J Neurogastroenterol Motil, Vol. 23 No. 1 January, 2017
pISSN: 2093-0879 eISSN: 2093-0887
https://doi.org/10.5056/jnm16135
Journal of Neurogastroenterology and Motility

Review

Biomarkers of Irritable Bowel Syndrome

Jae Hak Kim,[1] Eugenia Lin,[2] and Mark Pimentel[2]*

[1]Department of Internal Medicine, Ilsan Hospital, Dongguk University, Goyang, Gyeonggi-do, Korea; and [2]GI Motility Program, Division of Gastroenterology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Traditionally, irritable bowel syndrome (IBS) has not been regarded as an organic disease, and the pathophysiology of IBS is heterogeneous. Currently, the diagnosis of IBS is based upon the Rome diagnostic criteria. The performance of these criteria is only modest in predicting IBS, and moreover their validation is lacking. Additionally, as functional symptoms are common in the general population, healthy controls or volunteers are difficult to define and there is currently no definition of "normal" in the Rome criteria. Due to the weaknesses of the current diagnostic criteria, patients and doctors expect new gold standard diagnostic tools. Various etiologic mechanisms result in potential biomarkers. The focus of this research has been to find non-invasive biomarkers from serum, breath gas, and fecal materials. Though biomarkers should be based on biological and pathogenic processes, most biomarkers for IBS have been developed to identify organic diseases and therefore eliminate IBS. To date, these types of biomarkers for IBS have been disappointing. The purposes of developing biomarkers include improvement of diagnosis, differentiation from other organic diseases, and discrimination of IBS subtypes. A true mechanistic biomarker would make it possible to rule in IBS, rather than to rule out other organic diseases. New serologic biomarkers for diarrhea-predominant IBS have been introduced based on the pathophysiologic findings from a rat model and validation in a large-scale clinical trial. Further investigations of abnormal organic findings from each subtype of IBS would enable the development of new, simple subtype-specific biomarkers.
(J Neurogastroenterol Motil 2017;23:20-26)

Key Words
Biomarkers; Constipation; Diarrhea; Irritable bowel syndrome

Introduction

Irritable bowel syndrome (IBS) is traditionally diagnosed using the Rome diagnostic criteria, a symptom-based criteria standard, currently revised as the Rome IV criteria.[1] The Rome III criteria for IBS had a modest diagnostic ability with a sensitivity of 75% in primary care,[2] and a sensitivity of 69% and specificity of 80% in secondary care.[3] However, validation of the Rome criteria is lacking and most of the validations of these criteria compare the criteria to normal subjects and not organic gastrointestinal (GI) illness. In addition, diagnosis based on the Rome criteria starts with excluding other organic GI diseases with inevitably expensive investigations. For example, more than 70% of patients with inflammatory bowel disease (IBD) would meet the Rome criteria for IBS.[4] The indefinite clinical definition of IBS also makes it difficult to determine "healthy" controls.[5] In clinical practice, as well as in research, it is hard to determine normal subjects relative to patients with IBS since the Rome criteria does not provide a strict definition of "normal" or "healthy." Therefore, biomarkers for IBS are still highly necessary.

Received: August 21, 2016 Revised: September 16, 2016 Accepted: October 2, 2016

*Correspondence: Mark Pimentel, MD, FRCP(C)
GI Motility Program, Division of Gastroenterology, Cedars-Sinai Medical Center, 8730 Alden Drive, Suite 240E, Los Angeles, CA 90048, USA
Tel: +1-310-423-6143 Fax: +1-310-423-8356, E-mail: pimentelm@cshs.org

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.[6] Up-to-date biomarkers for IBS have been developed with several purposes: (1) to improve the diagnosis,[7-9] (2) to differentiate from other organic diseases,[9,10] and (3) to discriminate between IBS subtypes.[8] Though the markers should be associated with a possible pathophysiologic mechanism of IBS, some biomarkers for other diseases such as IBD are used for differentiating IBS from non-IBS.[7,9,10] Various materials for developing biomarkers have been introduced, including serologic markers,[7-9] fecal markers,[10] cellular/molecular markers, breath tests, scintigraphic markers, and colonic mucosal immune markers. The most significant issues when developing biomarkers for IBS are the small population sample size and limiting comparisons between IBS patients and healthy subjects or subjects with other diseases.

In this article, we discuss the biomarkers for IBS, including those for specific IBS subtypes, from various materials.

Biomarkers for "Not Irritable Bowel Syndrome"

One of the common themes in the development of biomarkers for IBS are panels or components that identify IBS based on finding results consistent with other disorders. An example would be a high fecal calprotectin. By having this level high, the test essentially rules in IBD and thus eliminates IBS. So a positive test is "not IBS." This type of diagnostic approach which is being suggested to diagnose IBS as a negative test increases the probability that the patient has IBS only.

Though IBS has a heterogeneous pathophysiology, most researchers recruit all IBS subjects to be in the study population, resulting in decreased sample sizes for subgroup analyses such as diarrhea-predominant IBS (IBS-D) and constipation-predominant IBS (IBS-C). The first attempt to validate serum biomarkers in diagnosing IBS was the use of a 10-biomarker algorithm.[7] Healthy controls and patients with various GI conditions (256 IBS subjects, 71 normal subjects, 125 IBD subjects, 47 functional GI disorders, and 17 celiac disease) were tested with a biomarker panel (IL-1β, growth-related oncogene-α, brain-derived neutrophic factor, anti-*Saccharomyces cerevisiae* antibody, anti-CBir1, anti-human tissue translutaminase, TNF-like weak inducer of apoptosis, anti-neutrophil cytoplasmic antibody, tissue inhibitor of metalloproteinase-1, and neutrophil gelatinase-associated lipocalin). The sensitivity was 50% and the specificity was 88% for differentiating IBS subjects from non-IBS subjects, and the overall accuracy was 70%. However, these were primarily IBD markers rather than IBS markers, as this study was not designed to confirm IBS, but rather designed to diagnose other diseases and by doing so, establish "not IBS."

Another study presented the performance of a combination of 34 serologic and gene expression markers and psychological measurements in differentiating 168 IBS subjects (60 IBS-C, 57 IBS-D, and 51 mixed) from 76 healthy volunteers (HV).[8] Ten serological markers including histamine, tryptase, serotonin, and substance P, and 14 gene expression markers from analysis of differentially expressed genes in IBS and HV including CBFA2T2, CCDC147, and ZNF326 were added to the original 10 biomarker panel. This panel had a sensitivity of 81% and a specificity of 64%. Good discrimination was also obtained between IBS subtypes, with the best discrimination being observed for IBS-C vs IBS-D. However, the definition of HV, which was characterized as adults without any illness, active infection, or significant medical condition was vague and excluded any comment on the functional symptoms. Additionally, comparisons with other organic diseases were not provided. It is difficult to think that a test is needed to discriminate IBS from healthy subjects since they have no symptoms and do not seek care. A biomarker would best discriminate IBS from other organic GI disorders.

A recent study with 196 IBS subjects and 160 healthy controls (HC) without GI symptoms demonstrated that a panel of 8 biomarkers had a sensitivity of 88.1% and a specificity of 86.5% in discriminating IBS subjects from HC.[9] These populations were extracted from the Maastricht IBS cohort. Validation of this biomarker panel for the discrimination between organic GI disorders was not performed.

Other non-invasive biomarkers studied include fecal biomarkers. Fecal markers in general have been developed to reflect inflammation of the intestinal mucosa, which means that their primary purpose is to identify IBD and therefore "not IBS." The most frequently studied marker is calprotectin. Calprotectin is a heterodimer of S100A8 and S100A9 and the overexpression of S100A8/A9 is associated with inflammatory and neoplastic disorders.[11] Recently, pooled analysis demonstrated that fecal calprotectin had a sensitivity of 93% and a specificity of 94% at a cut-off value of 50 μg/g in differentiating IBS from IBD.[10] The cut-off level is low and calprotectin is not related to the pathogenesis of IBS but is rather a test for IBD.

Biomarkers for Ruling in Irritable Bowel Syndrome Compared to Healthy Humans

Biomarkers in this category use new techniques that might rule in IBS based on comparison to HC. However, testing is limited to IBS and healthy subjects, but not comparisons to other GI organic disorders. Furthermore, their links to IBS pathophysiology remain unclear in most cases.

Fecal short-chain fatty acids (SCFA) and granins are biomarkers for the discrimination of IBS from HC. SCFA are derived from non-digestible carbohydrates through gut microbial fermentation.[12] SCFA include acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid, and iso-valeric acid. A study with a small population size (25 IBS subjects and 25 HC) aimed to diagnose IBS by measurement of fecal SCFA.[13] Differences in the levels of propionic and butyric acid had the best diagnostic properties, with a sensitivity of 92% and a specificity of 72% at a cut-off value > 0.015 mmol/L. However, diet was not controlled for, and because of the exploratory design of the study, subjects were not consistent. Granins (chromogranins [Cg] and secretogranins [Sg]) are proteins distributed ubiquitously in vesicles of secretory cells of the enteric, endocrine, and immune system, and may serve as markers for activity of the enteric neuroendocrine system.[14] A separate analysis of fecal CgA, CgB, SgII, SgIII, and calprotectin in 82 IBS subjects and 29 HC demonstrated that SgII, SgIII, and CgB had discriminative validity to identify IBS patients.[14] SgII had a sensitivity of 80% and a specificity of 79%. Both SgIII and CgB had fairly good discriminative validity to positively identify IBS patients. However, calprotectin in this research failed to discriminate IBS subjects from HC. To date, the role of granins in the pathophysiology of IBS is not clear and the reason why levels of granins are different in IBS subjects has not been elucidated.

A novel non-invasive metabolomic approach in the diagnosis of IBS is the analysis of the breath. In one study, a set of 16 volatile organic compounds (VOCs) from 170 IBS patients and 153 HC were analyzed.[15] Among hundreds of VOCs, n-hexane, 1,4-cyclohexadiene, n-hepane, and aziridine were elevated in the IBS group. Butane, tetradecanol, 6-methyloctadecane, nonadecatetraene, methylcyclohexane, 2-undecene, benzyl-oleate, 6,10-emethyl-5,9-undecadine-2-one, and 1-ethyl-2-methyl-cyclohexane were increased in HC. The Random Forest classification model based on these VOCs had a sensitivity of 89.4% and a specificity of 73.3%. These VOC biomarkers should be further investigated, as this study represented an initial step in the development of biomarkers and the metabolism of these compounds in the human body and potential relationship to IBS is poorly understood.

Although studies have divergent reports of the presence of visceral hypersensitivity in IBS, such as one study that showed that 21% of subjects with IBS had increased rectal pain sensations and 17% had decreased,[16] studies assessing visceral hypersensitivity by barostat have been conducted.[17-19] A study (86 IBS patients, 78 non-IBS patients, and 25 normal controls) suggested that rectal barostat testing to discriminate IBS patients from normal subjects

Table 1. Performances of Biomarkers for Irritable Bowel Syndrome to Identify Irritable Bowel Syndrome

Biomarkers	Comparison population	Sensitivity (%)	Specificity (%)	Positive LR	Negative LR	AUC
10 marker panel[7]	Non-IBS	50.0	88.0	4.17	0.57	0.76
34 marker panel[8]	HC	81.0	64.0	2.25	0.30	0.81
Combination of 34 marker panel and psychological measurement[8]	HC	85.0	88.0	7.08	0.17	0.93
8 marker panel[9]	HC	88.1	86.5	6.53	0.14	0.89
Fecal calprotectin[10]	IBD	93.0	94.0	15.50	0.07	NR
Fecal SCFA[13]	HC	92.0	72.0	3.29	0.11	0.89
Fecal SgII[14]	HC	80.0	79.0	3.81	0.25	0.86
Fecal SgIII[14]	HC	80.0	68.0	2.50	0.29	0.79
Fecal CgB[14]	HC	78.0	69.0	2.52	0.32	0.78
Fecal VOC[15]	HC	89.4	73.3	3.35	0.14	0.83
Rectal hypersensitivity ≥ 40 mmHg[17]	HC and non-IBS	95.5	71.8	3.39	0.06	NR
Rectal hypersensitivity ≥ 26 mmHg[19]	HC	63.0	90.0	6.30	0.41	0.77

LR, likelihood ratio; AUC, the area under the curve; SCFA, short chain fatty acids; SgII, secretogranin II; SgIII, secretogranin III; CgB, chromogranin B; VOC, volatile organic compounds; IBS, irritable bowel syndrome; HC, healthy control; IBD, inflammatory bowel syndrome; NR, not reported.

and non-IBS patients had a sensitivity of 95.5% and a specificity of 71.8% at the level of 40 mmHg.[17] In other study with a total of 126 IBS patients and 30 HC, optimal discrimination between IBS patients and HC at 26 mmHg with a visual analogue scale cut-off of ≥ 20 mm had a sensitivity of 63% and a specificity of 90%.[19] However, no consensus has been reached regarding the definition of visceral hypersensitivity. The repetitive stimulus of balloon distension may also be less sensitive. The performance of each of these biomarkers is presented in Table 1.

In addition to these biomarkers, another study assessed 3 quantitative traits including colonic transit time by scintigraphy, fecal bile acid (BA), and intestinal permeability which sought to discriminate between 64 IBS-D, 30 IBS-C, and 30 HV.[20] Total 48-hour fecal BA was significantly increased in IBS-D compared to HV (2495 ± 382 vs 957 ± 185 μM/48 hr). Colonic transit geometric center at 48 hours was significant in discriminating HV from IBS-C (3.86 ± 0.17 vs 3.22 ± 0.17). Small intestinal permeability could not be used to discriminate between the groups. The model of fecal BA excretion and colonic transit geometric center at 48 hours had a sensitivity of 60% and a specificity of 75% for discrimination between IBS-D and HV. Using the same model, IBS-C could be differentiated from HV with a sensitivity of 60% and a specificity of 80%. Alteration of colonic transit was only identified in one-third of IBS patients,[16] and about one-fourth of patients with lower functional GI disorders and diarrhea had BA malabsorption.[21] Finally, there have been studies attempting to find colonic mucosal immune markers, but these are still being debated.[22]

Specific Biomarkers for Diarrhea-predominant Irritable Bowel Syndrome

IBS-D occupies a special position amongst the IBS subtypes. The predominant symptom of diarrhea in IBS should be distinguished from IBD or celiac diseases. Moreover, about 10% of patients who have suffered from acute gastroenteritis subsequently develop post-infectious IBS.[23] Cytolethal distending toxin B (CdtB) is commonly produced by bacterial pathogens that cause gastroenteritis, including *Campylobacter jejuni*, which causes post-infectious phenotypes in a rat model which are similar to those in human IBS subjects.[24] The levels of circulating host antibodies to CdtB correlated with levels of small intestine bacterial overgrowth, and these anti-CdtB antibodies cross-reacted with the enteric neural protein, vinculin, likely through molecular mimicry.[24] A recent large scale study including a total of 2681 subjects (2375 IBS-D subjects, 43 healthy subjects, 121 celiac, and 142 IBD subjects) demonstrated that anti-CdtB antibodies had a sensitivity of 43.7% and a specificity 91.6% at a cut-off value of ≥ 2.80 to discriminate IBS-D from IBD.[25] Anti-vinculin antibodies had a sensitivity of 32.6% and a specificity of 83.8% at a cut-off value of ≥ 1.68 to distinguish IBS-D from IBD. This important finding acknowledges the possibility of ruling in IBS in contrast with previous serum-based biomarkers,[7,8] which is a big leap forward in ascertaining an organic basis approach, rather than a symptom-based criteria approach. This test establishes the possibility that IBS is an organic disease with a significant pathophysiology-based biomarker distinct from IBD.

Another research study distinguished IBS-D from active IBD using fecal volatile organic metabolites (VOMs).[26] Thirty IBS-D, 62 active Crohn's disease, 48 active ulcerative colitis, and 109 HC participants were recruited. Using the 11 key VOMs, the discriminatory model showed a sensitivity of 96% and a specificity of 80%. Diet and medication were not controlled. The study population was small in number and analysis of fecal VOMs was standardized.

Specific Biomarkers for Constipation-predominant Irritable Bowel Syndrome

Lactulose breath testing (LBT) measures methane and hydrogen in breath samples obtained at baseline and every 15 to 20 minutes after ingestion of 10 g lactulose until 2 hours or even later using gas chromatography.[27] The definition for a methane-positive test or a methane producer varies in the literatures (Table 2).[28-38] However, a breath methane level ≥ 3 ppm at any point during the test has been recently used to define methane producers.[34,36] Methane production as a diagnostic test has been shown to be very accurate in predicting IBS-C, with a sensitivity of 91% and a specificity of 81.3%.[33] Two earlier studies support that methane is associated with the severity of IBS-C,[33,39] and although methane does not account for all IBS-C patients, a meta-analysis including a total of 1277 subjects (319 methane producers and 958 methane non-producers) showed that methane is significantly associated with IBS-C.[40] Another study demonstrated that methane-producing IBS subjects had small bowel movements, straining, lactose intolerance, and weight loss.[34] Furthermore, objective measures of constipation tracking stool habits showed that the degree of methane production on LBT correlated with the severity of constipation.[39] The quantity of methane on LBT was directly proportional to the severity of constipation, and moreover, greater methane production correlated with lower stool frequency and a lower Bristol stool score. Though LBT did not discriminate patients with IBS from healthy controls, methane-producing patients with IBS were significantly more likely

Table 2. Definition of Methane-positive Test or a Methane Producer on Breath Test

Authors	Sugar	Dose	Interval (min)	Duration (hr)	Definition	Published year
Pimentel et al[28]	Lactulose	10 g in 1-2 ounces water	15	3	Any rise before 90 min or > 20 ppm during test	2003
Pimentel et al[29]	Lactulose	10 g of syrup	15	3	> 20 ppm within 90 min	2003
Bratten et al[30]	Lactulose	10 g in 240 mL	20	3	≥ 1 ppm at baseline or any level during test	2008
Parodi et al[31]	Glucose	50 g in 250 mL	15	2	> 10 ppm in basal condition or after administration of glucose	2009
Attaluri et al[32]	Glucose	75 g in 250 mL	15	2	≥ 3 ppm on 2 separate breath samples	2010
Hwang et al[33]	Lactose	10 g in 240 mL	15	2	> 5 ppm at any point	2010
Makhani et al[34]	Lactulose	10 g of syrup	15	3	> 3 ppm at any point	2011
Sachdeva et al[35]	Glucose	100 g in 200 mL	15	2	fasting level of > 10 ppm	2011
Kim et al[36]	Lactulose	10 g in solution	15	3	> 3 ppm at any point	2012
Lee et al[37]	Lactulose	10 g in 200 mL	15	3	≥ 1 ppm during test	2013
Melchior et al[38]	Glucose	75 g in 250 mL	15	2	> 20 ppm or above 10 ppm in 2 samples by comparison with baseline level	2014

Table 3. Double-blind, Randomized, Placebo-controlled Trials of Antibiotic Treatments of Irritable Bowel Syndrome

Authors	Setting	Sample size	Subjects	Treatment methods	Primary outcome	Follow-up (wk)
Pimentel et al[28]	Single tertiary center	111	IBS	Neomycin 500 mg bid for 10 days	≥ 50% reduction in a composite score from 3 IBS symptoms	1
Chatterjee et al[39]	Single tertiary center	32	Constipation-predominant IBS	Neomycin 500 mg bid for 14 days vs neomycin 500 mg bid and rifaximin 550 mg tid for 14 days	Constipation severity on a visual analog scale	4
Pimentel et al[45]	Multi centers	1260	IBS without constipation	Rifaximin 550 mg tid for 2 weeks	Adequate relief of global IBS symptoms	12
Pimentel et al[46]	Two tertiary centers	87	IBS	Rifaximin 400 mg tid for 10 days	Global improvement in IBS	10

IBS, irritable bowel syndrome; bid, 2 times a day; tid, 3 times a day.

than non-methane-producing patients to report constipation, and significantly less likely to report diarrhea as a major symptom.[30]

However, other studies argue that methane production is not restricted to constipation-predominant diseases.[37,41,42] In a study of 1372 subjects with functional GI disorders, including 212 IBS patients, diarrhea was more common than constipation in patients with high methane levels on LBT/fructose breath tests. Furthermore, two-thirds of IBS-C patients did not have elevated methane levels after either lactose or fructose.[41] Another study demonstrated that the amounts of hydrogen and methane gas produced during LBT were not associated with IBS symptoms, except for a weak correlation between total gas amounts and a few IBS symptoms such as bloating, flatulence, and abdominal pain only in LBT-positive patients with IBS.[37] A more recent study revealed that IBS-C, which was associated with prolonged gut transit times, did not show an increase in positive testing for breath methane.[42] The authors explained the discrepancy with previous studies by variations in the definition of constipation, type of sugar, or proportion of patients with diarrhea.

In contrast, measuring breath methane to determine therapeutic response to non-absorbable antibiotics such as neomycin and rifaximin has been well established. Since eradication of small intestine bacterial overgrowth was shown to reduce symptoms of IBS,[43] double-blind, randomized, placebo-controlled studies using these antibiotics have been conducted (Table 3).[28,44-46]

Table 4. Irritable Bowel Syndrome Biomarkers

"Not IBS" markers	IBS vs HC markers	IBS-D markers	IBS-C markers
Serum[7,9] and fecal[9] panels	Fecal SCFA and granin[13]	Anti-CdtB antibodies[25]	LBT and methane production[33]
Serum panel, gene expression, and psychological measurement[8]	Breath test VOCs[15]	Anti-vinculin antibodies[25]	
Fecal calprotectin[11]	Visceral hypersensitivity/rectal barostat[17]	Fecal VOMs[26]	
	Colonic transit time, fecal BA, and intestinal permeability[20]		

IBS, irritable bowel syndrome; HC, healthy controls; IBS-D, diarrhea-predominant IBS; IBS-C, constipation-predominant IBS; SCFA, short-chain fatty acids; CdtB, cytolethal distending toxin B; LBT, lactulose breath test; VOCs, volatile organic compounds; BA, bile acid; VOMs, volatile organic metabolites.

Conclusions

For more than half of a century, IBS has not been considered an organic disease. The multifactorial pathophysiology of IBS made development of a single biomarker difficult (Table 4). To date, biomarkers for IBS were disappointing due to small study populations and the challenges of ruling out other organic diseases with only modest accuracy. To introduce accurate biomarkers, it could be necessary to break down IBS into each subtype and these biomarkers should come from the biological and mechanistic findings. Changing the current standard concept of IBS, to the idea that IBS is indeed an organic disease, is a key cornerstone. Studies validating biomarkers that identify IBS as a distinct entity, are linked to the pathophysiology of the disease, determine the organic nature of IBS and are important in predicting the type of IBS (constipation or diarrhea) appear to be emerging.

Financial support: Jae Hak Kim was supported by the Dongguk University Research Fund of 2014.

Conflicts of interest: Cedars-Sinai has licensing agreements with Valeant Pharmaceuticals, Commonwealth Laboratories, and Synthetic Biologics, Inc. Mark Pimentel is a consultant for Valeant Pharmaceuticals, Commonwealth Laboratories, Synthetic Biologics Inc, Microphama Inc, and Naia Pharmaceuticals, and is on the advisory boards for Valeant Pharmaceuticals and Commonwealth Laboratories. The remaining authors have no conflicts to disclose.

Author contibutions: Jae Hak Kim and Eugenia Lin performed the research, compiled the data, and drafted the manuscript; and Jae Hak Kim, Eugenia Lin, and Mark Pimentel edited the manuscript and approved submission of the final version.

References

1. Mearin F, Lacy BE, Chang L, et al. Bowel Disorders. Gastroenterology 2016;150:1393-1407.
2. Engsbro AL, Begtrup LM, Kjeldsen J, et al. Patients suspected of irritable bowel syndrome--cross-sectional study exploring the sensitivity of Rome III criteria in primary care. Am J Gastroenterol 2013;108:972-980.
3. Ford AC, Bercik P, Morgan DG, Bolino C, Pintos-Sanchez MI, Moayyedi P. Validation of the Rome III criteria for the diagnosis of irritable bowel syndrome in secondary care. Gastroenterology 2013;145:1262-1270, e1.
4. Stanisic V, Quigley EM. The overlap between IBS and IBD: what is it and what does it mean? Expert Rev Gastroenterol Hepatol 2014;8:139-145.
5. Ghorbani S, Nejad A, Law D, Chua KS, Amichai MM, Pimentel M. Healthy control subjects are poorly defined in case-control studies of irritable bowel syndrome. Ann Gastroenterol 2015;28:87-93.
6. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89-95.
7. Lembo AJ, Neri B, Tolley J, Barken D, Carroll S, Pan H. Use of serum biomarkers in a diagnostic test for irritable bowel syndrome. Aliment Pharmacol Ther 2009;29:834-842.
8. Jones MP, Chey WD, Singh S, et al. A biomarker panel and psychological morbidity differentiates the irritable bowel syndrome from health and provides novel pathophysiological leads. Aliment Pharmacol Ther 2014;39:426-437.
9. Mujagic Z, Tigchelaar EF, Zhernakova A, et al. A novel biomarker panel for irritable bowel syndrome and the application in the general population. Sci Rep 2016;6:26420.
10. Waugh N, Cummins E, Royle P, et al. Faecal calprotectin testing for differentiating amongst inflammatory and non-inflammatory bowel diseases: systematic review and economic evaluation. Health Technol Assess 2013;17:xv-xix, 1-211.
11. Kim JH, Sh O, Kim E-J, et al. The role of myofibroblasts in upregulation of S100A8 and S100A9 and the differentiation of myeloid cells in the colorectal cancer microenvironment. Biochem Biophys Res Commun 2012;423:60-66.
12. Natarajan N, Pluznick JL. From microbe to man: the role of microbial short chain fatty acid metabolites in host cell biology. Am J Physiol Cell Physiol 2014;307:C979-C985.
13. Farup PG, Rudi K, Hestad K. Faecal short-chain fatty acids - a diagnostic biomarker for irritable bowel syndrome? BMC Gastroenterol

2016;16:51.
14. Öhman L, Stridsberg M, Isaksson S, Jerlstad P, Simrén M. Altered levels of fecal chromogranins and secretogranins in IBS: relevance for pathophysiology and symptoms? Am J Gastroenterol 2012;107:440-447.
15. Baranska A, Mujagic Z, Smolinska A, et al. Volatile organic compounds in breath as markers for irritable bowel syndrome: a metabolomic approach. Aliment Pharmacol Ther 2016;44:45-56.
16. Camilleri M, McKinzie S, Busciglio I, et al. Prospective Study of Motor, Sensory, Psychological and Autonomic Functions in Patients with Irritable Bowel Syndrome. Clin Gastroenterol Hepatol 2008;6:772-781.
17. Bouin M, Plourde V, Boivin M, et al. Rectal distention testing in patients with irritable bowel syndrome: sensitivity, specificity, and predictive values of pain sensory thresholds. Gastroenterology 2002;122:1771-1777.
18. Posserud I, Syrous A, Lindström L, Tack J, Abrahamsson H, Simrén M. Altered rectal perception in irritable bowel syndrome is associated with symptom severity. Gastroenterology 2007;133:1113-1123.
19. Ludidi S, Conchillo JM, Keszthelyi D, et al. Rectal hypersensitivity as hallmark for irritable bowel syndrome: defining the optimal cutoff. Neurogastroenterol Motil 2012;24:729-733, e345-e346.
20. Camilleri M, Shin A, Busciglio I, et al. Validating biomarkers of treatable mechanisms in irritable bowel syndrome. Neurogastroenterol Motil 2014;26:1677-1685.
21. Valentin N, Camilleri M, Altayar O, et al. Biomarkers for bile acid diarrhoea in functional bowel disorder with diarrhoea: a systematic review and meta-analysis. Gut Published Online First: 7 Sep 2015. doi: 10.1136/gutjnl-2015-309889.
22. Ford AC, Talley NJ. Mucosal inflammation as a potential etiological factor in irritable bowel syndrome: a systematic review. J Gastroenterol 2011;46:421-431.
23. Shah ED, Riddle MS, Chang C, Pimentel M. Estimating the contribution of acute gastroenteritis to the overall prevalence of irritable bowel syndrome. J Neurogastroenterol Motil 2012;18:200-204.
24. Pimentel M, Morales W, Pokkunuri V, et al. Autoimmunity links vinculin to the pathophysiology of chronic functional bowel changes following campylobacter jejuni Infection in a rat model. Dig Dis Sci 2015;60:1195-1205.
25. Pimentel M, Morales W, Rezaie A, et al. Development and validation of a biomarker for diarrhea-predominant irritable bowel syndrome in human subjects. PLoS One 2015;10:e0126438.
26. Ahmed I, Greenwood R, Costello Bde L, Ratcliffe NM, Probert CS. An investigation of fecal volatile organic metabolites in irritable bowel syndrome. PLoS One 2013;8:e58204.
27. Triantafyllou K, Chang C, Pimentel M. Methanogens, methane and gastrointestinal motility. J Neurogastroenterol Motil 2014;20:31-40.
28. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome. a double-blind, randomized, placebo-controlled study. Am J Gastroenterol 2003;98:412-419.
29. Pimentel M, Mayer AG, Park S, Chow EJ, Hasan A, Kong Y. Methane production during lactulose breath test is associated with gastrointestinal disease presentation. Dig Dis Sci 2003;48:86-92.
30. Bratten JR, Spanier J, Jones MP. Lactulose breath testing does not discriminate patients with irritable bowel syndrome from healthy controls. Am J Gastroenterol 2008;103:958-963.
31. Parodi A, Dulbecco P, Savarino E, et al. Positive glucose breath testing is more prevalent in patients with IBS-like symptoms compared with controls of similar age and gender distribution. J Clin Gastroenterol 2009;43:962-966.
32. Attaluri A, Jackson M, Valestin J, Rao SS. Methanogenic flora is associated with altered colonic transit but not stool characteristics in constipation without IBS. Am J Gastroenterol 2010;105:1407-1411.
33. Hwang L, Low K, Khoshini R, et al. Evaluating breath methane as a diagnostic test for constipation-predominant IBS. Dig Dis Sci 2010;55:398-403.
34. Makhani M, Yang J, Mirocha J, Low K, Pimentel M. Factor analysis demonstrates a symptom cluster related to methane and non-methane production in irritable bowel syndrome. J Clin Gastroenterol 2011;45:40-44.
35. Sachdeva S, Rawat AK, Reddy RS, Puri AS. Small intestinal bacterial overgrowth (SIBO) in irritable bowel syndrome: frequency and predictors. J Gastroenterol Hepatol 2011;26(suppl 3):135-138.
36. Kim G, Deepinder F, Morales W, et al. Methanobrevibacter smithii is the predominant methanogen in patients with constipation-predominant IBS and methane on breath. Dig Dis Sci 2012;57:3213-3218.
37. Lee KN, Lee OY, Koh DH, et al. Association between symptoms of irritable bowel syndrome and methane and hydrogen on lactulose breath test. J Korean Med Sci 2013;28:901-907.
38. Melchior C, Gourcerol G, Dechelotte P, Leroi AM, Ducrotté P. Symptomatic fructose malabsorption in irritable bowel syndrome: a prospective study. United European Gastroenterol J 2014;2:131-137.
39. Chatterjee S, Park S, Low K, Kong Y, Pimentel M. The degree of breath methane production in IBS correlates with the severity of constipation. Am J Gastroenterol 2007;102:837-841.
40. Kunkel D, Basseri RJ, Makhani MD, Chong K, Chang C, Pimentel M. Methane on breath testing is associated with constipation: a systematic review and meta-analysis. Dig Dis Sci 2011;56:1612-1618.
41. Wilder-Smith CH, Materna A, Wermelinger C, Schuler J. Fructose and lactose intolerance and malabsorption testing: the relationship with symptoms in functional gastrointestinal disorders. Aliment Pharmacol Ther 2013;37:1074-1083.
42. DuPont AW, Jiang ZD, Harold SA, et al. Motility abnormalities in irritable bowel syndrome. Digestion 2014;89:119-123.
43. Pimentel M, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. Am J Gastroenterol 2000;95:3503-3506.
44. Pimentel M, Chang C, Chua KS, et al. Antibiotic treatment of constipation-predominant irritable bowel syndrome. Dig Dis Sci 2014;59:1278-1285.
45. Pimentel M, Lembo A, Chey WD, et al. Rifaximin therapy for patients with irritable bowel syndrome without constipation. N Engl J Med 2011;364:22-32.
46. Pimentel M, Park S, Mirocha J, Kane SV, Kong Y. The effect of a nonabsorbed oral antibiotic (rifaximin) on the symptoms of the irritable bowel syndrome: a randomized trial. Ann Intern Med 2006;145:557-563.