Can fecal calprotectin accurately identify histological activity of ulcerative colitis? A meta-analysis

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Abstract

Background and Aims: Elevated fecal calprotectin (FC) levels have been reported to correlate with histological activity in patients with ulcerative colitis (UC). However, the accuracy of FC for evaluating histological activity of UC remains to be determined. The aim of this study was to determine the accuracy of FC for evaluating histological activity of UC, based on updated definitions.

Methods: Related studies were retrieved from the PubMed, Web of Science, Embase, and Cochrane databases. Adult participants diagnosed with UC were included when sufficient data could be extracted to calculate the accuracy of FC for evaluating histological activity. The primary outcome was histological response, and the secondary outcome was histological remission, defined according to a recently updated position paper of European Crohn's and Colitis Organization. Statistics were pooled using bivariate mixed-effects models. The area under the curve was estimated by summary receiver-operating characteristic curves.

Results: Nine studies were included, from which 1039 patients were included for the analysis of histological response and 591 patients for histological remission. For the evaluation of histological response, the pooled sensitivity, specificity, and the area under the curve were 0.69 [95% confidence interval (CI): 0.52–0.82], 0.77 (95% CI: 0.63–0.87), and 0.80 (95% CI: 0.76–0.83), respectively. For the evaluation of histological remission, the corresponding estimates were 0.76 (95% CI: 0.71–0.81), 0.71 (95% CI: 0.62–0.78), and 0.79 (95% CI: 0.75–0.82), respectively. FC had a higher accuracy in studies using Nancy Index. For histological response, the cut-off values of FC ranged from 50 to 172 µg/g, and the sensitivity was higher in studies with FC cut-off values >100 µg/g (0.77 *versus* 0.65).

Conclusion: FC is a valuable biomarker for assessing histological activity in patients with UC. A cut-off value of 100–200 µg/g is more appropriate to spare patients from an unnecessary endoscopy and biopsy.

Keywords: calprotectin, histological remission, histological score, histology, inflammatory bowel disease, meta-analysis, ulcerative colitis

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Introduction

Ulcerative colitis (UC) is a chronic, idiopathic inflammatory disease involving the colon, characterized by relapsing and remitting mucosal inflammation. The therapeutic target of UC was recommended as clinical and endoscopic remission.[1] Recently, accumulating evidence suggests that histological remission more effectively reduces relapse, corticosteroid use, and hospitalization.[2,3] Better long-term outcomes are associated with histological remission than with mucosal healing.[2,3] Therefore, histological remission has also been proposed as a target for the treatment of UC.[4]

Biopsy is the gold standard for detecting and grading histological activity of UC. However, biopsy is invasive and may cause bleeding complications.[5] Since the heterogenous distribution of inflammatory changes exists among different sample sites, the risk of sampling error creates additional challenges.[1] Therefore, a non-invasive biomarker capable of surveillance of histological activity of UC would be helpful in clinical practice.

Calprotectin is a protein derived predominantly from neutrophils and accounts for approximately 60% of the cytosolic protein in neutrophils.[6] Since neutrophil infiltration into the mucosa correlates with endoscopic severity and systemic inflammation,[7] fecal calprotectin (FC) might be an appropriate marker to assess gastrointestinal inflammation.[8] FC is expected to support or replace histological assessment as a non-invasive biomarker because of its proven strong correlation with histological activity.[9,10] Recently, a systematic review attempted to summarize the relationship between FC and histological remission of UC.[11] However, the definitions of histological remission are inconsistent in different studies and that review could not explore the threshold effect caused by various FC cut-off values. Moreover, that review could not summarize the quantitative effect of different patient characteristics, FC assays, histological activity criteria, and other factors. Since the European Crohn's and Colitis Organization (ECCO) has recently updated the definitions of histological activity of UC, we applied the updated definitions to perform a meta-analysis to better assess the accuracy of FC for evaluating histological activity in UC.

Materials and methods

Search strategy
This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[12] A review protocol was created before the search. We searched the PubMed, Web of Science, Embase, and Cochrane databases up to 31 December 2020. We used the following MeSH headings, keywords, and text words: "ulcerative colitis," "UC," "inflammatory bowel disease," "IBD," "fecal calprotectin," "faecal calprotectin," "calprotectin," "FC," "histologic activity," "histologic score," "histologic remission," "histologic healing," "Nancy index," "Geboes score," and "Robarts histopathologic score." No language or publication date restrictions were applied. We also screened abstracts from the Digestive Disease Week and the ECCO from the past 5 years. Selected articles' reference lists were checked for revision and screened for additional relevant publications.

Definition
The primary outcome was histological response, and the secondary outcome was histological remission. According to the position paper of ECCO,[4] histological response is defined as continuous Geboes score (GS) $\leqslant 12$, GS < 3.0, Robarts histopathology index (RHI) $\leqslant 9$ (with subscores of 0 for neutrophils in the epithelium and without erosions or ulcers), Nancy Index (NI) $\leqslant 1$.[13–15] Histological remission is defined as continuous GS $\leqslant 6$ GS, GS $\leqslant 2.0$, NI $= 0$, RHI $\leqslant 3$ (with subscores of 0 for lamina propria neutrophils and neutrophils in the epithelium and without ulcers or erosions).

Since both GS < 3.0 and GS < 3.1 indicate no neutrophils in the epithelium,[13] studies with GS < 3.1 were also included in the analysis of histological response. Since both GS $\leqslant 2.0$ and GS < 2.0 indicate neither an increase in eosinophils nor neutrophils in the lamina propria,[13] studies with GS < 2.0 were also included for the analysis of histological remission.

Selection criteria
The inclusion criteria were as follows: adult patients diagnosed with UC; detailed histological scores described; and the relationship between FC and histological activity of UC assessed. Studies were excluded for the following reasons: histological outcomes not matched with the definition of histological response or histological remission or those using a strict definition for a combined histological score and endoscopic score; lack of, or insufficient, data to calculate true-positive, false-positive, false-negative, or true-negative values; case reports, reviews, and editorials; and fewer than 20 samples. When patient data were reported more than once, only the most recent article with the most information was included.

Data extraction and quality assessment
The titles and abstracts were reviewed by two investigators (XY and SZ) independently. After

the eligibility assessment, data were extracted, including the first author, publication year, study design, study region, patient sex, patient age, endoscopic activity, FC assay, median of FC, FC cut-off, interval between fecal sampling and endoscopy, and definition of histological remission or histological response. The absolute number of true-positive, false-positive, false-negative, and true-negative cases was collected or calculated to develop a 2×2 contingency table. When multiple cut-off values existed, we selected the pre-specified one or manufacturer's reference first. If there is no pre-specified cut-off value or manufacturer's reference, we select the optimal cut-off value with the highest Youden index.

Two authors (XY and SZ) independently assessed the study quality by using the Quality Assessment of Diagnostic Accuracy Studies 2 tool.[16] In any case of discrepancies, we consulted with the senior reviewer (MC) to reach consensus.

Statistical analyses
A bivariate mixed-effects regression model was used to pool the diagnostic accuracy and calculate the sensitivity, specificity, likelihood ratio, diagnostic odds ratio, and confidence intervals (CIs). The summary receiver-operating characteristic (SROC) curve was plotted. An area under the SROC curve near 0.50 indicated low accuracy and that near 1.0 indicated perfect accuracy. Heterogeneity was measured by the I^2 inconsistency test. Heterogeneity was ranked low (25%), moderate (50%), or high (75%).[17] Two methods were used to identify the sources of heterogeneity: (1) the squared correlation coefficient of the proportion of heterogeneity due to threshold effects; and (2) univariate meta-regression and subgroup analyses (including study region, study design, endoscopic activity, definition of histological score, number of patients, FC assay, interval between fecal sampling and endoscopy and cut-off values). Sensitivity analysis was performed to assess the effect of individual studies on the summary estimates. Cook's distance was used to identify outlier studies. As the number of included studies was below 10, publication bias was not explored.

Quality assessments were conducted using RevMan software (version 5.4, the Cochrane Collaboration, Oxford, UK). All statistical analyses were performed using Stata software (version 15, StataCorp, College Station, TX, USA) with the MIDAS module.[18] A *p* value < 0.10 was considered to indicate statistical significance in meta-regression.

Results

Study selection and characteristics
We searched databases and other sources and identified 517 and 22 records, respectively. After the titles and abstracts were screened, the full text of 63 articles were reviewed. Finally, nine studies were included for quantitative synthesis (Figure 1).

We included 1039 patients from six studies for the analysis of histological response and 591 patients from four studies for the analysis of histological remission (Tables 1 and 2). The studies were from various regions, with three from Asia,[19–21] one from North America,[22] and five from Europe.[23–27] Eight studies were designed prospectively. The mean or median age of patients varied from 42 to 50 years. The FC assay differed between studies. The median FC levels were between 56.2 to 91 µg/g in patients with histological response or remission. The cut-off value of FC ranged from 50 to 237 µg/g. The interval between fecal sampling and endoscopy was within 14 days in all studies except one,[23] which did not report the maximal interval (the FC sampling time in this study was before bowel preparation or at least 5 days after endoscopy to avoid false positive results, with unchanged therapy).

Quality assessment
The methodological quality assessment and risk of bias are summarized in Figure 2. All studies had low applicability concerns. In one study,[24] the bias of patient selection was unclear owing to incomplete reporting on consecutive patient enrollment. The bias risk of the index test was high in four studies because they did not have a pre-specified FC cut-off and used the optimal cut-off value from the receiver operating characteristic curve.[19,22,23,27] FC test was blinded to the histological result in one study,[20] and the other studies did not report this blind method. In five studies,[19–23] the risk of bias in the reference standard was unclear because of unclear blinding to the FC results. Although seven studies did not include all patients in the analysis of histological activity owing to the existence of other outcomes, the majority of overall patients (91%,[21] 97%,[22]
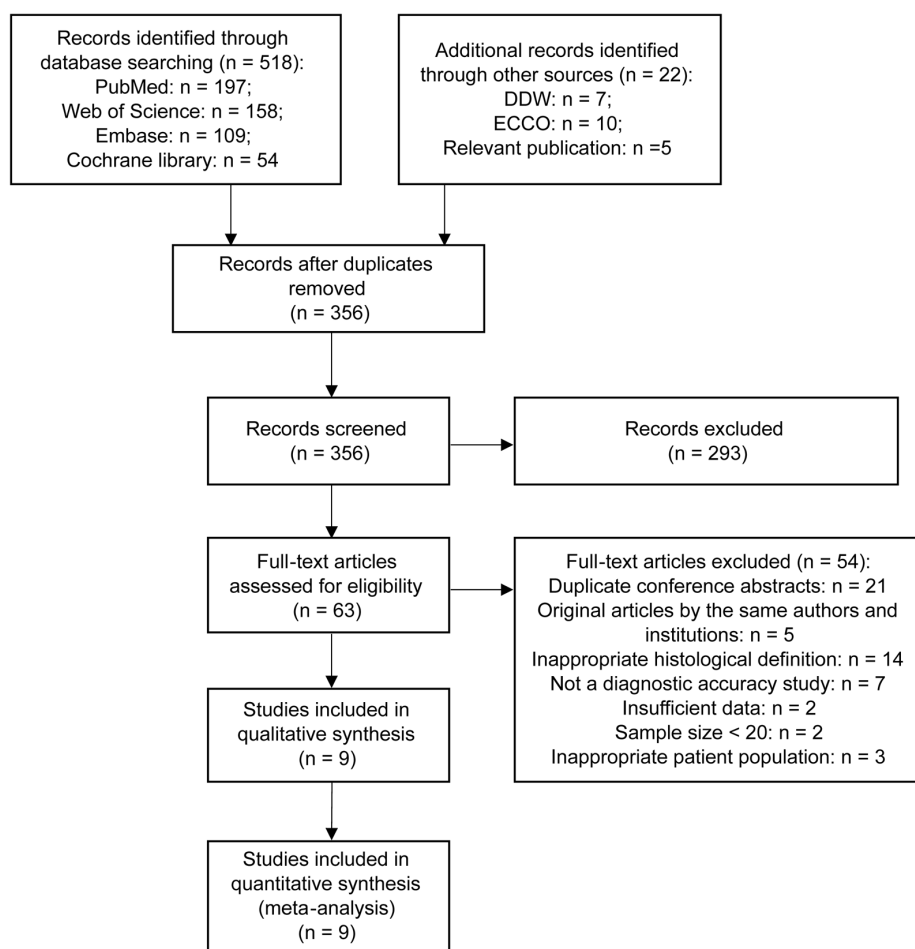
Figure 1. Flow chart for the study selection procedure following the statement of PRISMA.
DDW, Digestive Disease Week; ECCO, European Crohn's and Colitis Organization; PRISMA, preferred reporting items for systematic review and meta-analysis.

Table 1. General characteristics of all included studies.

Reference	Region	Publication year	Study design	Sex: male	Age	Endoscopic activity: MES ≤ 1
Cannatelli et al.[23]	UK	2020	Prospective	50%	44 (mean)	62%
Hart et al.[22]	Canada	2020	Prospective	53%	48 (mean)	86%
Kawashima et al.[19]	Japan	2020	Prospective	57%	47 (median)	100%
Langhorst et al.[24]	Germany	2019	*Post hoc*	40%	45 (mean)	Not reported
Magro et al.[25]	Portugal	2017	Prospective	47%	47 (median)	96%
Magro et al.[26]	Portugal	2020	Prospective	47%	45 (mean)	78%
Sagami et al.[21]	Japan	2020	Prospective	74%	42 (median)	46%
Shi et al.[20]	China	2017	Prospective	48%	50 (median)	71%
Walsh et al.[27]	UK	2019	Prospective	50%	46 (median)	UCEIS ≤1: 40%

MES, Mayo endoscopic score; UCEIS, ulcerative colitis endoscopic index of severity; UK, United Kingdom.

Table 2. Summary of results of fecal calprotectin in included studies.

Reference	FC assay	FC value (histological response / remission vs activity)	FC cut-off value (µg/g)	Intervals between FC and endoscopy	Definition Histological response	Histological remission	No. of patients	TP	FP	TN	FN
Cannatelli et al.[23]	ELISA (Buhlmann)	68.7 vs 810.9 (mean)	172	Before bowel preparation or at least 5 days after endoscopy	NI ⩽1		76	27	13	35	1
Hart et al.[22]	ELISA (Buhlmann)	79.5 vs 148.5 (median)	135	2 days	GS < 3.1		180	54	25	55	46
Kawashima et al.[19]	Not reported	56.2 vs 118.1 (median)	82.7	Within 3 days		GS < 2.0	74	29	12	22	11
Langhorst et al.[24]	Not reported	Not reported	50	Within 7 days	NI ⩽ 1		228	68	62	82	16
Magro et al.[25]	QB (Buhlmann)	81.5 vs 231.0 (median)	100	Within 24 h	GS <3.1		364	157	15	63	129
Magro et al.[26]	QB (Buhlmann)	73.5 vs 510.0 (median)	237	Within 24 h		RHI ⩽3[a]	339	178	38	70	53
Sagami et al.[21]	Colloidal gold aggregation (Alfresa)	405 (overall median, IQR: 13 – 12517)	100	Within 10 days		GS ⩽2.0	39	9	4	21	5
Shi et al.[20]	ELISA (Inova)	40 (overall median, IQR: 6 – 242)	50	Within 3 days	NI ⩽ 1		139	64	11	40	24
			50			GS < 2.0	139	60	15	48	16
Walsh et al.[27]	Fcal test (Buhlmann)	91 vs 985 (median)	72	Within 14 days	NI ⩽1		52	12	0	27	13

ELISA, enzyme-linked immunosorbent assay; FC, fecal calprotectin; FN, false-negative; FP, false-positive; GS, Geboes score; IQR, interquartile range; NI, Nancy Index; QB, Quantum Blue; RHI, Robarts histopathology index; TN, true-negative; TP: true-positive.
[a]With subscores of 0 for lamina propria neutrophils and neutrophils in the epithelium and without ulcers or erosions.
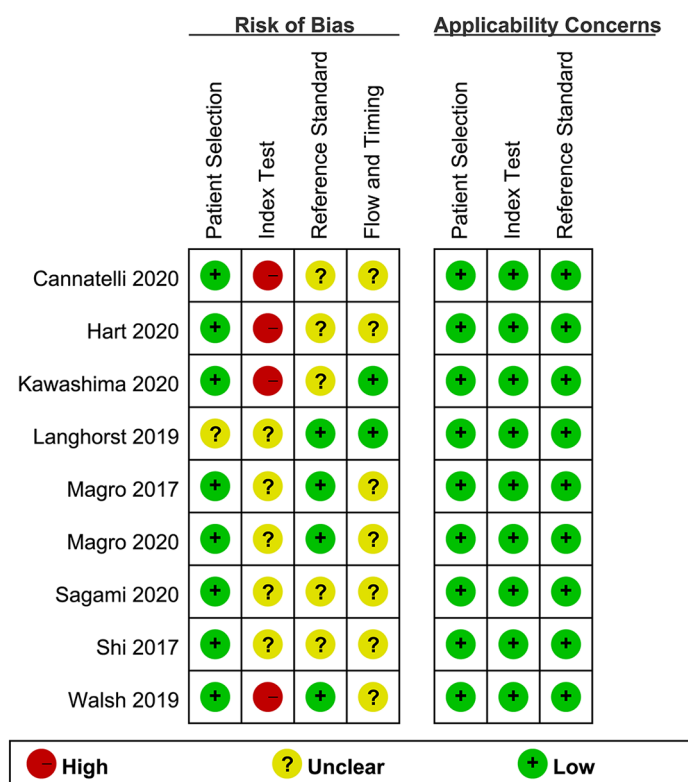
Figure 2. Summary of the methodological quality of the included studies. Assessed by the Quality Assessment of Diagnostic Accuracy Studies-2.

Table 3. Meta-analysis of pooled diagnostic statistics.

Parameter	Estimate	
	Histological response (95% CI)	**Histological remission (95% CI)**
Sensitivity	0.69 (0.52–0.82)	0.76 (0.71–0.81)
Specificity	0.77 (0.63–0.87)	0.71 (0.62–0.78)
Positive likelihood ratio	3.0 (1.9–4.8)	2.6 (2.0–3.4)
Negative likelihood ratio	0.40 (0.26–0.62)	0.33 (0.27–0.42)
Diagnostic odds ratio	8 (4–16)	8 (5–12)
Area under the SROC curve	0.80 (0.76–0.83)	0.79 (0.75–0.82)

CI, confidence interval; SROC, summary receiver-operating characteristic.

98%,[25] 80%,[26] 93%,[28] 99%,[20] 79%[27]) in these studies were still included in the analysis of the FC test and histological score.

Quantitative synthesis

The primary outcome was histological response. The pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratios were 0.69 (95% CI: 0.52–0.82), 0.77 (95% CI: 0.63–0.87), 3.0 (95% CI: 1.9–4.8), 0.40 (95% CI: 0.26–0.62), and 8 (95% CI: 4–16), respectively (Table 3, Figure 3a). The area under the SROC curve was 0.80 (95% CI: 0.76–0.83) (Table 3, Figure 4a). High heterogeneity was detected in these estimates (overall
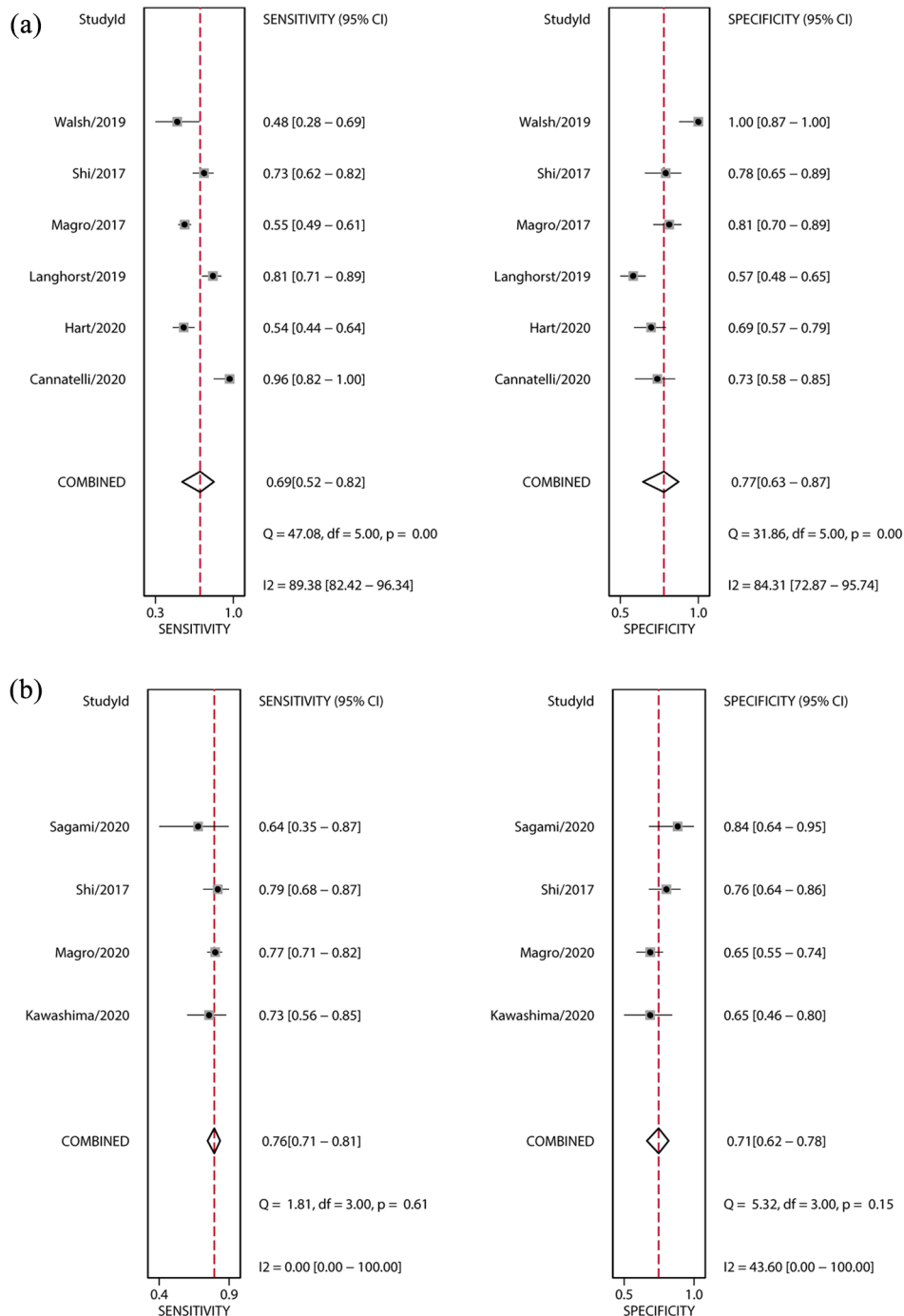
Figure 3. Forest plots of the sensitivity and specificity of FC for histological activity of UC. (a) Histological response; (b) histological remission. Bivariate mixed-effects models were applied. ■ point estimates; ◇ pooled estimates; error bars indicate 95% CI; data in parentheses are 95% CIs.
CI, confidence interval; FC, fecal calprotectin; UC, ulcerative colitis.

heterogeneity: $I^2 = 95\%$, sensitivity: $I^2 = 89\%$, specificity: $I^2 = 84\%$).

The secondary outcome was histological remission. The pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were 0.76 (95% CI: 0.71–0.81), 0.71 (95% CI: 0.62–0.78), 2.6 (95% CI: 2.0–3.4), 0.33 (95% CI: 0.27–0.42), and 8 (95% CI: 5–12), respectively (Table 3, Figure 3b). The area under
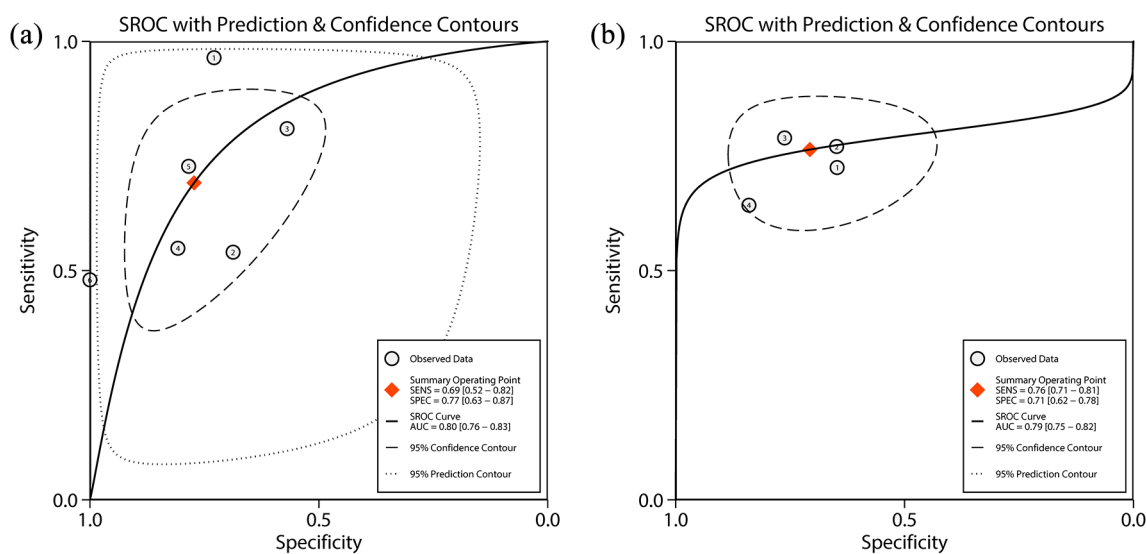
Figure 4. SROC curve plots of FC for histological activity of UC. (a) Histological response; (b) histological remission. Numbered circles represent the respective individual studies. The square represents the point estimate of pooled sensitivity and specificity. The solid line indicates the SROC curve. The dashed line and dotted line represent 95% confidence contour and 95% prediction contour, respectively.
AUC: area under the curve; FC, fecal calprotectin; SENS: sensitivity; SPEC: specificity; SRAC, summary receiver-operating characteristic; UC, ulcerative colitis.

the SROC curve was 0.79 (95% CI: 0.75–0.82) (Table 3, Figure 4b). Low heterogeneity was detected in these estimates (overall heterogeneity: $I^2=0\%$, sensitivity: $I^2=0\%$, specificity: $I^2=44\%$)

Exploration of source of heterogeneity
The squared correlation coefficient of the primary outcome indicated that 37% of heterogeneity came from the threshold effect. The study of Walsh *et al.* may have been an outlier because it selected a cut-off value to attain the highest specificity (100%) and lowest sensitivity (48%).[27]

The results of univariate meta-regression and subgroup analyses are summarized in Table 4. Two pre-specified subgroups could be combined into one subgroup because they had the identical division of studies (subgroups 6: interval between fecal sampling and endoscopy/number of patients). The sensitivity was significantly higher in studies using NI score (0.74 *versus* 0.56, $p=0.03$). The specificity was significantly lower in studies with intervals within 7 days/number of patients $\geqslant$ 100 (0.71 *versus* 0.90, $p=0.07$). The sensitivity was higher in studies with FC cut-off values >100 μg/g (0.77 *versus* 0.65, $p=0.38$), although the *p* value is not significant. The squared correlation coefficient of the secondary

outcome indicated that 100% of heterogeneity came from the threshold effect.

Sensitivity analysis
The sensitivity analysis is shown in Table 5. One article was removed each time to evaluate the impact of a single study on this meta-analysis. The outcome remained stable no matter which document was deleted. In the influence analysis (Figure 5), the study by Walsh *et al.* was an outlier.[27] After removal of this study, the summary statistics were only slightly altered.

Discussion
To our knowledge, this is the first meta-analysis to assess the accuracy of FC for evaluating histological activity in patients with UC. Assessment of the histological activity may indicate the prognosis of UC and guide treatment decisions.[29] Patients with UC in histological remission are more likely to be symptom-free and have a reduced risk of relapse, surgery, hospitalization, and colorectal cancer.[30–32] Nevertheless, invasive endoscopy and biopsy are unpleasant for patients. FC, as a non-invasive marker, correlates well with histological activity and is expected to be a surrogate marker for histological evaluation.[10] Therefore, we explored the

Table 4. Univariate meta-regression and subgroup analysis for the primary outcome (histological response).

Covariate	κ	Sensitivity (95% CI)	p value	Specificity (95% CI)	p value
Study design characteristics					
Region					
Asian	1	0.73 (0.41–1.00)	0.87	0.79 (0.54–1.00)	0.94
Western	5	0.68 (0.51–0.85)		0.77 (0.64–0.90)	
Study design					
Prospective	5	0.66 (0.50–0.83)	0.32	0.79 (0.71–0.87)	0.48
Post-hoc	1	0.81 (0.58–1.00)		0.57 (0.38–0.76)	
Histological score					
NI	4	0.74 (0.64–0.84)	0.03*	0.81 (0.64–0.98)	0.61
GS	2	0.56 (0.40–0.72)		0.75 (0.49–1.00)	
MES ⩾ 1					
⩾70%	3	0.61 (0.42–0.79)	0.11	0.76 (0.57–0.96)	0.59
<70%	3	0.77 (0.61–0.93)		0.81 (0.61–1.00)	
FC test characteristics					
FC assay					
ELISA	3	0.75 (0.57–0.94)	0.71	0.73 (0.56–0.90)	0.26
Not ELISA or unclear	3	0.62 (0.41–0.84)		0.81 (0.65–0.96)	
Intervals between fecal sampling and endoscopy/number of patients					
Within 7 days/⩾100	4	0.67 (0.46–0.87)	0.40	0.71 (0.56–0.87)	0.07*
Over 7 days or unclear/<100	2	0.80 (0.56–1.00)		0.90 (0.77–1.00)	
FC cut-off value					
⩽100 μg/g	4	0.65 (0.47–0.84)	0.38	0.80 (0.67–0.93)	0.91
>100 μg/g	2	0.77 (0.53–1.00)		0.71 (0.49–0.92)	

CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; FC, fecal calprotectin; GS, Geboes score; MES, Mayo endoscopic score; NI, Nancy Index.
*$p < 0.10$.

accuracy of FC to evaluate the histological activity of UC. For histological response, the pooled sensitivity, specificity and the area under the SROC curve were 0.69, 0.77, and 0.80, respectively. For histological remission, the corresponding estimates were 0.76, 0.71, and 0.79, respectively. The area under the SROC curve indicated that FC had a good diagnostic accuracy. High heterogeneity was detected in the primary outcome, and

low heterogeneity was detected in the secondary outcome. Then, the source of heterogeneity was explored for the primary outcome. A sensitivity analysis was further conducted to assess the robustness of the findings.

FC has been widely studied in inflammatory bowel disease for its ability to predict histological activity. It has a higher predictive value

Table 5. Sensitivity analysis for the primary outcome (histological response).

| Studies excluded	Sensitivity (95%CI)	Specificity (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)	Diagnostic odds ratio (95% CI)	Area under the curve (95% CI)
Cannatelli *et al.*[23]	0.62 (0.49– 0.74)	0.78 (0.62–0.89)	2.9 (1.7–5.0)	0.48 (0.37–0.62)	6 (3–12)	0.75 (0.71–0.79)
Hart *et al.*[22]	0.71 (0.53– 0.85)	0.79 (0.62–0.90)	3.5 (2.0–6.2)	0.36 (0.22–0.58)	10 (5–20)	0.82 (0.79– 0.85)
Langhorst *et al.*[24]	0.67 (0.47–0.83)	0.80 (0.68–0.89)	3.4 (2.0–5.7)	0.41 (0.24–0.70)	8 (3–20)	0.82 (0.78–0.85)
Magro *et al.*[25]	0.73 (0.53–0.86)	0.78 (0.58–0.90)	3.3 (1.7–6.3)	0.35 (0.20–0.62)	9 (4–24)	0.82 (0.78–0.85)
Shi *et al.*[20]	0.69 (0.48–0.84)	0.78 (0.59–0.90)	3.1 (1.7–5.8)	0.40 (0.23–0.70)	8 (3–20)	0.80 (0.77–0.84)
Walsh *et al.*[27]	0.73 (0.56–0.86)	0.71 (0.62–0.79)	2.5 (1.9–3.4)	0.38 (0.22–0.65)	7 (3–14)	0.77 (0.73–0.80)

CI, confidence interval.
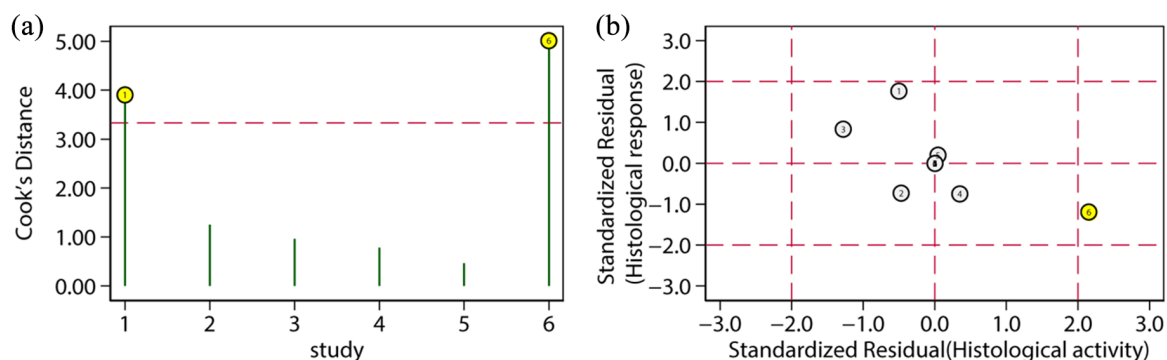
Figure 5. Plots of Cook's distance for histological response. (a) Spike plot: each study is presented as a vertical line, with its corresponding study number labeled along the *x*-axis. Studies above the dotted horizontal line are influential studies. (b) Scatter plot: Numbered circles represent the respective individual studies. Outlier studies are in yellow.

for histological remission in UC than in Crohn's disease.[33] FC also has a higher predictive value for histological remission than for endoscopic remission.[34] FC can predict histological remission in adults and relates closely to histological activity in pediatric patients with UC.[35] As for therapeutic monitoring, a low FC level showed good correlation to histological remission in patients treated with adalimumab for UC.[36] Moreover, with technique improvement, FC could also be measured at home. The FC levels obtained through home-based measurement were in agreement with the result of enzyme-linked immunosorbent assay (ELISA).[37] Therefore, FC is suitable and convenient for surveillance of histological activity in UC.

There are various histological scoring systems for UC. Neutrophil infiltration is a significant marker among these scores.[38] According to the position paper of ECCO, histological activity is defined by neutrophil infiltration of the epithelium and/or lamina propria.[4] Since FC is derived predominantly from neutrophils, it can reflect the neutrophilic granulocyte migration through the gut wall and reflect histological inflammation.[39] Histological remission is defined by more specific values considered to indicate neutrophil infiltration than is histological response, which might partly explain the higher pooled sensitivity of histological remission in this study, in addition to the threshold effect. Histological response has also been reported to have a higher cut-off value for FC than does histological remission.[40] However, a small portion of FC may also come from monocytes and macrophages rather than from neutrophils or from the proximal gastrointestinal tract due to neoplasia or infection, which

can cause bias in the interpretation of FC related to histological activity of the colon.[41]

There are numerous histological scores for UC. Although NI, RHI, and GS showed a good concordance of identifying histological activity in patients with UC,[42] different histological scores use different cut-off values of FC to identify histological activity.[26] In the subgroup analysis of histological scores, studies using NI had a significantly higher sensitivity than did studies using GS (0.74 *versus* 0.56, $p = 0.03$). Moreover, studies using NI also had a higher specificity than did studies using GS, although the p value is not significant (0.81 *versus* 0.75, $p = 0.61$). NI is a fully validated score. It is the only histological score that is recommended by ECCO for both clinical trials and observational studies.[4] Since FC correlated more closely with NI in this study, NI may be more suitable for evaluating histological activity when FC is used for disease surveillance.

The heterogeneity was also caused by the intervals between fecal sampling and endoscopy, and the number of patients. The specificity was significantly lower in studies with intervals within 7 days/number of patients $\geqslant 100$. There is no recommendation for the maximal interval between fecal sampling and endoscopy, but studies with longer or unclear intervals did not show a lower accuracy of FC than did studies with intervals within 7 days. Since studies with longer or unclear intervals also had a smaller number of patients, the difference in specificity should be carefully explained, considering both the effect of intervals and the number of patients.

We also assessed the heterogeneity of FC assays. The ELISA assay had a higher sensitivity and lower specificity than did other assays, which is not significant though. There are various FC assays from various manufacturers. While there was a qualitative correlation between assays from different manufacturers, quantitative agreement was reported to be suboptimal.[41] In a previous study, different FC assays had obviously different sensitivity and specificity in distinguishing histological activity of UC when using the same cut-off values.[42]

The cut-off values of FC ranged from 50 to 172 μg/g in the analysis of histological response. Usually, FC levels less than 50 μg/g indicate normal or remission while FC levels over 200 μg/g indicate abnormality or inflammation. Uncertainty remains in patients who have FC levels of between 50 and 200 μg/g. Raising the cut-off value to 100 μg/g would have little effect (4%) on specificity but much more (14%) on sensitivity for non-inflammatory bowel disease.[43] Therefore, we also explored whether raising the cut-off value to over 100 μg/g would get a better outcome. Studies with cut-off values >100 μg/g had a higher sensitivity than did studies with cut-off values $\leqslant 100$ μg/g (0.77 *versus* 0.65). Because the threshold effect also explained a small source (37%) of heterogeneity, the specificity decreases as the cut-off value increases. Studies with cut-off values >100 μg/g had a correspondingly lower, but acceptable, specificity (0.71 *versus* 0.80). Considering the range of FC cut-off values and the balance between sensitivity and specificity in this study, one should consider 100–200 μg/g for the cut-off value of FC. A higher sensitivity can identify more patients in histological response, which can spare these patients from unnecessary endoscopy and biopsy,[25] but the exact cut-off value should be further decided according to the prevalence of histological activity and different FC assays.

The robustness of the results was assessed in the sensitivity analysis. The summary statistics changed only slightly no matter which study was removed, which suggested that the results were stable and reliable.

This study has several limitations. First, both histological scores and FC assays were inconsistent among studies, which might have increased the risk of bias of the index test and reference standard. Second, blinding is important for diagnostic accuracy, but most studies did not report whether blinding was adopted for the index test and reference standard, thereby increasing the risk of measurement bias. Third, individual patient characteristics (such as age and medication usage), sampling time, and laboratory storage conditions varied among studies. Heterogeneity could not be eliminated completely. Furthermore, the likelihood ratio was small, indicating a small change in the probability of the pretest rather than a large change.[44] Thus, FC should be interpreted carefully and cannot completely replace endoscopy and biopsy. However, FC is still an acceptable and valuable biomarker for the continuous surveillance of histological activity.

Conclusion

FC has a good diagnostic accuracy for histological response and remission in patients with UC. A cut-off value of 100–200 µg/g is more appropriate to identify patients in histological response and spare patients from unnecessary endoscopy and biopsy. The exact cut-off value should be further decided according to the prevalence of histological activity and different FC assays. Further studies are required to confirm these findings.

Authors Contributions

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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