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Systematic review with meta-analysis: Diagnostic performance of faecal calprotectin in distinguishing inflammatory bowel disease from irritable bowel syndrome in adults

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Summary

Background: Symptoms of inflammatory bowel disease (IBD) often overlap with those of irritable bowel syndrome (IBS).

Aim: To evaluate the diagnostic performance of faecal calprotectin in distinguishing patients with IBD from those with IBS

Methods: We searched MEDLINE, Embase, Scopus, and Cochrane Library databases up to 1 January 2023. Studies were included if they assessed the diagnostic performance of faecal calprotectin in distinguishing IBD from IBS (defined according to the Rome criteria) using colonoscopy with histology or radiology as reference standard in adults. We calculated summary sensitivity and specificity and their 95% confidence intervals (CI) using a random-effect bivariate model. The risk of bias was assessed using the Quality Assessment of Diagnostic Accuracy Studies II.

Results: We included 17 studies with a total of 1956 patients. The summary sensitivity was 85.8% (95% CI: 78.3–91), and the specificity was 91.7% (95% CI: 84.5–95.7). At a prevalence of IBD of 1%, the negative predictive value was 99.8%, while the positive predictive value was only 9%. Subgroup analyses showed a higher sensitivity in Western than in Eastern countries (88% vs 73%) and at a cut-off of ≤50 μg/g than at >50 μg/g (87% vs. 79%), with similar estimates of specificity. All studies were at "high" or "unclear" risk of bias.

Conclusions: Faecal calprotectin is a reliable test in distinguishing patients with IBD from those with IBS. Faecal calprotectin seems to have a better sensitivity in Western countries and at a cut-off of ≤50 μg/g.

As part of AP&T's peer-review process, a technical check of this meta-analysis was performed by Dr Y Yuan. The Handling Editor for this article was Professor Richard Gearry, and it was accepted for publication after full peer-review.

1 | INTRODUCTION

The inflammatory bowel disease (IBD) is a disorder that includes two forms of chronic intestinal inflammation: ulcerative colitis (UC) and Crohn's disease (CD).[1] The incidence of IBD has increased over the last decades leading to a substantial social and economic burden on governments and health systems in the coming years.[2,3] Irritable bowel syndrome (IBS) is a disabling functional bowel disorder characterised by recurrent abdominal pain associated with a change in stool frequency or form.[4] The global prevalence of IBS is 11%, with all regions of the world suffering from this disorder at similar rates, causing a significant burden to healthcare systems worldwide.[5] There is considerable overlap between symptoms in patients with IBD and IBS, including abdominal pain and diarrhoea; thus, it is not always possible to distinguish between the two conditions based solely on symptoms.[6] Endoscopy with histopathological sampling is often considered indispensable in the investigation of patients with suspected IBD, but for many patients, endoscopy with the necessary bowel preparation is uncomfortable,[7] and in most cases it will result negative.[8]

Faecal calprotectin has been proposed as a non-invasive test that may help distinguish patients with IBD from those with IBS. Calprotectin is a stool marker of inflammation derived from neutrophils and released into the gut during inflammation[9] that can be measured with several commercially available methods.[10,11] This test could play a relevant role in clinical practice reducing the number of unnecessary endoscopic procedures.[12] However, the diagnostic reliability of faecal calprotectin in identifying patients with IBD among those with IBS remains uncertain, especially in adults. Several studies provided estimates of sensitivity and specificity that ranged from 50% to 100%, and in addition, most of them had a case–control design with a high risk of selection bias. Finally, not all studies used the Rome criteria to define IBS. Only two previous meta-analyses[13,14] assessed the diagnostic performance of faecal calprotectin in discriminating IBD from IBS; the former[13] published in 2007 showed a low sensitivity and specificity ≤80% in adults, while the latter reported negative and positive predictive values, but not the estimates of sensitivity and specificity. On the other hand, two further meta-analyses included patients with other gastrointestinal organic diseases and this may have biased the estimates of sensitivity and specificity in discriminating patients with IBD from those with IBS.[15,16] Clarifying the diagnostic performance of this test is essential for its use in patients with IBS-like symptoms, to distinguish between those who do and do not need endoscopic and radiologic investigations.

We carried out a systematic review with meta-analysis to evaluate the diagnostic performance of faecal calprotectin in distinguishing adult patients with IBD from those with IBS defined according to the Rome criteria.

2 | METHODS

We performed a systematic review with a meta-analysis following the recommendations of the Cochrane Collaboration's Diagnostic Test Accuracy Group.[17]

2.1 | Search strategy and study selection

We searched MEDLINE via PubMed, Embase via Embase.com, Scopus, and the Cochrane Library databases up to 1 January 2023. The electronic search of the literature was performed using the following keywords: "calprotectin", "fecal calprotectin", "inflammatory bowel disease", "IBD", "enteritis", "colitis", "Crohn", "irritable bowel syndrome", "IBS". The search strategies are reported in Appendix S1. We did not restrict language or publication status. Two authors (ED and MS) did the initial selection based on titles and abstracts. Subsequently, they independently performed a detailed full-text assessment of potentially relevant studies, with any disagreement resolved through discussion or arbitration by a third reviewer (RMZ).

Selected studies were included in the review if they met the following pre-specified criteria: diagnostic studies evaluating the accuracy of faecal calprotectin in distinguishing IBD from IBS in adults using colonoscopy with histology or radiology of the small bowel for diagnosis of IBD[18] as reference standard, and the Rome criteria for the diagnosis of IBS. In particular, in cohort studies the diagnosis of IBD was based on colonoscopy and histology with or without radiology, while in case–control studies it could be based on radiology of small bowel only. We excluded studies that did not meet the inclusion criteria or if essential information, including data for construction of a 2×2 table, was missing and could not be obtained by the authors.

2.2 | Data extraction and risk of bias assessment

Two authors (ED and MS) extracted independently the following items from each study, when available: study design, country, inclusion and exclusion criteria, total number of participants, average age and number of males, number of patients with IBD, UC, CD, and IBS, the Rome criteria for IBS, faecal calprotectin assay and cut-off value, reference standard, and data for construction of a 2×2 table. When multiple articles for a single study were found, the latest publication was considered and supplemented, if necessary, with data from the previous publications.

Two authors (ED and LF) independently assessed the risk of bias and concerns regarding applicability to the review question of the included studies using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool (Appendix S2).[19] We evaluated the presence of potential bias in four domains: patient selection, index test, reference standard, and flow and timing. Any disagreement was resolved through discussion and, if necessary, arbitration by a third reviewer (RMZ).

2.3 | Statistical analysis

The primary outcome was the diagnosis of IBD regardless of the type of disease. The secondary outcome was the diagnosis of UC and CD, separately.

We created 2×2 tables that contained the number of cases found to be true positives (subjects with positive faecal calprotectin who had IBD), true negatives (subjects with negative faecal calprotectin who did not have IBD), false positives (subjects with positive faecal calprotectin who did not have IBD), and false negatives (subjects with negative faecal calprotectin who had IBD). Using 2×2 tables, we calculated sensitivity and specificity with 95% confidence interval (CI) for each study and created coupled forest plots for each set of data. We calculated summary estimates of sensitivity, specificity, and positive and negative likelihood ratios (LRs) using a random-effect bivariate model, and we fit a summary hierarchical receiving-operating characteristic (HSROC) curve. We used the summary estimates of sensitivity and specificity to estimate negative and positive predictive values based on the median prevalence (pre-test probability) of IBD across the studies and the prevalence of 1% and 5% as the prevalence of IBD in primary and secondary care settings. We selected these prevalence values based on previous reports.[16]

We explored heterogeneity between studies through visual examination of the forest plot and the prediction region in HSROC space.[17] We planned to explore sources of heterogeneity adding them as covariates, if appropriate, to the bivariate regression model: geographic region (West vs East), study design (cohort vs case–control studies), study period (before 2010 vs. after 2010), number of participant centres (monocentre vs multicentre studies), inclusion of teenagers (no vs yes), inclusion of a subgroup of patients with non-active IBD (no vs yes), the Rome criteria (Rome I–II vs Rome III–IV), exclusion of celiac disease for the diagnosis of IBS (no vs yes), type of assay (ELISA vs immunochromatographic assay) and cut-off of faecal calprotectin (≤50 vs. >50 μg/g), and risk of bias (low risk vs high risk or some concerns) in the study. We performed subgroup analyses for any covariates that showed a statistically significant association with summary estimate of accuracy. In addition, we performed separate meta-analyses to assess the performance of faecal calprotectin in distinguishing UC and CD from IBS.

We used Cook's distance to check for particularly influential studies and produced a scatter plot of the standardised level 2 residuals to check for outliers.[20] Cook's distance is a measure of the influence of a study on the diagnostic accuracy parameters and can be used to check for influential studies that may distort the pooled estimates of sensitivity and specificity in diagnostic accuracy meta-analyses. Standardised level 2 residuals give some insight into why suggesting if outliers influence pooled sensitivity, specificity, or both.

We did not investigate publication bias as tests for publication bias and standard funnel plot are not recommended in meta-analysis of diagnostic test accuracy studies. All analyses were performed with STATA version 16 (StataCorp).

3 | RESULTS

The electronic search identified 7555 records after duplicates were removed, of which 80 full-text articles were assessed for eligibility. Of the 80 articles, 17 met the inclusion criteria and were included in the meta-analysis.[21–37] Figure 1 shows the flow chart of references through the selection process and the reasons for study exclusion.

3.1 | Study characteristics

The 17 included studies involved a total of 1956 participants. Of these, 1083 (55.4%) had a diagnosis of IBD, including 585 with UC and 498 with CD, and 873 had IBS. All studies, but three that included a subgroup of teenagers,[21,23,27] enrolled only adult patients. Eleven studies were conducted in Europe,[21–28,30,31,34] four in Asia,[32,33,35,37] one in the USA,[36] and one in Africa.[29] Twelve studies were performed in a single centre,[22–26,29–31,33,34,36,37] while five were multicentre studies.[21,27,28,32,35] Three studies[31,34,36] included also patients with indeterminate colitis, but we were able to exclude these patients from the 2×2 table. Nine studies enrolled only patients with active IBD,[21,23,24,29–34] while eight studies[22,25–28,36,37] enrolled both patients with active IBD and non-active IBD.

The diagnosis of IBS was based on the Rome II criteria in 10 studies[21–28,30,33] and Rome III criteria in 7 studies.[29,31,32,34–37] Only 6 studies[22,23,26,28,30,31] excluded coeliac disease for the diagnosis of IBS. Regarding the index test, 13 studies measured faecal calprotectin by ELISA[21–29,31,33,35,36] and 4 studies by immunochromatographic assay.[30,32,34,37] The cut-off of faecal calprotectin was ≤50 μg/g in 10 studies[21,22,25–29,31,32,35] and >50 μg/g in 6 studies,[23,24,30,33,36,37] while it was not available in one study.[34] Table 1 shows the characteristics of the included studies.

Ten studies reported data on sensitivity and specificity of faecal calprotectin separately for UC and CD: 4 studies only for UC,[29,30,33,37] 3 studies[21,23,24] only for CD, and 3 studies for both UC and CD.[22,27,35]

Tables S1 and S2 show the results of the assessment of the risk of bias and concerns regarding applicability to the review question of the included studies. All studies were at "high risk" or "unclear risk" of bias in one or more domains. In particular, ten studies[22–25,27,30,32,34–36] were at high risk of selection bias because of their case–control design, whereas in 6 studies[23,26,27,33,34,37] the cut-off of faecal calprotectin was not pre-specified. In addition, there were unclear data for the blinding of the index test,[22,23,29,30,32,34,37] the intubation of the ileum,[24–27,30,33,34,36,37] and the time interval between index test and reference standard.[22,24,27,30,33,34,36]

3.2 | Diagnostic performance

Seventeen studies reported data on the performance of faecal calprotectin in distinguishing patients with IBD from those with IBS. Sensitivity estimates ranged from 57%[33] to 100%,[21,29,31,34] and the specificity ranged from 52%[31] to 100%.[24,26,28,30,32] Figure 2 shows the coupled forest plot of sensitivity and specificity with 95% CIs for

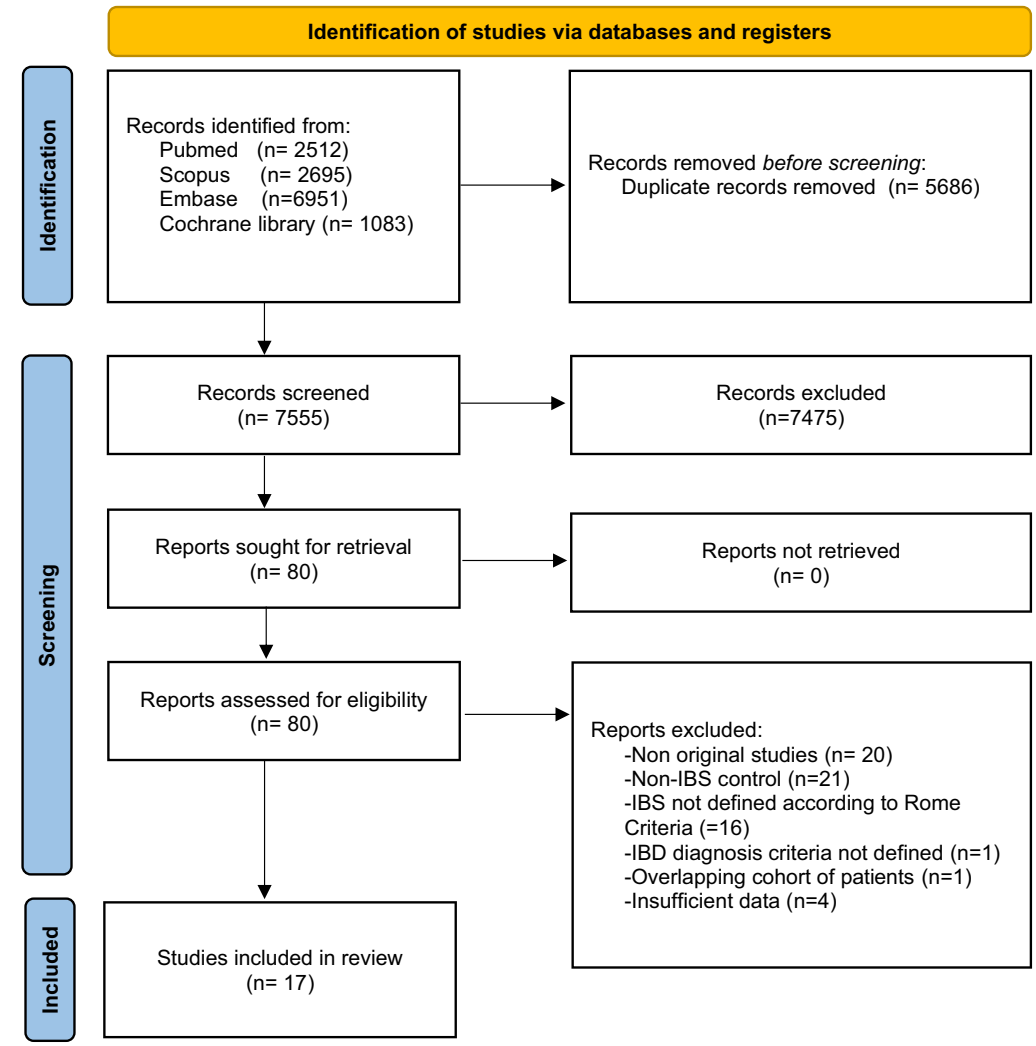

FIGURE 1 Flow chart of systematic literature search.

each included study. Pooling the results from the studies, the summary sensitivity was 85.8% (95% CI: 78.3%–91%) and the summary specificity was 91.7% (95% CI: 84.5%–95.7%); the summary positive LR was 10.3 (95% CI: 5.3–20.1) and the summary negative LR was 0.15 (95% CI: 0.09–0.24). The HSROC curve shows the summary sensitivity and specificity and the 95% confidence and prediction regions (Figure 3). Based on the median prevalence of IBD across the cohort studies of 50%, the negative predictive value was 91% (95% CI: 89%–93%) and the positive predictive value was 87% (95% CI: 84%–89%). At the pre-specified prevalence of IBD of 1% and 5%, the negative predictive value was 99.8% (95% CI: 99%–100%) and 99.2% (95% CI: 99%–100%) and the positive predictive value was 9% (95% CI: 7%–12%) and 34% (95% CI: 30%–41%), respectively.

The scatter of point estimates in the coupled forest plot (Figure 2) and the large prediction region in the HSROC space (Figure 3) showed a large heterogeneity between the studies. The meta-regression analysis showed that the geographic region ($p < 0.001$) and the cut-off value ($p < 0.001$) were possible sources of heterogeneity among the studies. After excluding the study by El-Badry[29] from Africa, the subgroup analysis by geographic region yielded a summary sensitivity of 88% (95% CI: 82%–93%) in Western countries (12 studies) and 73% (95% CI: 59%–87%) in Eastern countries (4 studies), and a summary specificity of 92% (95% CI: 86%–99%) and 91% (95% CI: 80%–100%) in Western and Eastern countries, respectively. The subgroup analysis by the cut-off value produced a summary sensitivity of 87% (95% CI: 80%–94%) for a cut-off of ≤50 μg/g (10 studies) and 79% (95% CI: 68%–91%) for a cut-off of >50 μg/g (6 studies), and a summary specificity of 92% (95% CI: 86%–99%) and 92% (95% CI: 84%–100%), respectively. The summary sensitivity of faecal calprotectin was lower in Eastern than in Western countries with both cut-off of ≤50 μg/g (East: 2 studies, sensitivity 84%, 95% CI: 68%–100% vs. West: 7 studies, sensitivity 87%, 95% CI: 79%–85%) and cut-off of >50 μg/g (East: 2 studies, sensitivity 63%, 95% CI: 55%–72% vs. West: 4 studies, sensitivity 87%, 95% CI: 81%–94%).

TABLE 1 Characteristics of the studies included.

Study, year	Study type and country	Study design	Patients (*n*)	Age range (years)	Sex, male (*n*, %)	Inflammatory bowel disease		Irritable bowel syndrome		Faecal calprotectin assay		Reference standard for inflammatory bowel disease
						Total patients (*n*)	UC/CD (*n*)	Total patients (*n*)	Rome criteria	Assay type (brand)	Cut-off value (μg/g)	
Tibble 2000[21]	Multicentre, Iceland and UK	Cohort	190	16–85	51 (26.8)	31	0/31	159	Rome II	ELISA kit (NA)	30	Endoscopy, histology, and radiology
Costa 2003[22]	Single centre, Italy	Case–control	179	20–79	111 (62)	131	82/49	48	Rome II	ELISA (Calprest, Eurospital)	50	Endoscopy, histology, and radiology
Dolwani 2004[23]	Single centre, UK	Case–control	49	16–71	11 (22.4)	25	0/25	24	Rome II	ELISA kit (Calprotech Ltd)	60	Radiology
Wassell 2004[24]	Single centre, UK	Case–control	50	25–69	11 (22)	25	0/25	25	Rome II	ELISA (Calprotech Ltd.)	90	Endoscopy and histology
Kaiser 2007[25]	Single centre, UK	Case–control	83	19–71	30 (36.1)	59	27/32	24	Rome II	ELISA (PhiCal, Calpro AS)	50	Endoscopy and histology
Schröder 2007[26]	Single centre, Germany	Cohort	76	20–75	33 (43.4)	45	20/25	31	Rome II	ELISA (Immundiagnostik)	24.3	Endoscopy and histology
Langhorst 2008[27]	Single centre, Germany	Case–control	139	15–70	39 (28.1)	85	42/43	54	Rome II	ELISA (Immundiagnostik)	48	Endoscopy and histology
Schoepfer 2008[28]	Multicentre, Switzerland	Cohort	94	20–79	40 (42.5)	64	28/36	30	Rome II	ELISA (Calpro AS)	50	Endoscopy and histology
El-Badry 2010[29]	Single centre, Egypt	Cohort	28	18–54	15 (53.6)	8	8/0	20	Rome III	ELISA (PhiCal, Nycomed)	50	Endoscopy and histology
Tursi 2011[30]	Single centre, Italy	Case–control	32	26–76	12 (37.5)	16	16/0	16	Rome II	Immunocromatographic assay (CalDetect, SOFAR)	60	Endoscopy and histology
Caviglia 2014[31]	Single centre, Italy	Cohort	38	18–78	20 (52.6)	17	5/12	21	Rome III	ELISA (Calprest, Eurospital)	50	Endoscopy and histology
Chang 2014[32]	Single centre, Taiwan	Case–control	84	20–70	N/A	58	22/36	26	Rome III	Immunocromatographic assay (Buhlmann Laboratories)	48.5	Endoscopy, histology, and radiology
Kalantari 2014[33]	Single centre, Iran	Cohort	88	18–65	50 (56.8)	44	44/0	44	Rome II	ELISA kit (N/A)	164	Endoscopy and histology
David 2015[34]	Single centre, Romania	Case–control	134	36–60	52 (38.8)	18	15/3	116	Rome III	Immunocromatographic assay (CalDetect, SOFAR)	N/A	Endoscopy and histology
Fu 2017[35]	Multicentre, China	Case–control	120	24–48	77 (64.2)	93	49/44	27	Rome III	ELISA (Buhlmann fCal kit)	50	Endoscopy, histology, and radiology
Berinstein 2019[36]	Multicentre, USA	Case–control	179	21–65	73 (40.8)	92	45/47	87	Rome III	ELISA (Buhlmann fCal kit)	160	Endoscopy and histology
Gür 2020[37]	Single centre, Turkey	Cohort	393	26–56	201 (51.1)	272	182/90	121	Rome III	Immunocromatographic assay (Calfast, Eurospital)	55	Endoscopy and histology

Abbreviations: CD, Crohn's disease; N/A, not available; UC, ulcerative colitis.

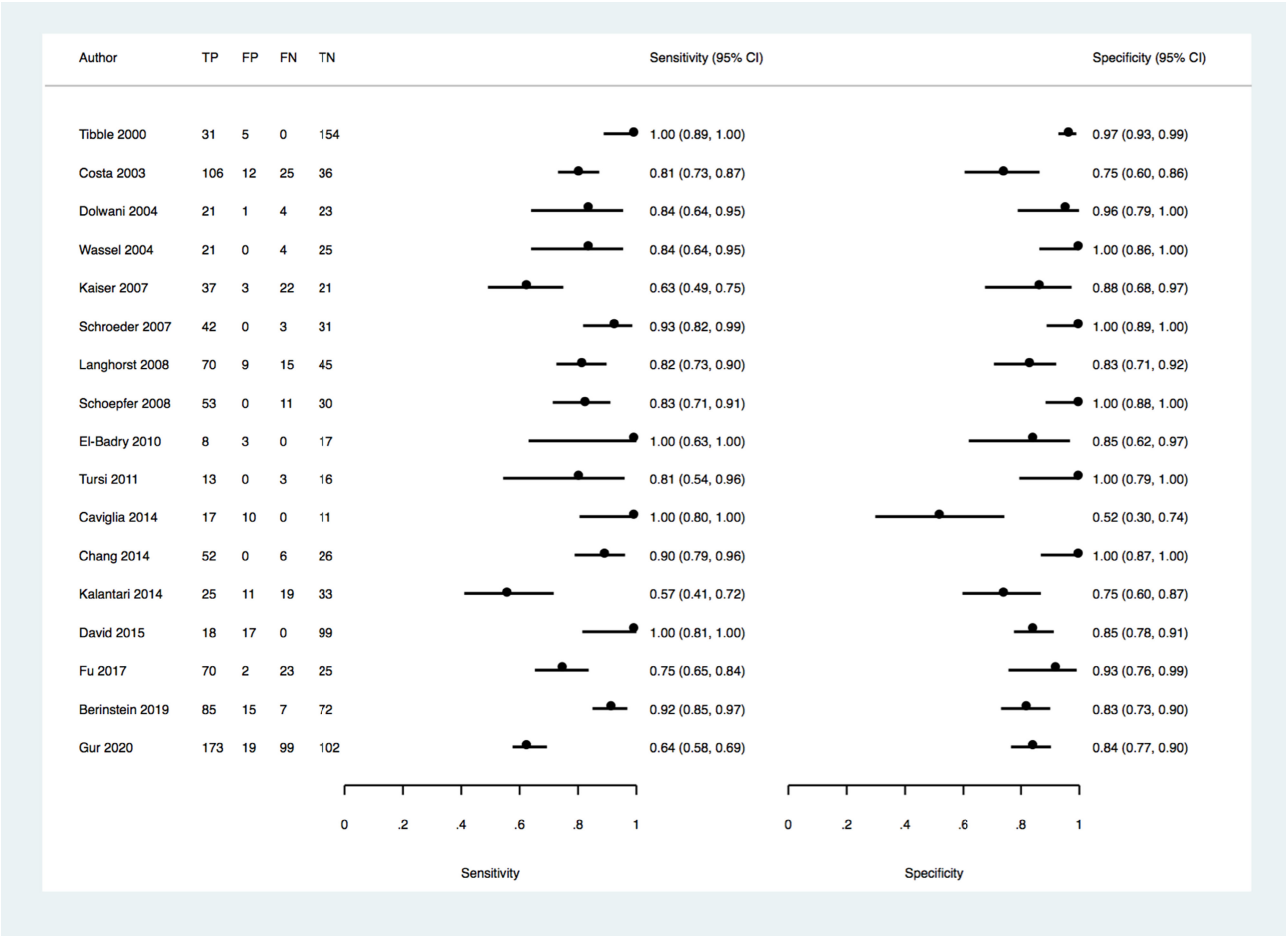

FIGURE 2 Coupled forest plot of sensitivity and specificity of faecal calprotectin for distinguishing inflammatory bowel disease from irritable bowel syndrome in each study. TP, true positive; FP, false positive; FN, false negative; TN, true negative.

Pooling the 4 studies using a cut-off value of <50 μg/g yielded a summary sensitivity and specificity of 91% (95% CI: 85%–98%) and 97% (95% CI: 92%–100%) respectively, while pooling the 6 studies that used a cut-off value of 50 μg/g produced a sensitivity and specificity of 82% (95% CI: 71%–93%) and 87% (95% CI: 74%–99%), respectively.

The performance of faecal calprotectin did not significantly vary according to the study design ($p = 0.86$), study period ($p = 0.36$), number of participant centres ($p = 0.18$), inclusion of teenagers ($p = 0.61$), inclusion of a subgroup of patients with non-active IBD ($p = 0.28$), the Rome criteria ($p = 0.15$), exclusion of celiac disease for the diagnosis of IBS ($p = 0.77$), and type of assay ($p = 0.85$) (Table S3).

After the calculation of summary estimates, we produced Cook's distance and standardised residuals to identify influential studies (Figure 4). Cook's distance showed that the study by Caviglia et al[31] could be influential, and standardised residuals reported that this study was an outlier for specificity. No clinical or methodological characteristics could explain why this study was an outlier. However, after the exclusion of the study by Caviglia et al. the sensitivity (from 85.8% to 84.6%) and specificity (from 91.7% to 92%) did not change, likely due to the very small sample size of the study.

Pooling data from the 7 studies[22,27,29,30,33,35,37] that assessed the performance of faecal calprotectin in the diagnosis of UC in a total of 753 patients, of whom 423 had UC and 330 had IBS, the summary sensitivity was 83.1% (95% CI: 63.8%–92.2%) and the summary specificity was 83.3% (95% CI: 78.6%–87.2%) (Figure 5).

Pooling data from 6 studies[21–24,27,35] including a total of 554 patients, of whom 217 had CD and 337 had IBS, the summary sensitivity and specificity for the diagnosis of CD were 92.4% (95% CI: 78.5%–97.6%) and 93.1% (95% CI: 83.3%–97.4%), respectively (Figure 6).

4 | DISCUSSION

This meta-analysis included 17 studies that assessed the diagnostic performance of faecal calprotectin in distinguishing patients with IBD from those with IBS defined according to the Rome criteria. Pooling data from these studies yielded a summary sensitivity of 85.8% and a summary specificity of 91.7%. Assuming a prevalence of IBD of 1% in primary care and 5% in secondary care, the negative predictive value of faecal calprotectin was very high, 99.8% in primary care and 99.2% in secondary care, whereas the

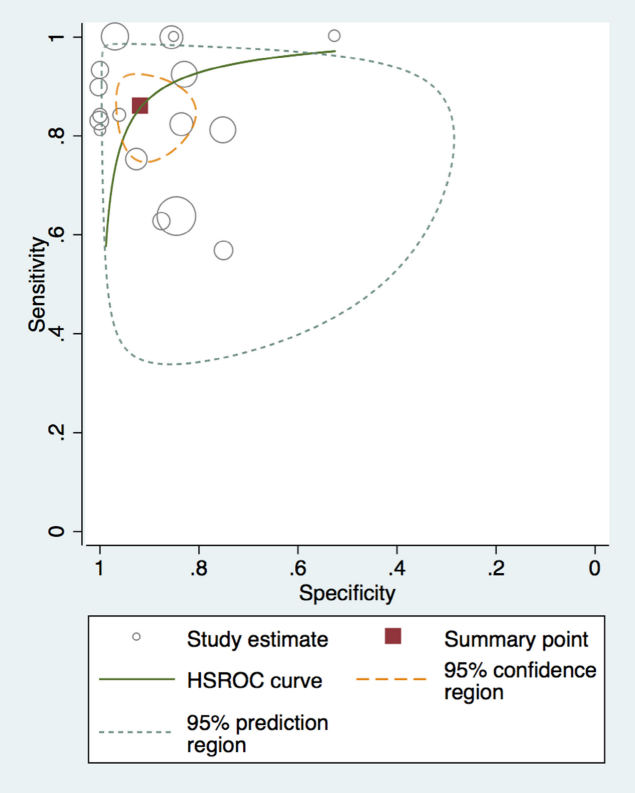

FIGURE 3 Hierarchical summary receiver-operating characteristic plot of faecal calprotectin for distinguishing inflammatory bowel disease from irritable bowel syndrome. Each circle indicates an individual study, and it is sized according to the total number of subjects; the solid spot in middle is the summary sensitivity and specificity; and the inner and outer ellipses indicate the 95% confidence region and prediction region, respectively.

positive predictive value was low, 9% and 34%, respectively. This implies that 99 out of 100 patients with a negative test result will not have IBD, whereas only 9 in primary care and 34 in secondary care of 100 patients with a positive test result will have IBD. In other words, for example, for every 1000 patients with IBS symptoms of whom 10 have IBD (prevalence of IBD = 1%), faecal calprotectin would identify 9 patients (true positive) and would miss 1 patient (false negative) with IBD; of the remaining 990 patients without IBD, 908 would be correctly identified (true negative) and 82 would be evaluated incorrectly as having IBD when they do not (false positive) (Table 2).

We found that the pooled sensitivity and specificity of faecal calprotectin were slightly higher for the diagnosis of Crohn's disease (92% and 93%) compared to ulcerative colitis (83% and 83%).

4.1 | Strengths and weaknesses of the study

A strength of this review is the comprehensive search of literature without restrictions on the language and type of publications. As there is not a powerful method for testing for publication bias in a meta-analysis of diagnostic accuracy studies,[17] we are not able to assess the likely impact of unpublished studies on our results. However, unpublished studies would require a very large sample size to change the findings of our meta-analysis. Another strength of this study is the use of a multilevel statistical approach with a bivariate model, which is recommended for meta-analysis of diagnostic accuracy studies.[17]

A weakness of our findings was the substantial heterogeneity between the studies. However, heterogeneity is a common place in meta-analysis of diagnostic test accuracy studies. The meta-regression analysis showed that the geographic region and the cut-off value of faecal calprotectin were sources of heterogeneity between the studies. The subgroup analysis based on geographic region showed that the sensitivity of faecal calprotectin was higher in Western than in Eastern countries (86% vs. 73%). This finding may be partially due to diversity in genetic and environmental factors and disease characteristics between Western and Eastern populations that may possibly influence the diagnostic performance of faecal calprotectin.[35,38] For example, Asian patients with IBD showed a higher proportion of proctitis among those with UC and perianal disease among those with CD than Caucasians,[38] which may partially affect the sensitivity of faecal calprotectin in diagnosing IBD. In addition, in our study population CD was less frequent in Eastern (36.4%, 170 CD and 297 UC) than in Western countries (53.9%, 328 CD and 280 UC), and this may contribute to the lower sensitivity of faecal calprotectin in Eastern countries. However, our result should be considered with caution as only 4 studies were carried out in Eastern countries, and in addition, possible problems with confounding factors cannot be excluded.

We also found that the cut-off of ≤50 μg/g provided a better sensitivity than the cut-off of >50 μg/g, with similar specificity. Previous meta-analyses provided inconsistent results on the best cut-off of faecal calprotectin for the diagnosis of IBD. The meta-analysis by Von Roon reported that the cut-off of 100 μg/g was better than that of 50 μg/g [13] for differentiating patients with IBD from those with IBS, while the meta-analysis by Menees[14] showed a lower negative predictive value with a cut-off of 50 μg/g in comparison with 100 μg/g. Our finding suggests that the cut-off of ≤50 μg/g could provide the best combination of sensitivity and specificity in differentiating patients with IBD from those with IBS. The cut-off value did not affect the difference in the sensitivity of faecal calprotectin between East and West.

Another limitation of our study is the inclusion of patients with both active and non-active IBD and the inclusion also of teenagers. However, meta-regression analyses showed that the inclusion of studies with a subgroup of patients with non-active IBD or teenagers did not significantly affect the estimates of sensitivity and specificity.

Unfortunately, only a few studies with a small sample size assessed the performance of faecal calprotectin for the diagnosis of UC or CD, separately. With this limitation, we found that sensitivity and specificity of faecal calprotectin were slightly higher for CD than UC. Indeed, 95% confidence intervals were quite large suggesting

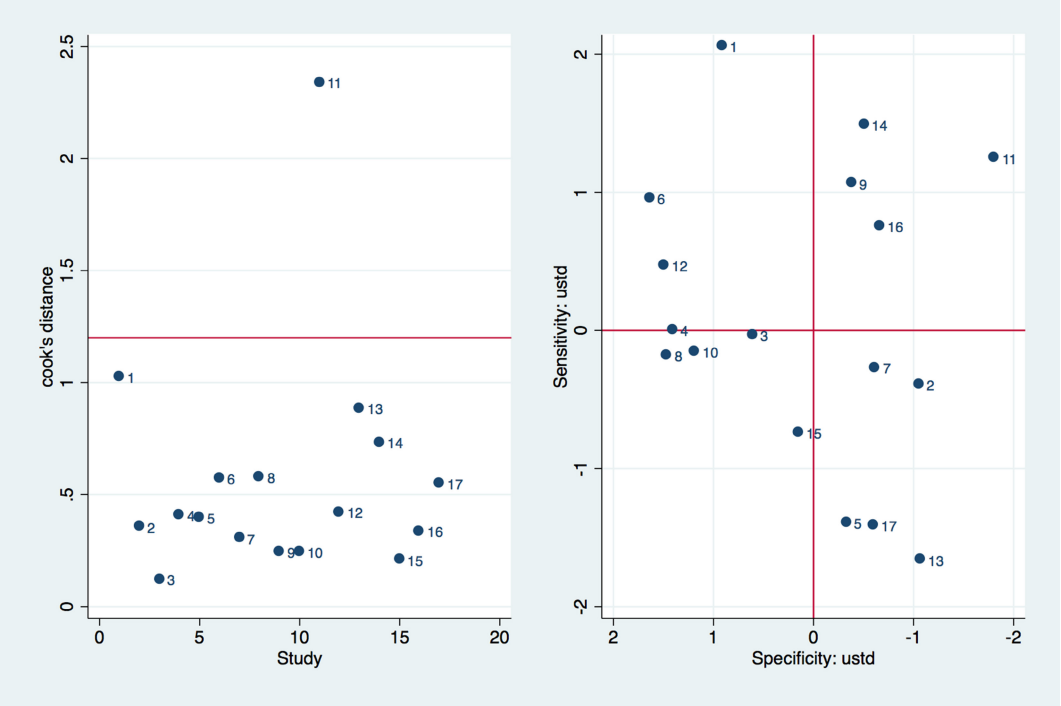

FIGURE 4 Influence analysis. Left panel: Cook's distance. Cut-off for declaring Cook's distance to be large = 1.2 (20, four times the number of parameters of the model (*n*: 5: sensitivity, specificity, variance of sensitivity, variance of specificity, variance correlation)/17, number of studies). Right panel: standardised residuals (standardised predicted random effects). ustd, standardised residuals. 11 = Caviglia.

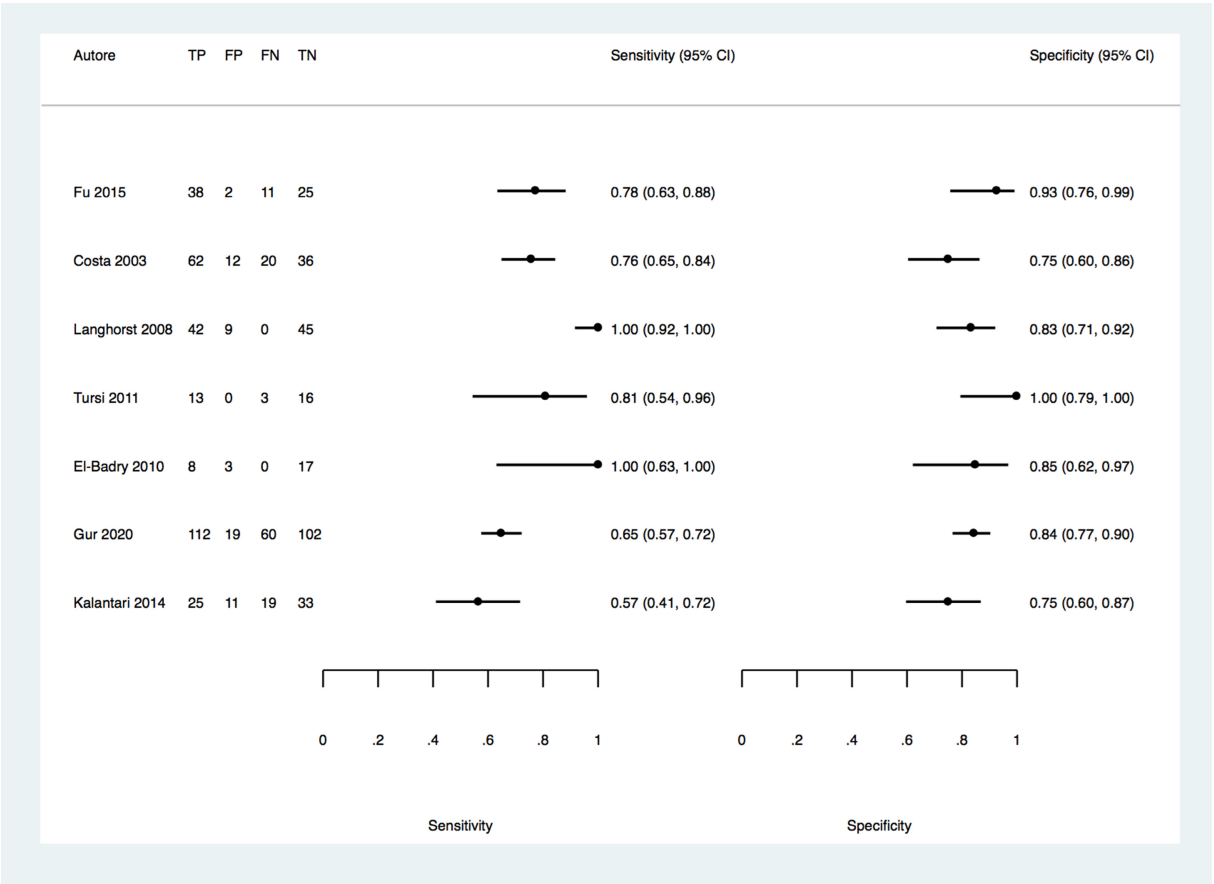

FIGURE 5 Coupled forest plot of sensitivity and specificity of faecal calprotectin for distinguishing ulcerative colitis from irritable bowel syndrome in each study. TP, true positive; FP, false positive; FN, false negative; TN, true negative.

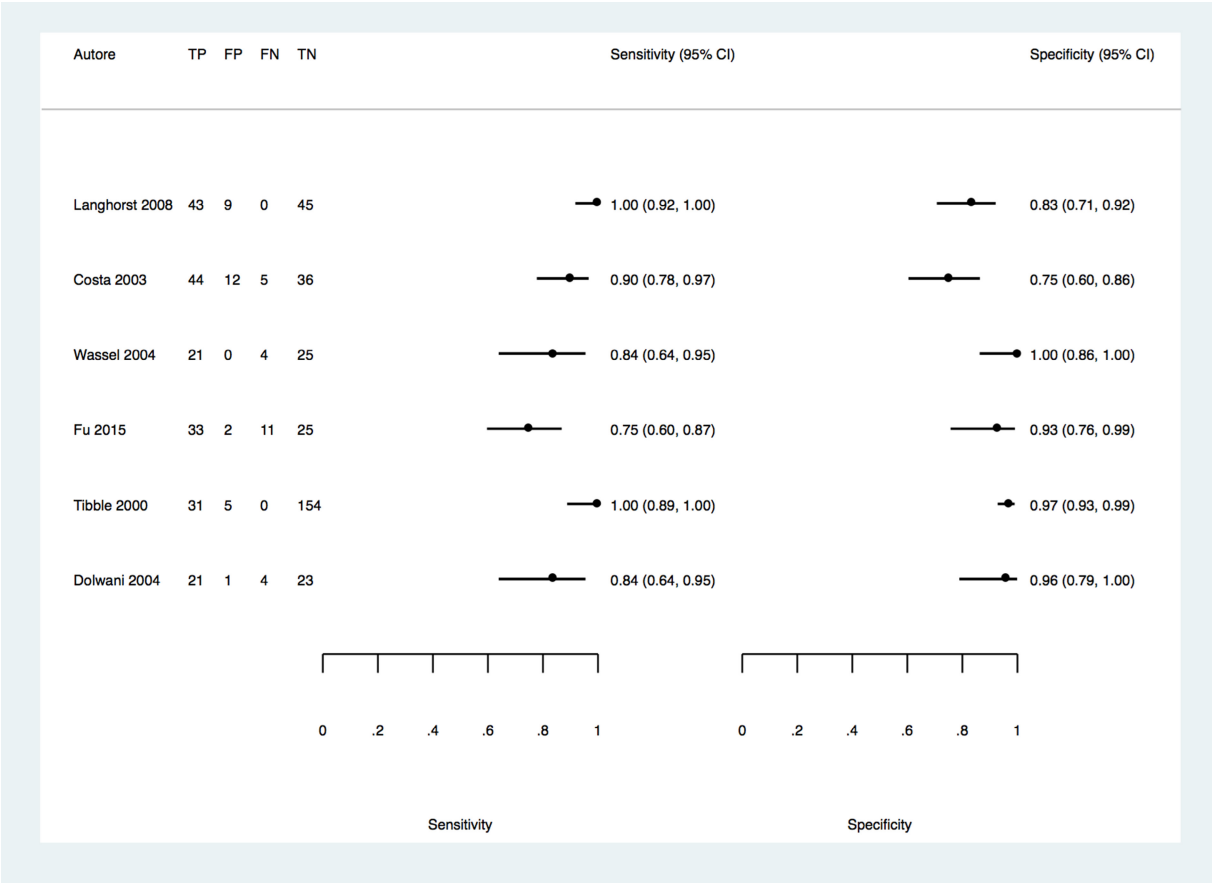

FIGURE 6 Coupled forest plot of sensitivity and specificity of faecal calprotectin for distinguishing Crohn's disease from irritable bowel syndrome in each study. TP, true positive; FP, false positive; FN, false negative; TN, true negative.

TABLE 2 Consequences of summary sensitivity and specificity of faecal calprotectin in 1000 tested patients with IBS symptoms.

	(*n*) 1000 with IBS symptoms	
	Primary care	**Secondary care**
Prevalence of IBD	1%	5%
Patients with IBD, *n*	10	50
Patients with IBS, *n*	990	950
Test result		
True positive	9	43
True negative	908	871
False negative	1	7
False positive	82	79

Abbreviations: IBD, inflammatory bowel disease; IBS, irritable bowel syndrome.

uncertainty in the pooled estimates of sensitivity and specificity for both CD and UC. However, our result is in line with a previous study that reported higher calprotectin levels in patients with CD than in those with UC.[13] Differences in the performance of faecal calprotectin might also be related to disease-specific differences in the localisation and extension of the inflammatory activity.

Another weakness of our meta-analysis is the low methodological quality of included studies. Most studies were case-controls and, thus, at high risk of selection bias. In addition, in about half of the studies the cut-off value of faecal calprotectin was not pre-specified, and it was unclear if the index test was performed in a blinded fashion. Most studies did not clearly state if patients underwent ileocolonoscopy, that is, if there was the intubation of the ileum, which may have introduced a misclassification bias. Finally, some studies did not report the time interval between stool sampling and endoscopy. If the interval was too long, a medical treatment or unknown factors may have affected faecal levels of calprotectin biasing the test results.

4.2 | Comparison with other studies

To our knowledge, this is the first systematic review with meta-analysis to estimate the summary sensitivity and specificity of faecal calprotectin in distinguishing patients with IBD from those with IBS using (a) a comprehensive literature search, (b) only patients with IBS as controls, (c) the Rome criteria for the diagnosis of IBS, and d) a multilevel statistical approach for meta-analysis. Two previous meta-analyses by Von Roon et al[13] and by Menees et al[14]

assessed the performance of faecal calprotectin in discriminating IBD from IBS. However, in the meta-analysis by Von Roon et al the control group included also healthy people in addition to patients with IBS. Furthermore, the analysis was performed using the traditional statistical model that consists of pooling sensitivities and specificities across the included studies, rather than the multilevel statistical approach that accounts for the correlation between sensitivity and specificity and provides less biased estimates of performance measures.[39] The meta-analysis by Von Roon et al reported in adults a sensitivity and specificity of 71% and 80%, respectively, while we found a higher sensitivity (85%) and specificity (91%) of faecal calprotectin in distinguishing patients with IBD from those with IBS. On the other hand, the meta-analysis by Menees et al provided negative and positive predictive values, but not the estimates of sensitivity and specificity of faecal calprotectin.[14] Indeed, sensitivity and specificity are important measures to calculate negative and positive predictive values of a diagnostic test in settings or populations with different prevalence of the target disease. Furthermore, because of the statistical method used to calculate predictive values, that is, Bayes' theorem, this meta-analysis was limited to using only studies that reported faecal calprotectin as median with interquartile ranges, thus excluding several other studies from the analyses. Finally, the old Manning criteria for the IBS diagnosis were also permitted in this review. However, the authors reported a high negative predictive value (<1% probability of having IBD at a prevalence of IBD of 1%) and a low positive predictive value of faecal calprotectin for distinguishing IBD from IBS, and our meta-analysis is in line with these findings.

Two further meta-analyses by van Rheenen et al[15] and Carrasco-Labra et al[16] reported the performance of faecal calprotectin for the diagnosis of IBD. However, both meta-analyses differ from our study as they also include patients with other gastrointestinal organic diseases. The meta-analysis by van Rheenen et al assessed the performance of faecal calprotectin in distinguishing patients with IBD from those with "no IBD," where the "no IBD" group contained a large spectrum of organic diseases (i.e., celiac disease, chronic infection, microscopic colitis, and colon cancer) in addition to IBS. This analysis reported sensitivity and specificity of faecal calprotectin of 93% and 96%, respectively. However, it is well known that the range of differential diagnoses present in non-diseased populations will affect the accuracy of the test.[40] Thus, this meta-analysis may have slightly overestimated the performance of faecal calprotectin in distinguishing patients with IBD from those with IBS. On the other hand, the meta-analysis by Carrasco-Labra et al evaluated the performance of faecal calprotectin in distinguishing patients with organic diseases, including IBD, from those with IBS reporting a sensitivity and specificity of faecal calprotectin of 81% and 87%, respectively.[16] Although most patients with organic diseases included in the meta-analysis had IBD, the presence of other organic diseases may have contributed to slightly underestimate the sensitivity of faecal calprotectin in detecting patients with IBD among those with IBS.

5 | CONCLUSIONS AND IMPLICATIONS

The results of our meta-analysis show that faecal calprotectin is a very reliable test in distinguishing patients with IBD from those with IBS symptoms defined according to the Rome criteria. Faecal calprotectin is a very powerful test for excluding IBD; applying faecal calprotectin to all patients with IBS symptoms in primary or secondary care, only a few will suffer for a missed diagnosis of IBD with a consequent delay in the treatment. Unfortunately, due to the low positive predictive value, faecal calprotectin is not powerful for the diagnosis of IBD; however, if we consider 1000 patients with IBS symptoms of whom 10 have IBD (prevalence of IBD = 1%) and 990 have IBS, only 82 of the 900 (8.3%) patients with IBS will have a false-positive faecal calprotectin and will undergo unnecessary endoscopic and imaging investigations (Table 2); thus, we provide evidence that faecal calprotectin would help avoid further diagnostic procedures in about 90% of patients with IBS.

A novel finding of our meta-analysis is that faecal calprotectin seems to have a higher sensitivity in Western than Eastern populations (86% vs. 73%). However, the lower sensitivity does not substantially affect the high negative predictive value of faecal calprotectin in Eastern countries; in fact, at a prevalence of IBD of 1% the negative predictive value remains high (99.7%) even with a sensitivity of 73%. However, the prevalence of IBD in Eastern countries seems to be even lower than in the West.[38] We also found that the cut-off of ≤50 μg/g provided a better sensitivity than at >50 μg/g without affecting the specificity. Thus, our finding would support the use of the cut-off of ≤50 μg/g for distinguishing patients with IBD from those with IBS in adults; this would allow to optimise the test for obtaining lower false-negative results without increasing false positives.

Therefore, our study provides evidence that faecal calprotectin is a useful screening test to rule out IBD and save endoscopic and radiologic procedures in patients with IBS symptoms defined according to the Rome criteria. However, the prevalence of IBD in our study population corresponds to that in tertiary care; thus, estimates of negative and positive predictive values are applicable to both primary and secondary care settings assuming that likelihood ratios remain constant across the spectrum of care.

Moreover, well-designed high-quality studies with a sample size large enough to allow stratification of the results by cut-off levels and type, activity, and distribution of IBD are needed to confirm the performance of faecal calprotectin in distinguishing patients with IBD from those with IBS symptoms, especially in patients <50 years and without alarm features.

AUTHOR CONTRIBUTIONS

Elton Dajti: Conceptualization (supporting); data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Leonardo Frazzoni:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal);

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