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TITLE PAGE

Title: Systematic Review and Meta-Analysis: Accuracy of Diagnosing Irritable Bowel Syndrome with Symptoms, Biomarkers, and/or Psychological Markers.

Short running head: Meta-analysis: Approaches to Diagnosing IBS.

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Cg	chromogranin
CI	confidence interval
ESR	erythrocyte sedimentation rate
FC	faecal calprotectin
GI	gastrointestinal
HADS	hospital anxiety and depression scale
HC	healthy control
IBS	irritable bowel syndrome
LR	likelihood ratio
PHQ-12	patient health questionnaire-12
PHQ-15	patient health questionnaire-15

ROC receiver operating characteristics

Sg secretogranin

VOM volatile organic metabolite

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SUMMARY

Background: Irritable bowel syndrome (IBS) is a complex, heterogeneous disease which can be challenging to diagnose. No study has identified and assessed the accuracy of all available methods of diagnosing IBS.

Aims: We conducted a systematic review of the literature to identify and assess accuracy of symptom-based diagnostic criteria, biomarkers, psychological markers, or combinations thereof.

Methods: MEDLINE, EMBASE and EMBASE Classic were searched (until April 2015) to identify studies reporting accuracy of available methods to diagnose IBS in adult populations. Eligible studies assessed accuracy of these diagnostic tests against an accepted reference standard. Data were extracted to calculate positive and negative likelihood ratios, with 95% confidence intervals (CIs), of the diagnostic test utilised. Where more than one study used the same test, data were pooled in a meta-analysis.

Results: 22 studies (7106 patients) were eligible. Positive and negative likelihood ratios of the current gold standard, the Rome III criteria, were 3.35 (95% CI 2.97-3.79) and 0.39 (95% CI 0.34-0.46), similar to other symptom-based criteria. Eleven biomarkers performed no better than symptom-based criteria. Psychological markers performed well in one study. Five different combinations were assessed. The best in terms of positive likelihood ratio was faecal calprotectin, intestinal permeability, and Rome I criteria (26.4; 95% CI 11.4-61.9), and in terms of negative likelihood ratio serum-based biomarkers and psychological markers (0.18; 95% CI 0.12-0.25).

Conclusions: Symptom-based diagnostic criteria, biomarkers, and psychological markers performed modestly in predicting IBS. Combining symptoms with markers appears more effective, and may represent the way forward in diagnosing IBS.

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder characterised by abdominal pain associated with a change in stool form and/or frequency.(1) The condition has an estimated prevalence of up to 20% in Western populations,(2) and the impact of IBS socially and economically is significant, with up to 12% of IBS patients having to stop work completely. There is an estimated direct cost per patient to the US economy of between \$742 and \$7547.(3) The pathogenesis of IBS is poorly understood and, as yet, no unifying structural or physiological cause has been identified. Furthermore, making a positive diagnosis of IBS is challenging as the symptoms often overlap with those of organic GI disease such as colorectal cancer,(4) inflammatory bowel disease,(5) bile acid malabsorption,(6) and coeliac disease.(7, 8)

Symptom-based diagnostic criteria were developed to aid in making a positive diagnosis of IBS and are the current gold standard, the latest iteration of these being the Rome III criteria.(1) However, these criteria have been criticised for being overly complex and impractical in a clinical setting, particularly in primary care where the majority of IBS is diagnosed and managed.(9, 10) Partly as a consequence of this, interest has grown in developing novel biomarkers, measurable biological characteristics including physiological responses, proteins, genes and metabolites, as a diagnostic tool.(11) Patients with IBS are more likely to have higher levels of anxiety, neuroticism, or mood instability when compared to healthy individuals and those with other lower GI disorders.(12-15) As a result, studies have also been conducted to assess whether measures of psychological wellbeing can aid in the diagnosis of IBS.

We, and others, have previously examined the accuracy of symptom-based diagnostic criteria in predicting IBS. (16, 17) However, since this meta-analysis was performed there have been more studies published, as well as the description of novel attempts to diagnose IBS. As the Rome IV process for functional GI disorders is due to report in 2016, a summary of the accuracy of all available approaches, including symptoms, biomarkers, psychological markers, and combinations of the above would seem timely. We have therefore conducted an updated systematic review and meta-analysis examining this issue.

METHODS

Search Strategy and Study Selection

The systematic review was performed according to the Cochrane Methods Group on screening and diagnostic tests guidelines.⁽¹⁸⁾ A search of the medical literature was conducted using MEDLINE (January 1946 to April 2015), EMBASE, and EMBASE classic (1947 to April 2015). Eligible studies were required to report prospectively on adult (≥ 16 years of age) patients with lower GI symptoms, and had to assess the accuracy of one or more of the available accepted symptom-based diagnostic criteria for IBS, biomarkers, psychological markers, or combinations thereof, in diagnosing IBS against an accepted reference standard, taken as being a physician's diagnosis of IBS, another set of accepted diagnostic criteria, or the absence of an organic explanation for these symptoms, such as IBD, microscopic colitis, or colorectal cancer, following lower GI endoscopy (Table 1). When assessing the accuracy of symptom-based diagnostic criteria, the reference standard was mandated as negative lower GI investigations, but when assessing the accuracy of novel biomarkers this could either be accepted symptom-based diagnostic criteria or a physician's diagnosis of IBS. We did not consider studies that applied an accepted test for organic disease, such as faecal calprotectin or lactoferrin, and therefore effectively reached a diagnosis of IBS by exclusion of the specific organic disease that the test was designed to detect, eligible for inclusion in this meta-analysis.

Search terms used to identify potentially relevant publications were: *irritable bowel syndrome, IBS, functional diseases, colon or functional adj5 bowel*. These were combined, using the set operator AND, with the following search terms: *Kruis, Manning, Rome 1, Rome I, Rome 2, Rome II, Rome3, Rome III, biomarker, f\$ecal biomarker, psychological marker,*

metabolite, transit time, colonic motility, small intestinal motility, visceral hypersensitivity, pain, bile acid, cytokine, mast cell, intestinal permeability, chromogranin or secretogranin.

These were again combined using the set operator AND with the search terms *sensitivity* or *specificity*. There were no language restrictions and abstracts of the papers identified by the initial search were evaluated by the lead author for appropriateness to the study question.

All potentially relevant papers were obtained and evaluated in detail. Foreign language papers were translated. Abstract books of conference proceedings between 2007 and 2014 were hand-searched to identify potentially eligible studies published only in abstract form.

The bibliographies of all identified relevant studies were used to perform a recursive search of the literature. Articles were assessed independently by two reviewers using pre-designed eligibility forms, according to the prospectively defined eligibility criteria. Any disagreement between investigators was resolved by consensus.

Data Extraction

All data were extracted independently by two reviewers on to a Microsoft Excel spreadsheet (XP professional edition; Microsoft Corp, Redmond, WA, USA) as total number of patients with IBS, total number of IBS patients testing positive for IBS using the diagnostic criteria, biomarker, psychological marker, or combination thereof under study, total number of non-IBS patients, and the total number of non-IBS patients testing positive for IBS using the same diagnostic test. In addition, the following clinical data were extracted for each study: setting (primary or secondary care), number of centres, country of origin, and diagnostic test applied.

Data Synthesis and Statistical Analysis

The degree of agreement between investigators, in judging study eligibility, was measured using the Kappa statistic. The accuracy of diagnostic tests is often summarised using sensitivity and specificity. Although these are useful measures of a test's performance, they provide the probability of the test being positive if the disease of interest is present, or the probability of the test being negative if the disease is absent. However, for a physician consulting with a patient it is more useful to know the probability of the patient truly having the disease if the test is positive, or truly not having the disease if the test is negative. These are known as the positive and negative predictive values of the test.

One of the limitations of positive and negative predictive values is that their magnitude varies according to the prevalence of the disease under study. For this reason, more useful summary measures of the diagnostic accuracy of a test are the positive and negative likelihood ratios (LR). These are derived from the sensitivity and specificity of a test, which are fixed, and therefore the advantage of using LRs over predictive values is that LRs do not vary to the same degree as predictive values with changes in the prevalence of the disease. (19)The positive LR is derived from the formula: $\frac{\text{sensitivity}}{1-\text{specificity}}$ while the negative LR is derived from the formula: $\frac{1-\text{sensitivity}}{\text{specificity}}$. The positive LR describes the likelihood of an individual having the disease if the diagnostic test is positive, and the negative LR the likelihood of an individual not having the disease if the test is negative. As a rule of thumb, positive LRs above 10 are very useful in ruling in a disease, and negative LRs below 0.1 are very useful in ruling out a disease. However, in rare diseases, which have a low prevalence in the population, a higher positive LR is required to cause a useful increase in the probability of disease that may result in a change in management, whereas in a disease of high

prevalence, a smaller negative LR is required to cause a useful decrease in the probability of disease that may result in a change in management.

For each study we identified in our literature search, we extracted the raw data from the paper, rather than summary statistics, in order to obtain true positives, true negatives, false positives, and false negatives, into a Microsoft Excel spreadsheet (XP professional edition; Microsoft Corp, Redmond, WA, USA), and calculated the sensitivity, specificity, positive, and negative predictive values of each of the diagnostic tests for IBS under study. The positive and negative LRs, and their 95% confidence intervals (CIs), were also calculated within the same spreadsheet, using the aforementioned formulas. Where data were not extractable, we attempted to contact the authors of the original paper in order to obtain further information.

Where the accuracy of identical symptom-based criteria, biomarkers, psychological markers, or combinations thereof, were reported by more than one study, we combined the LRs from each study using StatsDirect version 2.7.7 (StatsDirect Ltd, Sale, Cheshire, England), in order to generate pooled positive and negative LRs with 95% CIs. We also pooled results from all studies in order to obtain pooled positive and negative LRs with 95% CIs for each approach used to diagnose IBS, including symptom-based criteria alone, biomarkers alone, psychological markers alone, and combinations of these. We used a random effects model to provide a more conservative estimate of the accuracy of the various methods, allowing for heterogeneity between studies. QUADAS-2, a quality assessment tool for primary diagnostic accuracy studies, was used to assess the risk of bias and any applicability concerns in the eligible studies.⁽²⁰⁾ All eligible studies were judged against four key domains

covering patient selection, the diagnostic test applied, the reference standard, and the flow of patients through the study.

RESULTS

The search strategy identified 3361 citations, of which 33 studies appeared to be eligible and were retrieved for evaluation. Twenty-two of these met all eligibility criteria (Supplementary Figure 1). (21-42) Agreement between investigators when assessing eligibility was excellent (94% agreement, $K = 0.86$). The 22 included studies evaluated a total of 7106 patients. Eleven of the studies were conducted in Europe, six in North America, four in Asia, and one in Australasia. Thirteen of the studies were of cross-sectional design, and nine were case-control. As case-control studies are not representative of the true IBS prevalence, these were excluded when calculating the pooled prevalence of IBS, which was 43% (95% CI 37%-50%) in the remaining 13 studies. Twenty-one were conducted in secondary care, with one in both primary and secondary care. Individual study characteristics are summarised in Supplementary Table 1. The diagnostic tests utilised in the eligible studies are shown in Table 2, along with the number of studies assessing the accuracy of each test, total number of patients included, and the positive and negative LRs with 95% CIs (pooled where appropriate). Study bias and applicability outcomes assessed, according to the QUADAS-2 tool, are shown in Supplementary Table 2. Fourteen of the 22 studies were judged as high risk in one or more of the four key domains.

Symptom-based Diagnostic Criteria

Five studies evaluated ≥ 2 of the Manning criteria, (21-24, 27) six studies ≥ 3 , (21-24, 26, 27) and six studies ≥ 4 . (21-24, 26, 27) The Rome I criteria were evaluated in three studies, (25-27) the Rome II criteria in two studies (26, 27) and the Rome III criteria in only one study. (27) All studies were cross-sectional in design, collected symptom data using a

questionnaire completed by the patient, and utilised a reference standard of a normal colonoscopy or barium enema to confirm the diagnosis of IBS.

The Manning Criteria

Pooled positive and negative LR_s when using ≥ 2 of the Manning criteria in a total of 2452 patients were 2.20 (95% CI 1.54-3.14) and 0.18 (95% CI 0.10-0.32) respectively.(21-24, 27) In studies assessing ≥ 3 of the Manning criteria,(21-24, 26, 27) accuracy was best in the study conducted by Dogan *et al.*,(24) with a positive LR of 7.15 (95% CI 4.93-10.57) and a negative LR of 0.11 (95% CI 0.07-0.17). However, this was not replicated in the five other studies, including in the original validation study.(21) The pooled positive and negative LR_s, in a total of 2966 patients, were 2.85 (95% CI 1.95-4.15) and 0.36 (95% CI 0.24-0.53) respectively (Figure 1a and 1b). Finally, when data were pooled from the six studies assessing the accuracy of ≥ 4 of the Manning criteria,(21-24, 26, 27) positive and negative LR_s, in a total of 2986 patients, were 3.43 (95% CI 2.49-4.71) and 0.66 (95% CI 0.60-0.73) respectively (Figure 2a and 2b).

The Rome Criteria

Pooled positive and negative LR_s in the three studies,(25-27) containing 3006 patients, that reported on the Rome I criteria were 3.20 (95% CI 2.29-4.47) and 0.22 (95% CI 0.10-0.49) respectively. Pooled positive and negative LR_s of the two studies,(26, 27) containing 2402 patients, that evaluated the Rome II criteria were 2.56 (95% CI 1.64-4.00) and 0.25 (95% CI 0.08-0.85) respectively. The positive and negative LR_s in the one study,(27) containing 1848 patients, that reported on the Rome III criteria were 3.35 (95% CI 2.97-3.79) and 0.39 (95% CI 0.34-0.46).

Biomarkers

Visceral Hypersensitivity and Pain Perception as a Biomarker

One case control study and one cross-sectional study evaluated the role of visceral hypersensitivity using rectal barostat testing(29) and pain perception(30) during colonoscopy, in differentiating IBS from healthy controls (HCs) and miscellaneous gastrointestinal and medical conditions, in a total of 328 patients. Pooled positive and negative LRs in the two studies were 3.71 (95% CI 2.74-5.02) and 0.16 (95% CI 0.10-0.24) respectively. One case-control study, containing 138 patients, reported on colonic air insufflation to reproduce typical abdominal pain experienced in IBS as a biomarker in differentiating the disorder from colonic structural disease.(28) This test performed poorly, with positive and negative LRs of 0.98 (95% CI 0.80-1.60) and 1.09 (95% CI 0.40-3.98) respectively.

Serum-based Biomarkers

The diagnostic accuracy of a serum-based 10 biomarker panel, which were selected by examining differences in biomarker expression between IBS patients and HCs (among them interleukin-1 β , anti-tissue transglutaminase and anti-neutrophil cytoplasmic antibody), was reported in one cross-sectional study(32) and one case-control study(33) containing a total of 760 patients, with a pooled positive LR of 3.03 (95% CI 1.49-6.17) and pooled negative LR of 0.52 (95% CI 0.43-0.64). In the study conducted by Jones *et al.*,(33) an additional 24 serum biomarkers, selected through a combination of gene chip human array, gene array data analysis and real-time quantitative polymerase chain reaction, were added

to the original 10 biomarker panel. Positive and negative LRs of the 34 biomarker panel were 2.28 (95% CI 1.71-3.17) and 0.30 (95% CI 0.21-0.42) respectively.

Faecal Biomarkers

Volatile Organic Metabolites (VOMs)

The diagnostic accuracy of VOMs, chemicals released in faeces and that can undergo changes in the presence of organic disease or changes in microbiota, were assessed in one case-control study containing 30 IBS patients with diarrhoea, 62 patients with active Crohn's disease and 48 patients with active ulcerative colitis.(34) Using a receiver operator characteristics curve (ROC) to determine optimum performance, the positive and negative LRs in differentiating IBS from active inflammatory bowel disease were 4.83 (95% CI 3.36-7.14) and 0.04 (95% CI 0.01-0.21) respectively.

Chromogranins and Secretogranins

Chromogranins (Cg) and secretogranins (Sg) are proteins found in the secretory cells of the endocrine, enteric, and immune system. In one case-control study, CgB and SgII and SgIII levels were measured in faecal samples from 82 IBS patients and 29 HCs.(35) SgII and SgIII levels were higher in the IBS patients, and CgB levels were lower. SgII performed the most accurately using a cut-off of >0.16 nmol/g, with a positive LR of 3.89 (95% CI 2.07-8.23) and negative LR of 0.25 (95% CI 0.15-0.39).

Mucosal Intestinal Endocrine Cells as a Biomarker

Quantification of CgA cells was performed on biopsy samples taken from the duodenum during gastroscopy in one case-control study.(36) Using a cut-off of <200 cells/mm², the positive and negative LRs in differentiating 203 IBS patients from 86 HCs, were 18.5 (95% CI 7.58-47.3) and 0.14 (95% CI 0.10-0.20). In a similarly designed study,(37) rectal biopsies were taken from 50 patients with IBS and 27 HCs. Endocrine cell content was quantified and three endocrine cells, (peptide YY, oxyntomodulin and somatostatin) were validated as diagnostic tests. Using optimum performance determined on an ROC curve, peptide YY performed the best, with a positive LR of 7.56 (95% CI 2.96-21.9) and a negative LR of 0.18 (95% CI 0.09-0.33) at a cut-off of <30 cells/mm².

Sigmoid Muscularis Propria Thickness as a Biomarker

In a cross-sectional study of 175 female patients who were undergoing trans-vaginal ultrasound for investigation of gynaecological symptoms, sigmoid muscularis propria thickness was measured.(31) A diagnosis of IBS was made using a cut-off for abnormal muscularis propria thickness of ≥ 3 mm. A clinical diagnosis of IBS was confirmed with the primary physician and/or Gastroenterologist following ultrasound. Positive and negative LRs were 14.9 (95% CI 7.07-31.5) and 0.31 (95% CI 0.17-0.51) respectively.

Combinations of Biomarkers

Faecal Calprotectin (FC) and Intestinal Permeability Ratio as a Biomarker

In the previously cited study from Tibble *et al.*,(25) all patients provided a stool sample for measurement of FC levels, in addition to undergoing a lactulose/L-rhamnose

small intestinal permeability test. Using an FC level of <10 mg/L and a permeability ratio of <0.05, this biomarker combination was able to identify IBS patients with a positive LR of 8.64 (95% CI 5.76-13.1) and a negative LR of 0.34 (95% CI 0.28-0.39).

Bile Acid Secretion and Colonic Transit as a Biomarker

One case-control study used a 2-item model consisting of total faecal bile acid excretion and colonic transit to differentiate between 64 IBS patients with diarrhoea, 30 IBS patients with constipation, and 30 HCs.(38) Using the optimum cut-off on an ROC curve, the 2-item model was able to differentiate IBS from HCs with a positive LR of 2.78 (95% CI 1.55-5.58) and a negative LR of 0.46 (95% CI 0.33-0.65).

Psychological Markers

The use of psychological markers in differentiating IBS from health was evaluated in two case-control studies containing 714 patients.(33, 39) In the study conducted by Spiller *et al.* 319 IBS patients and 151 HCs completed the patient health questionnaire 12 (PHQ-12). The PHQ-12 differs from the patient health questionnaire-15 (PHQ-15) in that the three specific gastrointestinal-related questions are removed. Using a cut-off score of >6, the positive LR for the PHQ-12 in differentiating IBS from health was 12.5 (95% CI 6.55-24.6), and the negative LR was 0.35 (95% CI 0.30-0.41). Using a cut-off score of >7, the anxiety component of the hospital anxiety and depression scale (HADS) was reported to have positive and negative LR of 2.88 (95% CI 2.20-3.86) and 0.37 (95% CI 0.30-0.45) respectively. The depression component of the HADS demonstrated positive and negative LR of 5.44 (95% CI 3.01-10.1) and 0.68 (95% CI 0.62-0.75) respectively.

In the previously described study conducted by Jones *et al.*(33), participants were asked to complete the HADS, PHQ-15, and the perceived stress scale. Positive and negative LRs of these measures of psychological well-being combined in differentiating between IBS and HCs were 2.95 (95% CI 2.04-4.48) and 0.35 (95% CI 0.26-0.46) respectively.

Combinations of Symptoms, Biomarkers and Psychological Markers

Kruis Statistical Model

The accuracy of the Kruis statistical model, a scoring system that incorporates the clinical history, physical examination, and blood tests (erythrocyte sedimentation rate (ESR), leucocyte count, and haemoglobin level), was assessed in four cross-sectional studies, including a total of 1171 patients.(24, 40-42) A score of ≥ 44 was used as the cut-off to diagnose IBS. The pooled positive LR of these studies as assessed in a previous meta-analysis,(16) as there have been no studies published in the interim, was 8.63 (95% CI 2.89-25.8) and the pooled negative LR was 0.26 (95% CI 0.17-0.41).

Other Statistical Models

Frigerio *et al.*,(42) in a cross-sectional study, lowered the predetermined cut-off point of haemoglobin level in the Kruis statistical model from 14g/100ml to 13g/100ml in males and from 12g/100ml to 11g/100ml in females. Positive and negative LRs for this modified model, containing 253 patients, were 7.73 (95% CI 4.83-12.4) and 0.34 (95% CI 0.22-0.49) respectively.

Although differing from the Kruis model in the items included, the model validated in the cross-sectional study by Bellentani *et al.*,(41) also incorporated the clinical history,

physical examination, and an ESR and leucocyte count. Positive and negative LRs for this statistical model, containing 254 patients, were 4.29 (95% CI 2.86-6.66) and 0.30 (95% CI 0.22-0.39) respectively.

A Combination of FC, Intestinal Permeability Ratio, and the Rome I Criteria

In the previously described study by Tibble *et al.*(25), if a positive result for the Rome I criteria was incorporated with FC levels of <10mg/L and permeability ratio of <0.05, the positive and negative LRs were 26.4 (95% CI 11.4-61.9) and 0.51 (95% CI 0.45-0.56) respectively.

A Combination of Serum-based Biomarkers and Psychological Markers

Finally, in the study conducted by Jones *et al.*(33), the serum-based 34 biomarker panel and psychological measures were combined to ascertain if this improved accuracy in diagnosing IBS. Positive and negative LRs for this combined approach in differentiating IBS from health were 7.14 (95% CI 4.01-13.3) and 0.18 (95% CI 0.12-0.25) respectively.

Pooled Positive and Negative LRs for Each Approach Used to Diagnose IBS

When individual study results were combined to obtain pooled positive and negative LRs for each of the approaches to diagnose IBS, using all available studies for each of the approaches they assessed, there were significant differences in the pooled positive LR between studies using symptom-based criteria alone (positive LR 2.85; 95% CI 2.53-3.20), and studies that used a combination of symptoms, biomarkers, and psychological markers (positive LR = 8.48; 95% CI 4.64-15.5), but not between any of the other methods (Figure 3a). Negative LRs were not significantly different for any of the four approaches (Figure 3b).

DISCUSSION

This study has examined the accuracy of symptom-based diagnostic criteria, biomarkers, psychological markers or combinations thereof, in making a diagnosis of IBS. The Rome III criteria, the current gold standard for the diagnosis of IBS, have only been validated in one study to date, and performed modestly and similarly to the other symptom-based diagnostic criteria that have been described previously, with a positive LR >3 and a negative LR of approximately 0.4. Proposed biomarkers, with the exception of abnormal sigmoid muscularis propria thickness in female patients, intestinal mucosal endocrine cells, and faecal VOMs, and a combination of FC and intestinal permeability, all examined in single studies, appeared to perform no better than available symptom-based diagnostic criteria. The accuracy of psychological markers was also similar. Combining symptoms, biomarkers, and/or psychological markers in various permutations seemed to perform better generally in diagnosing IBS, and with a significantly greater pooled positive LR compared with symptom-based criteria.

Strengths of this study include a comprehensive search strategy, including a recursive search of the bibliographies of all eligible studies, and searching of conference proceedings to identify any potential studies published that may not have been included in the original search of the medical literature. This resulted in the identification of a wide range of potential methods for diagnosing IBS; specifically four different symptom-based diagnostic criteria evaluated in seven studies, eleven biomarkers evaluated in twelve studies, four psychological markers evaluated in two studies, and five different combinations of symptoms, biomarkers, and/or psychological markers evaluated in six studies. Pooling the data in some of our analyses resulted in a study population of 1800

patients or more for each of the symptom-based diagnostic criteria, and >1000 patients for the Kruis statistical model. Furthermore, this is the first study that has attempted to summarise data from all available methods, including novel approaches, to diagnose IBS.

There are some limitations to this study. When data were pooled, results varied between individual studies that evaluated the same diagnostic method in some analyses, which may be partly explained by differences in study design, recruitment, setting, and country, and differences in the reference standard used for diagnosing IBS. However, we used a random effects model when pooling study data in all these analyses, in order to provide a more conservative estimate of diagnostic accuracy. The cut offs we used to define presence of IBS for each of the diagnostic tests assessed in this meta-analysis was imposed by the reporting of the authors of the original studies. This is less relevant for studies employing diagnostic criteria, such as the Manning criteria, because we were able to obtain data for several thresholds, but is an issue for studies using laboratory tests, such as faecal chromogranins or VOMs, or the measures of psychological affect, which were not always used at the threshold recommended by the original authors. Additionally, the pooled IBS prevalence of all studies was high at >40%, as the majority of studies were conducted in referral populations in secondary care, meaning that some of the findings may not be applicable to a primary care setting, where the majority of patients are diagnosed and managed, as the prevalence of IBS may well be lower. The inclusion of case-control studies may lead to an overestimation of the diagnostic performance of the test being examined, compared to studies using a clinical cohort, because these are subject to spectrum bias as the study design often omits mild cases that are difficult to diagnose.⁽⁴³⁾ Finally, 14 of the 22 eligible studies were judged as high risk of bias, or had other applicability concerns, when

assessing quality using the QUADAS-2 tool, highlighting the limitations of some of the studies.

In terms of our use of LR, the advantage of these is that they provide the clinician with an intuitive feeling on ruling in or ruling out a given disease based on the magnitude of the positive and negative LR, respectively, and are less likely to be influenced by prevalence of the disease of interest than predictive values. However, there are some disadvantages to using LR. In particular, LR are not often quoted in the medical literature, the main reason being that clinicians are more used to dealing with probabilities as in the case of predictive values, whereas LR express their results in terms of odds.⁽¹⁹⁾ Another disadvantage is that LR can have wide confidence intervals, particularly in rare diseases with a low prevalence. However, this is of less relevance to IBS, which has a prevalence of 10% to 20% in the general population.⁽²⁾ We have provided sensitivities, specificities, and positive and negative predictive values for each of the diagnostic tests applied in Supplementary Table 3.

Guidelines for the management of IBS recommend making a positive diagnosis of IBS based on symptoms, and discouraging a “diagnosis of exclusion” approach.^(44, 45) Symptom-based diagnostic criteria were developed to aid in this, and therefore avoid unnecessary and potentially invasive investigations. However, one of the most consistent findings of this study is the modest performance of all the available symptom-based criteria in identifying IBS. This comparable performance between the symptom-based criteria is perhaps not surprising, considering they are derivatives of each other, and therefore share the same strengths and weaknesses.

In general, the performance of biomarkers in the studies we identified was similar to symptom based criteria, which is disappointing considering their potentially expensive nature. In some cases, the biomarkers would not be considered useful as a test outside of a

tertiary referral centre, due to the invasive nature or complexity of the test applied.

Furthermore, a number of the studies that assessed the accuracy of biomarkers used healthy volunteers as controls, whereas a biomarker that differentiated IBS from other organic disorders, in which the symptoms are likely to overlap with those of IBS, would be more clinically useful. Sigmoid muscularis propria measurement using trans-vaginal ultrasound, appeared to perform well with a positive LR of 15. However, this study had a number of limitations, including the failure to exclude other causes of abnormal muscularis propria thickness, such as colorectal cancer, inflammatory bowel disease, or diverticular disease, only 27 patients in the study population having a confirmed diagnosis of IBS, and the generalisability of the results, given that the test was applied in female patients only. Additionally, the results have yet to be validated by other investigators, despite the study being published 8 years ago. Duodenal mucosal CgA cell quantification also performed well in differentiating IBS from health, but only in a single study, and the test is invasive. In addition, the effect of coeliac disease, duodenitis, duodenal ulcers or inflammatory bowel disease on numbers of CgA cells in the duodenum has not been studied, and therefore further work in this area is required before any definitive conclusion can be made from this study. Faecal VOMs showed some promise in differentiating IBS from active inflammatory bowel disease in one small study, but again results of this study will require validating by others.

Given the known association of IBS with somatic symptoms and mood disorders demonstrated by others,(46) it is perhaps surprising that the performance of psychological markers were, in general, no better than that of symptom-based criteria. One possible reason that studies evaluating psychological markers performed poorly, was that these predominantly used markers of anxiety and depression to predict presence of IBS. In a

recently conducted study, where somatisation data were collected from more than 4000 patients referred to secondary care for investigation of their symptoms,(47) mean somatisation scores and mean number of somatic symptoms reported were significantly higher in 840 patients with IBS compared with 2137 patients without IBS, but who still reported GI symptoms. These findings support those of Spiller *et al.*,(39) where somatisation was found to be superior to either anxiety or depression. Somatisation as a marker may therefore be more accurate in predicting the presence of IBS than other measures of psychological well-being. More focused measures of psychological affect such as worry or rumination, which may also be associated with IBS,(48) could provide fruitful avenues for future research. An additional issue is the fact that the two studies reporting on the accuracy of psychological markers differentiated IBS from health, and therefore whether psychological markers can accurately discriminate between IBS and organic gastrointestinal disorders is unclear. This would seem less likely, as there is evidence to suggest that many organic GI disorders are also associated with psychological impairment.(49-51)

Combinations of symptoms, biomarkers, and/or psychological markers seemed to perform better, generally, in the six studies that assessed the accuracy of these approaches, and were superior to symptom-based criteria in terms of the pooled positive LR. This may be because IBS is a complex, heterogeneous disorder, for which there is no single unifying explanation, and for which numerous mechanisms have been proposed.(52-56) Combining symptoms or examination findings, biomarkers and/or psychological markers may therefore be a more useful approach to diagnosing IBS, and perhaps points the way forward for future iterations of the Rome process. However, this approach will likely result in increasing complexity of the diagnostic test, and may reduce its practicality in a clinical setting. This is probably the main reason that the Kruis statistical model was never adopted widely, despite

its apparently superior performance in predicting IBS accurately. Any future set of diagnostic criteria would also need to be easily administrable and interpretable in a primary care setting, and this again probably explains why the combination of FC, intestinal permeability ratio, and Rome I criteria did not progress beyond a research setting, despite its accuracy.

Our findings highlight that existing diagnostic approaches are unable to define IBS with any great accuracy. An alternative strategy may be to use latent class analysis methods. These are designed to use available information to create an appropriate pattern of measurements that most closely represents the latent construct IBS, and define groups, or classes, which could serve as a way of improving the accuracy of methods used to diagnose IBS, and therefore help to discriminate between IBS and non-IBS symptom profiles. However, at the time of writing, there are only a few instances of this type of modelling being used in functional GI disorders.⁽⁵⁷⁾ These complex statistical methods have the potential to explore a combination of patient-reported, endoscopic, and biochemical variables, in order to develop a stronger predictor of IBS, which could then be employed in everyday practice in the clinic by exploiting technological advancements, such as smartphone applications. These novel methods to aid the diagnosis of IBS could also be used to assess clinically meaningful dependent variables, such as response to therapy, health care consumption, and quality of life.

In conclusion, this meta-analysis has shown that symptom-based diagnostic criteria, biomarkers, and psychological markers perform only moderately well in diagnosing IBS, and in the case of biomarkers many of these are potentially expensive or invasive, and are not yet practical for clinical application. Combining symptoms with markers of organic disease or psychological affect, for instance using latent variable models, may represent the best way forward in improving the accuracy of diagnosing IBS.

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Guarantor of the article: RS is guarantor.

Specific author contributions: RS, DJG, GRL, and ACF conceived and drafted the study. RS, DJG, and ACF collected all data. RS, DJG, and ACF analysed and interpreted the data. RS and ACF drafted the manuscript. All authors contributed to and approved the final draft of the manuscript.

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Table 1: Eligibility criteria for studies to be included in the systematic review.

Eligibility criteria
Adult patients (aged ≥ 16 years) with lower GI symptoms
Cross-sectional design or case-control
Applied a diagnostic test for IBS to all patients, including one or more of: symptom-based diagnostic criteria [†] , biomarkers, psychological markers, or combinations thereof [†]
Confirmed presence of IBS using an accepted reference standard [‡]
Results of diagnostic test for IBS compared with the reference standard
≥ 50 patients included

[†]Manning, Rome I, Rome, II, or Rome III criteria

[‡]Normal colonoscopy, barium enema, CT colonography, physician's opinion that this was IBS, or accepted symptom-based diagnostic criteria for IBS

Table 2: Pooled Positive and Negative Likelihood Ratios (LRs) of Diagnostic Tests for Irritable Bowel Syndrome.

	Diagnostic test applied	No. of studies	No. of patients	Positive LR (95% CI)	Negative LR (95% CI)
Symptom-based diagnostic criteria	Manning ≥ 2 criteria	5	2452	2.20 (1.54-3.14)	0.18 (0.10-0.32)
	Manning ≥ 3 criteria	6	2966	2.85 (1.95-4.15)	0.36 (0.24-0.53)
	Manning ≥ 4 criteria	6	2986	3.43 (2.49-4.71)	0.66 (0.60-0.73)
	Rome I	3	3006	3.20 (2.29-4.47)	0.22 (0.10-0.49)
	Rome II	2	2402	2.56 (1.64-4.00)	0.25 (0.08-0.85)
	Rome III	1	1848	3.35 (2.97-3.79)	0.39 (0.34-0.46)
Biomarkers	Visceral hypersensitivity	2	328	3.71 (2.74-5.02)	0.16 (0.10-0.24)
	Pain perception	1	138	0.98 (0.80-1.60)	1.09 (0.40-3.98)
	Serum-based 10 biomarker panel	2	760	3.03 (1.49-6.17)	0.52 (0.43-0.64)
	Serum-based 34 biomarker panel	1	244	2.28 (1.71-3.17)	0.30 (0.21-0.42)
	Volatile organic metabolites in faeces	1	140	4.83 (3.36-7.14)	0.04 (0.01-0.21)
	<i>Chromogranins and secretogranins in faeces:</i>				
	Secretogranin II	1	111	3.89 (2.07-8.23)	0.25 (0.15-0.39)
	Secretogranin III	1	111	2.59 (1.61-4.70)	0.28 (0.17-0.47)
Chromogranin B	1	111	2.51 (1.56-4.56)	0.32 (0.20-0.51)	

	Duodenal chromogranin A	1	289	18.5 (7.58-47.3)	0.14 (0.10-0.20)
	<i>Rectal endocrine cells:</i>				
	Peptide YY	1	77	7.56 (2.96-21.9)	0.18 (0.09-0.33)
	Oxyntomodulin	1	77	4.32 (2.14-9.87)	0.25 (0.14-0.43)
	Somatostatin	1	77	7.20 (2.81-20.9)	0.23 (0.12-0.38)
	Sigmoid muscularis propria thickness	1	175	14.9 (7.07-31.5)	0.31 (0.17-0.51)
	FC and small intestinal permeability ratio	1	602	8.64 (5.76-13.1)	0.34 (0.28-0.39)
	Bile acid secretion and colonic transit	1	124	2.78 (1.55-5.58)	0.46 (0.33-0.65)
Psychological markers	PHQ-12 score	1	470	12.5 (6.55-24.6)	0.35 (0.30-0.41)
	Anxiety component of the HADS	1	470	2.88 (2.20-3.86)	0.37 (0.30-0.45)
	Depression component of the HADS	1	470	5.44 (3.01-10.1)	0.68 (0.62-0.75)
	Combination of HADS, PHQ-15, and the perceived stress scale	1	244	2.95 (2.04-4.48)	0.35 (0.26-0.46)
Combinations of symptoms, biomarkers, and/or psychological markers	Kruis <i>et al.</i> statistical model	4	1171	8.63 (2.89-25.8)	0.26 (0.17-0.41)
	Modified Kruis statistical model	1	253	7.73 (4.83-12.4)	0.34 (0.22-0.49)
	Bellentani <i>et al.</i> statistical model	1	254	4.29 (2.86-6.66)	0.30 (0.22-0.39)

	FC, small intestinal permeability ratio, and Rome criteria	1	602	26.4 (11.4-61.9)	0.51 (0.45-0.56)
	Serum-based 34 biomarker panel and psychological markers	1	244	7.14 (4.01-13.3)	0.18 (0.12-0.25)

Figure 1a: Pooled Positive Likelihood Ratio of ≥ 3 Manning Criteria.

Figure 1b: Pooled Negative Likelihood Ratio of ≥ 3 Manning Criteria.

Figure 2a: Pooled Positive Likelihood Ratio of ≥ 4 Manning Criteria.

Figure 2b: Pooled Negative Likelihood Ratio of ≥ 4 Manning Criteria.

Figure 3a. Pooled Positive Likelihood Ratios for All Approaches to the Diagnosis of IBS.

Figure 3b. Pooled Negative Likelihood Ratios for All Approaches to the Diagnosis of IBS.