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**TITLE PAGE**

**Title:** Enhancing Diagnostic Performance of Symptom-based Criteria for Irritable Bowel Syndrome by Additional History and Limited Diagnostic Evaluation.

**Short “running” head:** Enhancing Clinical Diagnosis of IBS.

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<b>Abbreviations:</b>	BMI	body mass index
	CI	confidence interval
	CRP	C-reactive protein
	GI	gastrointestinal
	HADS	hospital anxiety and depression scale
	IBS	irritable bowel syndrome
	IBS-D	irritable bowel syndrome with diarrhea
	LR	likelihood ratio
	PHQ-15	patient health questionnaire-15
	SD	standard deviation

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**ABSTRACT**

**Objectives:** Symptom-based criteria to diagnose irritable bowel syndrome (IBS) positively perform only modestly. Our aim was to assess whether including other items from the clinical history and limited diagnostic evaluation improves their performance.

**Methods:** We collected complete symptom, colonoscopy, and histology data from 318 consecutive, unselected adult patients with lower gastrointestinal (GI) symptoms in secondary care. All participants underwent colonoscopy, with relevant organic findings recorded. The reference standard used to define the presence of true IBS was patient-reported lower abdominal pain or discomfort associated with a change in bowel habit, in the absence of organic GI disease. Sensitivity, specificity, and positive and negative likelihood ratios (LRs), with 95% confidence intervals, were calculated for Rome III criteria, as well as for modifications, incorporating nocturnal stools, results of simple blood tests (hemoglobin and C-reactive protein (CRP)), measures of somatization, and/or affective disorders (hospital anxiety or depression scale (HADS) score).

**Results:** The sensitivity and specificity of the Rome III criteria for identifying IBS was 69.6%, and 82.0% respectively, with positive and negative LR of 3.87 and 0.37. Clinically useful enhancements in positive LR were provided by combining Rome III criteria with: (a) high level of somatization (7.27); (b) normal hemoglobin and CRP with HADS score of  $\geq 8$  (5.04); (c) normal hemoglobin and CRP with a high level of somatization (7.56), or; (d) no nocturnal passage of stool with a high level of somatization (17.3). Specificity was  $\geq 95\%$  with each of these modifications.

**Conclusions:** Incorporating nocturnal stools, somatization, and affective disorders from the clinical history, and hemoglobin and CRP measurements, enhances the positive LR and specificity of symptom-based Rome III criteria for IBS.

**What is current knowledge?**

- Current symptom-based diagnostic criteria, such as the Rome III criteria, for irritable bowel syndrome (IBS) perform only modestly.
- Biomarkers for IBS perform no better than symptom-based diagnostic criteria, and are probably more expensive.
- A recent systematic review and meta-analysis suggested that approaches to diagnosing IBS that used combinations of symptoms with biomarkers and/or measures of anxiety or depression were more accurate.

**What is new here?**

- The sensitivity and specificity of the Rome III criteria for identifying IBS was 69.6%, and 82.0% respectively, with positive and negative LRs of 3.87 and 0.37.
- The addition of various combinations of markers of either somatization or anxiety/depression, and normal blood results, led to clinically useful enhancements to the performance of the Rome III criteria.
- These findings could be used to inform future iterative processes to develop diagnostic criteria for the functional gastrointestinal disorders.

## INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal (GI) disorder, characterized by lower abdominal pain or discomfort in association with a change in stool form and/or frequency.<sup>1</sup> The condition has a prevalence of up to 20% in Western populations,<sup>2</sup> and is associated with significant morbidity. IBS results in reduced quality of life for the individual, and represents a considerable economic burden to society due, in part, to the costs to healthcare systems of managing the condition.<sup>3</sup> There is an expectation that physicians should try to reduce these costs by making a positive diagnosis of IBS, using symptom-based diagnostic criteria, whilst simultaneously minimizing invasive investigations.<sup>4,5</sup>

Physicians may be reluctant to adopt this approach in clinical practice, as GI diseases manifest as a limited repertoire of symptoms, and those of IBS can mimic organic diseases such as inflammatory bowel disease,<sup>6</sup> microscopic colitis,<sup>7</sup> bile acid diarrhea,<sup>8,9</sup> or celiac disease.<sup>10</sup> The current “gold standard” for symptom-based diagnosis of IBS are the Rome III criteria,<sup>1</sup> but these have only been validated in one large study from Canada,<sup>11</sup> and performed modestly in distinguishing IBS from organic GI disease. However, one of the issues in diagnostic test studies for IBS is the lack of an accepted reference standard. Most investigators have used a normal colonoscopy as confirmation of a diagnosis of IBS;<sup>12</sup> that is, physicians still regard IBS as a diagnosis of exclusion, which is perhaps justified by the modest performance of the different symptom-based criteria for IBS proposed over the last four decades.<sup>11,13</sup> Indeed, the current level of diagnostic confidence, based exclusively on these criteria, has not reduced the performance of testing such as colonoscopy and biopsies in some settings,<sup>14</sup> despite the desirability to enhance high-value care.

Recently, research has focused on the development of novel fecal, serum, and



imaging-based biomarkers that may more accurately predict a diagnosis of IBS, or subgroups of IBS.<sup>15–19</sup> Some of the proposed biomarkers (which are unfortunately not widely available) may also serve as therapeutic targets and enhance outcomes. Despite this, a systematic review and meta-analysis that evaluated all generally available approaches to diagnose IBS demonstrated that biomarkers alone performed similarly to symptom-based criteria,<sup>20</sup> while adding to the cost of care. Interestingly, studies using combinations of symptoms with biomarkers and/or measures of psychological wellbeing reported improved diagnostic accuracy.<sup>17,21</sup> Other investigators have reported that the absence of "red flag" features, such as nocturnal symptoms,<sup>22,23</sup> or incorporating the results of simple laboratory tests, including hemoglobin and erythrocyte sedimentation rate,<sup>24</sup> may increase the ability to distinguish between functional and organic lower GI diseases.

Our aim, based on these observations, was to conduct a diagnostic accuracy study to examine whether the performance of the current gold-standard in symptom-based criteria for IBS could be improved if combined with other relevant markers. We hypothesized that the inclusion of the results of simple laboratory tests, absence of nocturnal symptoms, identification of markers of either somatization or anxiety/depression, or combinations thereof, would increase the performance of the Rome III criteria in diagnosing IBS. Proof of enhancement in the diagnostic performance of symptom-based criteria could result in a reliable, inexpensive, and easily administrable clinical evaluation, and represent a considerable advance in enabling clinicians to make a positive diagnosis of IBS confidently in the clinic.

## METHODS

### Participants and Setting

We recruited unselected, consecutive patients aged  $\geq 16$  years newly referred from primary care to secondary care for consideration of investigation of GI symptoms. All patients were approached in six of the medical gastroenterology outpatient clinics of Leeds Teaching Hospitals Trust, West Yorkshire, United Kingdom. The hospitals provide secondary care services to a local population of almost 800,000 people in the North of England. The only exclusion criteria were an inability to understand written English, as the questionnaires utilized were self-administered. Potentially eligible subjects were given a study information sheet at their initial clinic visit, before consultation with a gastroenterologist. Those agreeing to participate provided written informed consent at that visit. The local ethics committee approved the study (reference 13/YH/0216), with recruitment commencing in January 2014, and continuing through to December 2015. During the 2-year recruitment period the six involved clinics saw approximately 2200 new outpatient referrals. As the study was conducted in routine clinical practice, the diagnostic evaluation of the recruited patients was not standardized, and was left at the discretion of the responsible physician. We did not mandate a minimum panel of blood tests, or collection of colonic biopsy specimens in all patients. However, all patients agreeing to participate were asked to complete the questionnaires detailed below. In addition, fecal calprotectin testing was not used routinely within our department during the time this study was conducted.

## Data Collection and Synthesis

### Demographic and Symptom data

All demographic and symptom data were collected prospectively at the initial clinic visit. Questionnaire data were entered into a database by trained researchers who were not involved in the clinical care of the patient, thus ensuring assessors were blinded to symptom status. Demographic data of interest included age, height (in meters), and weight (in kilograms), from which body mass index (BMI) was calculated, gender, tobacco and alcohol use, marital status, educational level, and ethnicity. The Rome III diagnostic questionnaire for adult functional GI disorders was used to collect data on GI symptoms.<sup>25</sup> We also asked patients whether they experienced nocturnal passage of stool, which was recorded as occurring never, rarely, sometimes, often, most of the time, or always, with a symptom frequency of sometimes or greater used to define its presence

### Mood and Somatization Data

We used the validated hospital anxiety and depression scale (HADS) to collect information about mood.<sup>26</sup> This 14-item instrument contains seven questions concerning anxiety, and another seven depression. Each of these questions is scored from 0 to 3, giving a total possible score of 21 for anxiety or depression separately. A score of  $\geq 8$  was used to define possible anxiety or depression.

We used the validated patient health questionnaire-15 (PHQ-15) to assess for evidence of somatization-type behavior.<sup>27</sup> The individual symptom items are provided in Supplementary Table 1. Each of these questions is scored on a scale from 0 to 2, giving a

total possible score of 30. A score of  $\geq 15$  is the validated threshold used to define high levels of somatization.

### Baseline Tests in Diagnostic Evaluation

We also collected information from patients' case notes and computerized records. We recorded hemoglobin level (normal for males  $\geq 13.5$ g/dL, normal for females  $\geq 11.5$ g/dL) and C-reactive protein (CRP) (normal  $< 5$ mg/L) at the initial clinic visit. We also recorded the initial diagnosis made by the physician who consulted with the patient, as well as the final diagnosis made after investigation to the level deemed appropriate by each individual consulting physician.

### Definition of IBS

The presence or absence of Rome III-defined IBS among individual patients was assigned according to the scoring algorithm proposed for use with the Rome III questionnaire (Supplementary Table 2).

### Colonoscopic and Histopathological Data

All included patients underwent complete colonoscopy to the cecum or terminal ileum. The endoscopy units in Leeds Teaching Hospitals Trust employ colonoscopes from both Olympus and Fujinon. Bowel preparation was either a combination of polyethylene glycol and sodium picosulfate, or polyethylene glycol alone, depending on renal function. All endoscopists performing colonoscopic examinations remained blinded to the questionnaire data of the patient. Findings were recorded using the ADAM reporting system (Fujifilm,

Europe), with reports accessed by study investigators in order to record the final colonoscopic diagnosis for each included patient. Findings classified as consistent with organic disease at colonoscopy are provided in Table 1.

Biopsy specimens were obtained at the discretion of the endoscopist performing the colonoscopy. Standard policy during these colonoscopies in any patient with chronic diarrhea and a macroscopically normal colon is to take two biopsies from the right colon, two from the left colon, and two from the rectum. All biopsies were interpreted by experienced GI histopathologists, who remained blinded to the questionnaire data of the patient. Histopathological findings were accessed using computerized records to record the final histopathological diagnosis. Findings classified as being consistent with organic disease after histopathological examination of biopsy specimens are also provided in Table 1.

Using these data, patients were classified according to the presence or absence of organic lower GI disease. Individuals had to have no evidence of an organic explanation for their symptoms at both colonoscopy and histopathological examination of biopsy specimens in order to be classified as exhibiting no organic lower GI disease.

### **Reference Standard to Define the Presence of True IBS**

The reference standard used to define the presence of true IBS was lower abdominal pain or discomfort occurring at least three days per month over the last 3 months, in association with a change in bowel habit, and in the absence of organic lower GI disease after colonoscopy and histopathological examination of colonic biopsies, if obtained, which would explain these symptoms. Exclusion of celiac disease with distal duodenal biopsy was also undertaken, if celiac serology was positive.

## Statistical Analysis

In order to assess whether those who underwent colonoscopy and provided complete symptom data were representative of all patients recruited, demographic data were compared between those undergoing colonoscopy who completed the symptom questionnaire, and those who completed the symptom questionnaire but did not undergo colonoscopy, using a  $\chi^2$  test for categorical data, and an independent samples t-test for continuous data, with a mean and standard deviation (SD). Due to multiple comparisons a 2-tailed P value of  $<0.01$  was considered statistically significant for these analyses. We compared organic findings in those meeting the Rome III criteria for IBS, with those who did not, using Fisher's exact test, as numbers in each cell were relatively small. These statistical analyses were performed using SPSS for Windows version 21.0 (SPSS Inc, Chicago, IL, USA).

The first aim of the study was to ascertain the performance of the Rome III criteria for IBS in determining the presence of true IBS versus the reference standard of symptoms suggestive of IBS and a negative colonoscopy described above. To that end, sensitivity, specificity, and positive and negative predictive values, and their 95% confidence intervals (CIs), were calculated for the Rome III criteria versus the reference standard using StatsDirect version 2.8.0 (StatsDirect Ltd, Sale, Cheshire, England). The positive likelihood ratio (LR) and negative LR, and their 95% CIs, were also calculated. The positive LR can be calculated from the formula: positive LR = sensitivity / (1-specificity). The negative LR is derived from the formula: negative LR = (1-sensitivity) / specificity. We performed these analyses for all individuals recruited who underwent colonoscopy for investigation of their lower GI symptoms, and provided complete Rome III symptom data. However, in clinical practice the challenge is often distinguishing between IBS-D and other potential organic GI causes of diarrhea. With this in mind, we performed post hoc analyses including only those participants reporting either  $\geq 4$  stools per day, or loose, mushy, or watery stools.

The advantage of using LRs over predictive values is that LRs do not vary to the same degree as predictive values with a change in disease prevalence. As a rule of thumb, a positive LR of more than 10 is useful for ruling in a disease, and a negative LR of less than 0.1 is useful for ruling out a disease. However, in diseases of higher prevalence, the positive LR threshold required to cause a useful increase in probability that will result in a change of management may be lower. In a recently published systematic review, the authors assumed “medical certainty” for a novel biomarker in diagnosing IBS as a post-test probability (derived from the pre-test probability and positive LR) of  $\geq 80\%$ .<sup>28</sup> At this threshold, in a secondary or tertiary care population with a prevalence of IBS of around 50%, a test with a positive LR of  $\geq 5$  would identify IBS with a post-test probability of 86.5%.

The second aim was to compare the performance of proposed modifications to the Rome III criteria, by including information on nocturnal passage of stools, the physician’s working diagnosis at the initial consultation, laboratory results of hemoglobin and CRP, and measures of anxiety/depression and somatization, against the reference standard described above. Again, sensitivities, specificities, positive and negative predictive values, and positive and negative LRs were calculated for each of these modifications individually, and as combinations.

## RESULTS

There were 1002 consecutive patients (mean age 54.4 years (range 16 to 92 years), 638 (63.7%) female) who gave informed consent and were recruited into the study between January 2014 and December 2015. Of these, 318 (31.7%) patients (mean age 54.0 years (range 18 to 92 years), 216 (67.9%) female) underwent colonoscopy for investigation of their lower GI symptoms, and provided complete Rome III symptom data (Figure 1, flow chart). Comparison of the demographic data of this group with those who did not undergo colonoscopy is provided in Table 2. Patients providing complete symptom data and undergoing colonoscopy had a higher BMI and were more likely to meet the Rome III criteria for IBS, but there were no other significant differences between the two groups. Patients with IBS with diarrhea (IBS-D) were more likely to undergo colonoscopy, but not patients with IBS with constipation or those with mixed stool pattern IBS.

Among the 318 individuals providing complete symptom and colonoscopy data, 98 (30.8%) met the Rome III criteria for IBS. The mean age of these 98 patients was 46.7 years, and 73 (74.5%) were female. There were 286 (89.9%) patients who had a hemoglobin check, 178 (56.0%) with a CRP measurement, 212 (66.7%) with celiac serology, and 215 (67.6%) who had colonic biopsy specimens obtained. Relevant organic findings after colonoscopy and histopathological interpretation of biopsy specimens, plus duodenal biopsy in those with positive celiac serology, in those that met the Rome III criteria compared with the 220 patients that did not are detailed in Table 3. There were no significant differences in the prevalence of any of these between the two groups.



### **Performance of the Rome III Criteria for IBS Against the Reference Standard**

Of 79 (24.8%) individuals meeting the reference standard of symptoms suggestive of IBS and a negative colonoscopy, 55 met the Rome III criteria, giving a sensitivity of 69.6% (Table 4). Among the 239 patients without IBS according to this reference standard, 196 did not meet the Rome III criteria, giving a specificity of 82.0%. Positive and negative LR of the Rome III criteria were 3.87 (95% CI 2.85 to 5.26) and 0.37 (95% CI 0.26 to 0.51) respectively.

### **Effect of Additional Factors from the History and Simple Laboratory Tests on the Diagnostic Performance of Rome III Criteria**

The effect of incorporating nocturnal passage of stools, a physician's working diagnosis at the initial consultation that this was IBS, the presence of anemia or a raised CRP, HADS score of  $\geq 8$ , or high levels of somatization into the Rome III criteria are also shown in Table 4. Sensitivities in diagnosing IBS ranged from 18.2% for presence of the Rome III criteria, no nocturnal passage of stool, and a high level of somatization to 50.0% for presence of the Rome III criteria and a physician's initial impression that the diagnosis was IBS. Specificities ranged from 79.7% for presence of the Rome III criteria and a physician's initial impression that the diagnosis was IBS, to 99.0% for presence of the Rome III criteria, no nocturnal passage of stool, and a high level of somatization.

Improved positive LR were obtained by combining the Rome III criteria with a high level of somatization alone (positive LR 7.27; 95% CI 3.74 to 14.2); a normal hemoglobin and CRP with a HADS score of  $\geq 8$  (positive LR 5.04; 95% CI 2.48 to 10.2); a normal hemoglobin and CRP with a high level of somatization (positive LR 7.56; 95% CI 2.63 to 21.7); and no nocturnal passage of stool with a high level of somatization (positive LR 17.3;

95% CI 4.45 to 67.6). Note that for all these combinations, positive LR<sub>s</sub> were above the threshold of  $\geq 5$  that has been recommended to define a potentially useful test, providing the prevalence of IBS in the population under study is 50% or more.<sup>28</sup> Specificity approached 95% or more with all these modifications; thus the risk of a missed diagnosis of organic GI disease would be small, as the false positive rate was extremely low.

When the analyses were restricted to participants who reported either  $\geq 4$  stools per day, or loose, mushy, or watery stools, there were similar enhancements of positive LR<sub>s</sub> (in some instances, almost two-fold those for the Rome III criteria alone) with the incorporation of additional factors from the clinical history and simple laboratory tests into the Rome III criteria (Table 5).

## DISCUSSION

This study validated the symptom-based Rome III criteria for IBS against an accepted clinical reference standard. These criteria performed modestly, with a positive and negative LR of 3.87 and 0.37 respectively. In addition, we examined the effect of addition of nocturnal symptoms, factors related to somatization, affective disorders, and hemoglobin and CRP measurements on the accuracy of the symptom-based Rome III criteria. A combination of the Rome III criteria with a high level of somatization, a normal hemoglobin and CRP with a HADS score of  $\geq 8$ , a normal hemoglobin and CRP with a high level of somatization, or no nocturnal passage of stool with a high level of somatization all provided positive LRs of  $\geq 5$ . In a secondary or tertiary referral population in a University Hospital practice with a prevalence of IBS of 50% or more, a positive LR of this magnitude would be clinically useful for the diagnosis of IBS, identifying IBS with a post-test probability of  $>85\%$ .<sup>28</sup>

The performance of the Rome III criteria in this study is remarkably similar to that observed in a previous validation study, which also used a reference standard of the combination of symptoms suggestive of IBS and a negative colonoscopy.<sup>11</sup> In that prior study from Canada,<sup>11</sup> which included  $>1800$  patients, the positive and negative LRs of the Rome III criteria were 3.35 (95% CI 2.97-3.79) and 0.39 (95% CI 0.39-0.46) respectively. Unlike the current study, the previous study did not incorporate other features of the clinical history or simple laboratory tests with the Rome III criteria. Sensitivity analyses were conducted in the Canadian study,<sup>11</sup> where individuals reporting lower GI alarm symptoms, including rectal bleeding, anemia, weight loss, or a family history of colorectal cancer were excluded. However, the addition of lower GI alarm symptoms resulted in only a small improvement in the positive LR. Few other studies have attempted to modify the symptom-based Rome criteria.<sup>22</sup> Vanner et al. examined the effect of excluding patients with “red flag” features, including nocturnal GI symptoms, on the Rome I criteria.<sup>22</sup> However, this was a

small retrospective study, and the investigators did not attempt to separate nocturnal GI symptoms from other alarm symptoms, which are reported frequently by patients without organic disease.<sup>29</sup>

Psychological or affective disorders have been shown to be strongly associated with IBS.<sup>30</sup> There was an improvement in diagnostic test accuracy when other investigators added these to a biomarker panel in a recent study.<sup>17</sup> Rates of somatoform-type behavior, in particular, have been shown to be significantly higher in patients with IBS,<sup>31</sup> and to differentiate IBS from health with greater accuracy, compared with markers of anxiety and depression.<sup>32</sup> The results of our study support this finding, with a greater accuracy when a combination of the Rome III criteria and high level of somatization was used, as compared with a combination of the Rome III criteria and HADS scores. Incorporating the presence of co-existent functional GI disorders into our modifications to the Rome III criteria may also have improved their performance. However, unlike in IBS, some other functional GI disorders are diagnoses of exclusion. For instance, a diagnosis of functional heartburn would not be made on symptoms alone, but only after a negative upper endoscopy and normal pH and impedance studies. As our study did not mandate the relevant investigations to confirm that, when the appropriate symptoms were reported, the cause was indeed another functional GI disorder we were therefore unable to examine this issue.

We propose that the performance of the modifications to the Rome III criteria used in the current study can be best appreciated by comparing them with the accuracy of biomarkers. In general, biomarkers have been shown to perform no better than symptom-based diagnostic criteria in IBS,<sup>20</sup> and in some cases are probably not clinically useful outside of a research or tertiary care setting, due to their complex or invasive nature e.g. brain imaging, or endoscopy and biopsy with specialized histopathology.<sup>33–36</sup> Furthermore, many of the studies that have validated biomarkers have been limited by the fact that their utility in

IBS was compared with healthy controls, when it would be more useful to assess the performance of the biomarker in distinguishing between IBS and organic disease.

Alternatively, other appraisals of biomarkers have used IBS-enriched populations, reducing their generalizability to a clinical setting.<sup>20</sup>

One biomarker that is available for use in clinical practice currently was examined for its ability to differentiate IBS-D from inflammatory bowel disease, celiac disease, or health.<sup>19</sup> In this study, antibodies to cytotoxin B, a toxin commonly produced by *Campylobacter jejuni*, and to vinculin, a cell adhesion protein, performed best when differentiating IBS-D from inflammatory bowel disease, with positive LRs of 5.2 and 2.0 respectively. However, the authors used an enriched sample of cases, that consisted of a cohort of patients enrolled in a large randomized clinical trial of rifaximin, with >80% of participants having IBS-D. Thus, the LRs may not be reproducible in other populations, or in those with IBS not associated with diarrhea. This underlines the importance of our findings in a consecutive, unselected secondary care population, where various combinations of the Rome III criteria, two routine blood tests, and a symptom-item checklist, appeared accurate and would be inexpensive to administer as a diagnostic test.

The improved performance of the Rome III criteria when combined with relevant blood tests and markers of somatization and anxiety/depression is perhaps not surprising given the findings of other investigators, summarized in a recent meta-analysis.<sup>20</sup> Studies that have used symptoms with clinical laboratory tests, biomarkers, and markers of psychological disorders, have shown improved differentiation of IBS from organic GI diseases. This direction was first suggested by Kruis and colleagues in a statistical model in 1984,<sup>24</sup> and outperformed symptom-based diagnostic criteria alone in a previous meta-analysis.<sup>12</sup> Tibble et al. also demonstrated a high diagnostic accuracy of the Rome I criteria in combination with both a fecal calprotectin and a small intestinal permeability ratio.<sup>21</sup>

However, the Kruis model may be limited by its complexity, and the approach of Tibble et al. is not clinically applicable, given the lack of availability of measures of small intestinal permeability. We suspect that the proposed models did not progress beyond a research setting because of their complexity. Other markers for measurement of small intestinal and colonic permeability have been proposed since the earlier study by Tibble et al., based on the ratio of saccharide excretion,<sup>37</sup> although there is still no generally available, clinically applicable, and universally accepted test of intestinal permeability at present.

There are methodological strengths of our study. First, it was conducted in a large, unselected population referred to secondary care, so the results are likely to be generalizable to patients with suspected IBS seen in usual clinical care by gastroenterologists. The sample size, although smaller than the previous validation study of the Rome III criteria,<sup>11</sup> is larger than most other studies that have assessed the accuracy of diagnostic tests for IBS.<sup>20</sup> Second, it was designed to adhere to the STARD guidelines for the reporting of studies of diagnostic accuracy, with consecutive patients recruited, assessors blinded, and accepted references standard used. Third, it used inexpensive factors to modify the symptom-based criteria, and these lend themselves to application in primary or secondary care.

There are some limitations to the study. Not all patients that underwent colonoscopy provided complete symptom data, and we were therefore unable to include these individuals in our analyses. However, this number was comparatively small, with almost 90% of patients providing full data. Most of the patients included in the study were White Caucasian, meaning that these results may not be applicable to other ethnicities. The mean age of included individuals was relatively high at 54 years, which probably reflects our use of a negative colonoscopy as a reference standard, meaning that there is some selection bias and that the results may therefore not be generalizable to a younger population. In addition, the reference standard we used in our analyses included symptom data from the questionnaire,

which may have resulted in an overestimation of the accuracy of the Rome III criteria and its modifications, and a negative colonoscopy. There are other conditions that may mimic IBS, such as bile acid diarrhea, small intestinal bacterial overgrowth, or fructose and lactose intolerance,<sup>38-40</sup> which are not excluded by a negative colonoscopy. These were not screened for routinely in this study, which was conducted within usual clinical practice. However, the prevalence of unequivocal small intestinal bacterial overgrowth in patients presenting with symptoms suggestive of IBS is probably less than 5%.<sup>41</sup> For similar reasons, we did not mandate a minimum diagnostic work up in terms of a panel of standardized blood tests or colonic biopsy specimens in all patients. The modifications to symptom-based criteria in our current study enhanced the diagnosis of IBS, but do not necessarily identify actionable features of the disorder. Thus, the recently validated additional measurements of colonic transit or of bile acid metabolism still provide the best biomarkers to individualize therapy in subsets of IBS patients.<sup>18,42</sup> Finally, the approaches suggested by our findings may not completely change physician behavior, due to uncertainty or fear of a missed organic diagnosis, which is reflected by the fact that significantly more patients who met the Rome III criteria for IBS were referred for colonoscopy in this study. However, further proof of the validity of this approach in prospective cohorts will enhance the confidence with which physicians can make a positive diagnosis of IBS, which was the intent of the original symptom-based criteria proposed by Manning et al.<sup>13</sup>

In summary, the performance of the Rome III criteria in diagnosing IBS was similar to that observed in a previous validation study from a cohort in Canada.<sup>11</sup> Important novel findings from this study were that modifying these criteria, with questionnaires concerning nocturnal symptoms, anxiety/depression, and somatization, in addition to simple laboratory tests, improved their diagnostic performance. An inexpensive clinical test that combines symptoms with clinical markers, which is easily administered in a routine care setting, and

accurate enough to allow the physician to confidently make a positive diagnosis of IBS would be highly desirable, and may have important implications for enhanced value care.



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**Guarantor of the article:** RS is guarantor.

**Specific author contributions:** RS, MC, DJG, GRL, and ACF conceived and drafted the study. RS, DJG, MJG, and NT collected all data. RS, MC, GRL, and ACF analyzed and interpreted the data. RS, MC, GRL, and ACF drafted the manuscript. All authors have approved the final draft of the manuscript.

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**Table 1. Findings Consistent with Organic Disease at Colonoscopy, or After Histopathological Interpretation of Colonic Biopsies.**

At Colonoscopy	After Histopathological Interpretation of Colonic Biopsies
<p>Evidence of colitis</p> <p>Evidence of terminal ileitis (inflammation or ulceration)</p> <p>Colorectal carcinoma</p> <p>Colonic stricture</p> <p>Evidence of radiation-induced colorectal disease</p>	<p>Colonic adenocarcinoma</p> <p>Rectal adenocarcinoma</p> <p>Ulcerative colitis</p> <p>Crohn's disease</p> <p>Inflammatory bowel disease-unclassifiable</p> <p>Microscopic colitis</p> <p>Ischemic colitis</p> <p>Radiation enteropathy</p> <p>Ulceration seen macroscopically at colonoscopy with non-specific inflammation on histological examination</p> <p>Neuroendocrine tumor</p>

**Table 2. Demographics and Baseline Characteristics of Patients Who Underwent Colonoscopy and Provided Complete Symptom Data Compared with Those That Did Not Undergo Colonoscopy.**

	<b>Underwent colonoscopy and provided complete Rome III symptom data  (n = 318)</b>	<b>Did not undergo colonoscopy  (n = 642)</b>	<b>P value*</b>
<b>Mean age in years (SD)</b>	54.0 (16.3)	54.6 (18.1)	0.57
<b>Mean BMI (SD)</b>	27.2 (6.0)	26.2 (5.3)	0.02
<b>Female gender (%)</b>	216 (67.9)	402 (62.6)	0.11
<b>Tobacco use (%)</b>	74 (23.3)	149 (23.2)	0.99
<b>Alcohol use (%)</b>	171 (53.8)	351 (54.7)	0.87
<b>Marital status (%)</b>			
Married or cohabiting	177 (55.7)	354 (55.1)	
Divorced or separated	44 (13.8)	74 (11.5)	
Never Married	59 (18.6)	116 (18.1)	
Widowed	26 (8.2)	73 (11.4)	0.38
<b>Educational level (%)</b>			
Elementary	2 (0.6)	3 (0.5)	
High school	144 (45.3)	277 (43.1)	
College or technical school	77 (24.2)	137 (21.3)	
University	47 (14.8)	91 (14.2)	
Postgraduate	29 (9.1)	55 (8.6)	0.98



<b>White Caucasian ethnicity (%)</b>	292 (91.8)	573 (89.3)	0.25
<b>Met Rome III criteria for IBS (%)</b>	98 (30.8)	126 (19.6)	<0.001
IBS-D	46 (14.6)	32 (5.0)	<0.001
IBS-C	5 (1.6)	25 (3.9)	0.08
IBS-M	45 (14.2)	60 (9.3)	0.03
<b>HADS score <math>\geq 8</math> (n = 829)</b>	144/292 (49.3)	278/537 (51.8)	0.50
<b>High level of somatization (n = 725)</b>	57/258 (22.1)	99/467 (21.2)	0.78

\*P value for independent samples t-test for continuous data and Pearson  $\chi^2$  for comparison of categorical data.

**Table 3. Prevalence of Organic Disease in Patients Meeting the Rome III Criteria Compared With Those Who Did Not.**

	<b>Met Rome III criteria for IBS (n = 98)</b>	<b>Did not meet Rome III criteria for IBS (n = 220)</b>	<b>P value*</b>
<b>Ulcerative colitis (%)</b>	2 (2.0)	2 (0.9)	0.59
<b>Crohn's disease (%)</b>	4 (4.1)	2 (0.9)	0.08
<b>Inflammatory bowel disease-unclassifiable (%)</b>	0 (0)	2 (0.9)	0.52
<b>Nonspecific GI ulceration (%)</b>	1 (1.0)	4 (1.8)	1.0
<b>Collagenous colitis (%)</b>	4 (4.1)	12 (5.5)	0.78
<b>Lymphocytic colitis (%)</b>	2 (2.0)	9 (4.1)	0.51
<b>Colorectal cancer (%)</b>	0 (0)	2 (0.9)	1.0
<b>Celiac disease (%)</b>	2 (2.0)	5 (2.3)	1.0

\*P value for Fisher's exact test for comparison of categorical data.

**Table 4. Diagnostic Accuracy of the Rome III Criteria, and Modifications to the Rome III Criteria with the Inclusion of No Nocturnal Passage of Stool, Physician's Initial Impression that this was IBS, Biomarkers or Markers of Affective Disorders, or a Combination Thereof, versus the Reference Standard.**

	Number of patients providing data in the analysis	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
<b>Rome III criteria alone</b>	<b>318</b>	<b>69.6%</b> (58.3% – 79.5%)	<b>82.0%</b> (76.5% – 86.7%)	<b>56.1%</b> (46.3% – 65.5%)	<b>89.1%</b> (84.3% – 92.6%)	<b>3.87</b> (2.85 – 5.26)	<b>0.37</b> (0.26 – 0.51)
<b>Rome III criteria and no nocturnal passage of stool</b>	311	33.3% (23.1% – 44.9%)	91.0% (86.6% – 94.3%)	55.3% (41.3% – 68.6%)	80.3% (75.1% – 84.7%)	3.70 (2.21 – 6.14)	0.73 (0.61 – 0.84)
<b>Rome III criteria and physician's initial impression that this was IBS</b>	112	50.0% (33.4% – 66.6%)	79.7% (68.8% – 88.2%)	55.9% (39.5% – 71.1%)	75.6% (65.1% – 83.8%)	2.47 (1.42 – 4.27)	0.63 (0.43 – 0.84)

<b>Rome III criteria and normal hemoglobin and CRP</b>	208	49.0% (34.8% – 63.4%)	89.2% (83.2% – 93.6%)	59.5% (44.5% – 73.0%)	84.3% (78.0% – 89.1%)	4.53 (2.67 – 7.64)	0.57 (0.42 – 0.73)
<b>Rome III criteria and HADS score <math>\geq 8</math></b>	292	47.2% (35.3% – 59.4%)	89.1% (84.2% – 92.9%)	58.6% (45.8% – 70.4%)	83.8% (78.5% – 87.9%)	4.33 (2.76 – 6.76)	0.59 (0.46 – 0.72)
<b>Rome III criteria and high level of somatization</b>	258	37.9% (26.2% – 50.7%)	94.8% (90.6% – 97.5%)	71.4% (55.0% – 83.7%)	81.6% (76.0% – 86.1%)	7.27 (3.74 – 14.2)	0.66 (0.53 – 0.77)
<b>Rome III criteria, normal hemoglobin and CRP, and HADS score <math>\geq 8</math></b>	195	34.0% (20.9% – 49.3%)	93.2% (87.9% – 96.7%)	61.5% (42.5% – 77.6%)	81.7% (75.1% – 86.8%)	5.04 (2.48 – 10.2)	0.71 (0.55 – 0.84)

<b>Rome III criteria, normal hemoglobin and CRP, and high level of somatization</b>	165	24.4%  (12.4% – 40.3%)	96.8%  (92.0% – 99.1%)	71.4%  (45.4% – 88.3%)	79.5%  (72.3% – 85.1%)	7.56  (2.63 – 21.7)	0.78  (0.63 – 0.90)
<b>Rome III criteria, no nocturnal passage of stool, and HADS score ≥8</b>	290	22.2%  (13.3% – 33.6%)	95.4%  (91.7% – 97.8%)	61.5%  (42.5% – 77.6%)	78.8%  (73.5% – 83.3%)	4.84  (2.33 – 10.0)	0.82  (0.70 – 0.91)
<b>Rome III criteria, no nocturnal passage of stool, and high level of somatization</b>	256	18.2%  (9.8% – 29.6%)	99.0%  (96.3% – 99.9%)	85.7%  (60.1% – 96.0%)	77.7%  (72.0% – 82.5%)	17.3  (4.45 – 67.6)	0.83  (0.72 – 0.90)

**Table 5. Diagnostic Accuracy of the Rome III Criteria, and Modifications to the Rome III Criteria with the Inclusion of No Nocturnal Passage of Stool, Physician’s Initial Impression that this was IBS, Biomarkers or Markers of Affective Disorders, or a Combination Thereof, versus the Reference Standard Among Patients Presenting with Diarrhea.**

	Number of patients providing data in the analysis	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	Positive likelihood ratio (95% CI)	Negative likelihood ratio (95% CI)
<b>Rome III criteria alone</b>	252	68.0% (56.2% – 78.3%)	76.8% (69.9% – 82.4%)	55.4% (44.7% – 65.8%)	85.0% (78.5% – 90.2%)	2.94 (2.16 – 4.01)	0.42 (0.29 – 0.57)
<b>Rome III criteria and no nocturnal passage of stool</b>	251	30.7% (20.5% – 42.4%)	89.2% (83.7% – 93.4%)	54.8% (38.7% – 70.2%)	75.1% (68.7% – 80.8%)	2.84 (1.65 – 4.85)	0.77 (0.65 – 0.89)
<b>Rome III criteria and physician’s initial impression that this was IBS</b>	100	54.3% (36.7% – 71.2%)	76.9% (64.8% – 86.5%)	55.9% (37.9% – 72.8%)	75.8% (63.6% – 85.5%)	2.35 (1.38 – 4.03)	0.59 (0.39 – 0.84)

<b>Rome III criteria and normal hemoglobin and CRP</b>	163	51.0% (36.3% – 65.6%)	85.1% (77.2% – 91.1%)	59.5% (43.3% – 74.4%)	80.2% (71.9% – 86.9%)	3.42 (2.05 – 5.72)	0.58 (0.41 – 0.75)
<b>Rome III criteria and HADS score <math>\geq 8</math></b>	237	46.4% (34.3% – 58.8%)	85.7% (79.5% – 90.6%)	57.1% (43.2% – 70.3%)	79.6% (72.9% – 85.2%)	3.25 (2.07 – 5.07)	0.63 (0.49 – 0.77)
<b>Rome III criteria and high level of somatization</b>	207	38.1% (26.2% – 51.2%)	93.1% (87.6% – 96.6%)	70.6% (52.5% – 84.9%)	77.5% (70.5% – 83.5%)	5.49 (2.83 – 10.7)	0.67 (0.53 – 0.79)
<b>Rome III criteria, normal hemoglobin and CRP, and HADS score <math>\geq 8</math></b>	158	34.8% (21.4% – 50.3%)	91.1% (84.2% – 95.6%)	61.5% (40.6% – 79.8%)	77.3% (69.2% – 84.1%)	3.90 (1.93 – 7.83)	0.72 (0.55 – 0.86)

<b>Rome III criteria, normal hemoglobin and CRP, and high level of somatization</b>	131	25.0% (12.7% – 41.2%)	95.6% (89.1% – 98.8%)	71.4% (41.9% – 91.6%)	74.4% (65.5% – 82.0%)	5.69 (2.00 – 16.3)	0.78 (0.62 – 0.91)
<b>Rome III criteria, no nocturnal passage of stool, and HADS score <math>\geq 8</math></b>	237	20.3% (11.6% – 31.7%)	94.1% (89.3% – 97.1%)	58.3% (36.6% – 77.9%)	74.2% (67.8% – 79.9%)	3.41 (1.61 – 7.16)	0.85 (0.73 – 0.94)
<b>Rome III criteria, no nocturnal passage of stool, and high level of somatization</b>	207	17.5% (9.1% – 29.1%)	98.6% (95.1% – 99.8%)	84.6% (54.6% – 98.1%)	73.2% (66.4% – 79.3%)	12.6 (3.23 – 49.5)	0.84 (0.72 – 0.92)



**FIGURE LEGENDS**

**Figure 1. Flow of Study Participants.**

