-	-	-	-	> 0.05
D'Incà *et al*[28]	31		Adult	Fazio			77%	80%	95%	-	0.477	0.009
Mixed inflammatory bowel disease studies												
Vieira *et al*[30]	38	40	Adults	Unique score		4-8	90%	92%	96%	83%	-	-
D'Incà *et al*[28]	31	46	Adults	Fazio; Floren (SES-CD; Mayo)		7	76%	67%	90%	-	-	-
Ulcerative colitis studies												
D'Incà *et al*[28]		46	Adults	Floren		7	75%	60%	87%	92%	0.544	0.0001

CD: Crohn's disease; UC: Ulcerative colitis; PPV: Positive predictive value; NPV: Negative predictive value; SES-CD: Simple endoscopic score for Crohn's disease[55]; Mayo: Mayo endoscopic sub-scoring of ulcerative colitis[57]; D'Haens: Non-standard histologic scoring system[66]; Fazio: Non-standard histologic scoring system[67]; Floren: Non-standard histologic scoring system[68].

polymorphonuclear elastase, lysozyme, faecal haemoglobin (FHb), haemoglobin-haptoglobin complex (Hb-Hp), neopterin and S100A12 (Tables 3 and 6).

Cellier *et al*[41] compared faecal α1-antitrypsin to CDEIS in 121 CD patients and found no correlation (r = 0.26). In contrast, Moran *et al*[68] demonstrated in 28 IBD patients a significant correlation between faecal α1-antitrypsin and an alternative endoscopic index (r = 0.83, P = 0.001)[68].

Faecal polymorphonuclear elastase (PMN-e) is significantly correlated with endoscopic severity in CD (r = 0.32) and UC patients (r = 0.36)[23]. Similar results for a mixed group of patients were found by Silberer *et al*[32].

Nakarai *et al*[39] assessed faecal haemoglobin concentrations in 152 UC patients and compared this with the Mayo Endoscopic Score (threshold of mucosal healing). FHb showed sensitivity 94%, specificity 74%, PPV 40%, and NPV 98%.

Of the studies included in this review, only Kaiser *et al*[42] investigated the faecal marker S100A12. The specificity for both CD and UC subgroups was 100%, whereas the sensitivity was 81% in CD and 91% in UC.

DISCUSSION

Faecal biomarkers such as FC and FL offer tremendous promise as non-invasive markers of mucosal inflammation. As therapeutic targets move from symptom control to mucosal healing, it is imperative that non-invasive markers of inflammation are firstly validated and then become available for routine clinical use. This could allow more regular assessment of inflammation with subsequent timely clinical decisions and possibly lead to a reduced requirement for follow-up endoscopies. Sensitive and specific biomarkers are essential if a true treat-to-target approach is to be adopted.

At best the currently available studies show a mixed picture with few findings strongly replicated across multiple studies. This variability is reflected in diverse study designs with a wide range of endoscopic and other indices employed. Even within studies using the same indices, variable scores have been used to define MH or remission. Additionally, a wide range of cut off concentrations for faecal biomarkers have been used, leading to difficulty in the interpretation of individual results. Until a clear target for treatment is defined, it is difficult to resolve many of the differences between these studies.

Correlation coefficients are a useful means of comparing the association between two sets of continuous data (such as faecal biomarker concentration and mucosal inflammation). However, once such correlations have been shown to be significant, it is essential that accurate cut-off concentrations are determined for biomarkers using categorical data for mucosal inflammation. This allows sensitivity, specificity, positive and negative predictive values (in addition to accuracy) to be determined. These parameters are clinically useful, whereas correlation coefficients provide limited clinical relevance. Unfortunately, few studies provided in depth statistical analysis including all the required parameters.

While there were a large number of studies that assessed the utility of faecal biomarkers in reflecting mucosal inflammation at a single point in time, few followed patients prospectively to determine the prognostic significance of elevated biomarkers. In clinical medicine, such prognostic data are essential in determining appropriate treatment escalation and de-escalation.

Future studies

We suggest a number of ways in which future studies

Table 6 Studies investigating the correlation between other faecal marker concentrations and histologic activity in subjects with inflammatory bowel diseases

Ref.	Number of participants		Population	Histology index used	Histology index cut off	Faecal marker measured	Faecal marker cut off	Outcome measures				Correlation	
	CD	UC						Sensitivity	Specificity	PPV	NPV	*r* value	*P* value
Crohn's disease studies													
Kaiser *et al*[42]	32	27	Adults	Unique score	0	S100A12		-	-	-	-	0.44	< 0.01
	32					S100A12	0.8	81	100	-	-	0.451	0.01
		27				S100A12	0.8	91	100	-	-	0.44	< 0.025

CD: Crohn's disease; UC: Ulcerative colitis; PPV: Positive predictive value; NPV: Negative predictive value.

may contribute to an improved understanding of the relationship between faecal biomarkers and mucosal inflammation and healing.

Firstly, treatment targets in IBD need to be defined and validated. This issue is much broader than the field of faecal biomarkers, but is a clinical and philosophical problem that needs to be urgently resolved. Once resolved, then studies can be performed using established and meaningful endoscopic or other endpoints against which faecal biomarkers can be measured. This includes the assessment of biomarkers against endoscopic and histologic indices, unless there appears to be lack of a validated grading system in IBD for the latter.

Secondly, studies should report their data in clinically meaningful ways including sensitivity, specificity, positive and negative predictive values and accuracy. This will allow comparison between the performances of individual biomarkers and may demonstrate specific advantages of one biomarker over another.

Thirdly, consideration should be made to combining non-endoscopic data to provide the best measure of mucosal inflammation. This could include combinations of clinical symptoms, serum and faecal biomarkers and is likely to be superior to one single parameter. Such analyses will require well-powered studies to enable appropriate analyses.

Finally, the cost-effectiveness of biomarker-driven treatment algorithms needs to be compared with symptoms and endoscopy driven approaches. While biomarker assays are cheaper than endoscopy, the assay costs are still not inconsequential and cost effectiveness must be measured in future studies. These costs should include both direct and indirect costs (the latter are often missed in such analyses and the effect of absenteeism for clinical investigations for patients and their carers should be captured).

In conclusion, Surrogate markers for endoscopic severity in IBD patients are needed for many reasons. Mucosal healing is an important and meaningful objective in the management of this incurable disease. At present, faecal markers seem promising as tools to reflect mucosal healing in IBD, however further research is needed to elucidate their definitive role(s). The variability of study design and endpoints described in this review make it difficult to recommend the routine use of faecal biomarkers in all patients. Nor can one biomarker be suggested to be superior to another given the lack of robust comparative studies. Future research should focus on large studies with clinically meaningful endpoints.

COMMENTS

Background
With regard to disease severity in Crohn's disease and ulcerative colitis patients, several endoscopic, clinical and histologic grading systems are being used. The gold standard for assessing mucosal inflammation and response to therapy in inflammatory bowel disease (IBD) is endoscopy.

Research frontiers
Emerging data suggest that mucosal healing (MH) may be a more reliable target for treatment than clinical and biochemical assessment. MH is associated with improved outcomes in clinical trials. Faecal markers have shown to have multiple advantages in assessing MH and have been suggested as the gold standard for remission.

Innovations and breakthroughs
In addition to MH, the concept of deep remission (encompassing both clinical remission and MH) has been developed, although is yet to be formally tested in clinical trials. Recently a working group of the International Organisation for the study of IBD published a detailed description of potential targets for the management of IBD. The process leading to a "treat to target" approach in IBD has mirrored that seen in other diseases where tight disease control has led to improved patient outcomes.

Applications
Current available studies show a mixed picture of the utility of faecal biomarkers due to a wide variability in study design and endpoints. According to these data, the authors cannot argue for the use or certain cut off values of these markers. If a true treat-to-target approach is to be adopted, accurate and validated data are needed in order to be able to recommend sensitive and specific biomarkers.

Peer-review
In the review, the authors aimed to review the available studies about fecal markers of mucosal healing in IBD. The manuscript is of great clinical importance.

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