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Review article: small intestinal bacterial overgrowth – prevalence, clinical features, current and developing diagnostic tests, and treatment

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SUMMARY

Background
The symptoms and signs of small intestinal bacterial overgrowth (SIBO) are often identical to a variety of diseases and can lead to diagnostic confusion.

Aims
To review the diagnostic options for SIBO and present new investigative options for the condition.

Methods
A literature search was performed on MEDLINE, EMBASE and Web of Science for English articles and abstracts. Search terms included free text words and combinations of the following terms 'small intestinal bacterial overgrowth', 'small bowel bacterial overgrowth', 'diagnostic tests', 'treatment', 'antibiotics', 'probiotics', 'metabonomics', 'proton nuclear magnetic resonance spectroscopy', 'electronic nose' and 'field asymmetric ion mobility spectrometry'.

Results
All of the available methods to test for SIBO have inherent limitations and no 'gold-standard' diagnostic test for the condition exists. Accurate diagnosis of SIBO requires identification of bacterial species growing inappropriately within the small intestine and symptom response to antibiotics. Proton nuclear magnetic resonance spectroscopy, electronic nose technology and/or field asymmetric ion mobility spectrometry may represent better investigative options for the condition.

Conclusions
Novel diagnostic options are needed to supplement or replace available tests.

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INTRODUCTION

In the healthy human host, there are intrinsic mechanisms that control the number and composition of the microbiota in different regions of the gastrointestinal (GI) tract. Gastric acid destroys many bacteria before they leave the stomach. Once in the small intestine, biliary and pancreatic secretions limit bacterial growth; antegrade peristalsis in the small intestine reduces luminal growth potential; the intestinal mucus layer traps bacteria and the ileo-caecal valve inhibits retrograde translocation of bacteria from the colon into the ileum. Clinical conditions associated with small intestinal bacterial overgrowth (SIBO) are shown in Table 1.

There is no consensus as to a definition for SIBO. As a result, its true prevalence and relationship with other clinical disorders remain uncertain. The most commonly cited definition is quantitative: 10^5 or more colony-forming units per millilitre (CFU/mL) of bacteria grown from a small intestinal aspirate.[1] However, many patients with a wide range of GI conditions and symptoms have increased bacterial counts in the small intestine compared with healthy controls and older age also correlates with rising counts of small intestinal strict anaerobes, although total bacterial counts generally remain below 10^5 CFU/mL.[2]

Some authors suggest that the presence of upper respiratory bacteria in SIBO is clinically significant, but the presence of these organisms is not clearly associated with abnormal GI symptoms.[3] To date, it is only intestinal overgrowth with microbiota that commonly colonise the colon (mainly Gram-negative, strict anaerobes and Enterococci) that is clearly linked to a pathological state characterised by abnormal GI symptoms. If these bacteria are eradicated by antibiotics, then the symptoms resolve.[48, 49]

There are three common approaches towards diagnosing the condition: the first is the traditional approach of classifying it in quantitative terms in a microbiological context; the second is the breath testing technique using carbohydrates (e.g. glucose and lactulose); the third uses the symptomatic response to a trial of antibiotics. Two or three of these techniques are often combined for a more robust approach.

Table 1 | Reported prevalence of small intestinal bacterial overgrowth in normal populations and disease states

	Reported prevalence of SIBO	(references)
Normal populations		
Healthy study controls	0–20%	4–12
Dysmotility/gut wall injury		
Coeliac disease	9–67%	13–15
Connective tissue diseases, e.g. scleroderma	43–55%	16, 17
Crohn's disease	25–88%	18–20
Diabetes mellitus	8–44%	10, 21
Hypothyroidism	54%	22
Nonspecific dysmotility	76%	23
Radiation enteropathy	26%	24
Ulcerative colitis	81%	25
Miscellaneous		
Chronic fatigue syndrome	81%	20
Chronic pancreatitis	34–92%	26, 27
Drug-induced inhibition of acid secretion	26–75%	4, 23, 28
End-stage renal failure	36%	29
Fibromyalgia	93%	20
Irritable bowel syndrome	4–78%	6, 11, 30–33
Immunodeficiency syndromes	30–50%	34, 35
Liver cirrhosis	17–36%	36, 37
Obesity	17–41%	5, 38
Parenteral nutrition	70%	39
Rosacea	46%	40
Neuromuscular diseases		
Muscular dystrophy	65%	41
Parkinson's disease	54%	42
Surgery		
Abdominal surgery	82%	43
Bilateral truncal vagotomy	93%	44
Gastrectomy	63–78%	45, 46
Ileocaecal valve resection	32%	19
Roux-en-Y reconstruction	86%	47

LITERATURE SEARCH

A literature search was performed using the MEDLINE, EMBASE and Web of Science databases. The search was not date-restricted but was limited to abstracts and articles published in English. Search terms included free text words and combinations of the following terms 'small intestinal bacterial overgrowth', 'small bowel bacterial overgrowth', 'diagnostic tests', 'treatment', 'antibiotics', 'probiotics', 'metabonomics', 'proton nuclear magnetic resonance spectroscopy', 'electronic nose' and 'field asymmetric ion mobility spectrometry'.

CLINICAL FEATURES OF SIBO

Patients with SIBO may be clinically asymptomatic or have symptoms that fit the diagnostic criteria of irritable bowel syndrome (IBS). There are few studies, which have focused on identifying the predominant clinical symptoms in patients with SIBO. Those that have, suggest that the most common symptom caused by SIBO is diarrhoea, followed by abdominal pain and then bloating (Table 2).

Table 2 | Studies that have identified symptoms associated with small intestinal bacterial overgrowth

Reference	Study type and patient group	Sample size	Test(s) for SIBO	GI symptoms assessed	Patients with a positive test for SIBO		
					Number	% Patients with symptoms	*P* value†
28	Case series, G-O reflux on PPIs	*n* = 42	Glucose hydrogen breath test	*n* = 7	*n* = 11	Bloating (59%)	<0.001
						Diarrhoea (50%)	<0.001
						Flatulence (55%)	<0.001
						Pain (50%)	<0.001
4	Prospective, G-O reflux on PPIs and IBS patients	*n* = 450	Glucose hydrogen breath test	*n* = 5	*n* = 152	Bloating (50%)	N/A
						Constipation (10%)	N/A
						Diarrhoea (30%)	N/A
						Pain (10%)	N/A
58	Case reports, elderly	*n* = 5	14C-glycocholic acid breath test	Not described	*n* = 5	Diarrhoea (60%)	N/A
						Weight loss (100%)	N/A
59	Case reports, children	*n* = 9	Aspirate and culture lactulose breath test	Not described	*n* = 9	Diarrhoea (89%)	N/A
						Pain (11%)	N/A
16	Case series, systemic sclerosis	*n* = 51	Glucose hydrogen methane breath test	*n* = 11	*n* = 22	Bloating (86%)	<0.001
						Constipation (77%)	0.02
						Diarrhoea (59%)	<0.001
						Fever (18%)	0.03
						Pain (50%)	0.003
						Tenderness (55%)	0.003
60	Case series, gastroparesis	*n* = 740	Lactulose breath test	*n* = 20	*n* = 79	Bloating (13%)	N/A
						Constipation (13%)	N/A
						Diarrhoea (13%)	N/A
						Nausea (27%)	N/A
						Pain (20%)	N/A
19	Case series, Crohn's disease	*n* = 150	Glucose hydrogen breath test	*n* = 1	*n* = 38	Stool frequency >6/day (29%)	0.012
30	Prospective, IBS	*n* = 202	Lactulose breath test	*n* = 8	*n* = 157	Diarrhoea (37%)	N/A
						Pain (47%)	N/A
61	Retrospective, mixed patients	*n* = 675	Aspirate and culture	*n* = 6	*n* = 54	Steatorrhoea (11%)	N/A
15	Prospective, coeliac disease*	*n* = 15	Lactulose breath test	*n* = 4	*n* = 10	Gastric stasis (20%)	N/A
						Diarrhoea (30%)	N/A
						Pain (50%)	N/A

GI, gastrointestinal; G-O, gastro-oesophageal; IBS, irritable bowel syndrome; PPI, proton pump inhibitor; N/A, not applicable; SIBO, small intestinal bacterial overgrowth.

* Coeliac disease patients with persistent symptoms despite adherence to a gluten-free diet for >6 months.

† *P* value refers to the significance of the symptom in patients with a positive test for SIBO vs. those with a negative test.

Although many other symptoms have been described in SIBO, the significance of these symptoms is difficult to ascertain, given that the majority of studies have not used validated symptom questionnaires. Moreover, some studies describe patients with one symptom, where others describe patients with up to 20 abnormal symptoms. Other features of SIBO have also been identified, namely signs of nutrient malabsorption: weight

loss, fat-soluble vitamin deficiencies and deficiencies of vitamin B12, iron, serum bile acids and red blood cell folate.

Weight loss

Weight loss resulting from SIBO has been described.[19] Fat, protein and carbohydrate malabsorption may lead to reduced availability of nutrients to the host and subsequent weight loss. Steatorrhoea (fat malabsorption) may result from SIBO and is principally due to bacterial deconjugation of bile acids and subsequent deficiency of intraluminal conjugated bile acids.[50] It has been postulated that in SIBO, the microbiota are responsible for deaminating dietary protein in the lumen of the GI tract. As a consequence, there is a diversion of dietary nitrogen into urea formation, with the result that it becomes unavailable for protein anabolism by the human host.[51]

Carbohydrate malabsorption can result from SIBO due to reduced disaccharidase function and increased intraluminal carbohydrate degradation by bacteria.[52, 53] This macronutrient malabsorption coupled with chronic GI symptoms, which often include bloating, cramps and diarrhoea, may result in reduced dietary intake secondary to disease-related anorexia and subsequent weight loss.

Fat-soluble vitamin deficiencies

With vitamin D deficiency, osteomalacia or hypocalcaemia can occur and osteoporosis is a recognised complication of SIBO. Bone mineral density in the femoral neck and lumbar spine has been reported to be lower in patients with SIBO than in a reference population.[54]

There have been reports of vitamin E deficiency syndromes (neuropathy, T-cell abnormalities) in SIBO [55, 56] and a single case-report of night blindness caused by vitamin A deficiency secondary to SIBO.[57] Levels of vitamin K, however, are usually normal or raised in the context of SIBO as a result of bacterial synthesis of menaquinone.

Vitamin B12 and iron deficiency

Megaloblastic, macrocytic anaemia can occur in SIBO and is due to vitamin B12 (cobalamin) deficiency.[62] Polyneuropathy due to vitamin B12 deficiency has also been described and is attributed to greatly reduced absorption of both free and intrinsic-factor-bound vitamin B12.[63] Facultative Gram-negative aerobes and anaerobes are shown to be capable of competitively utilising vitamin B12.[64]

Iron deficiency anaemia can occur in SIBO. Although the exact mechanism is not known, it is likely due to injury to the mucosa caused by bacterial toxins, short-chain fatty acids and/or unconjugated bile acids. Such injury may inhibit iron absorption.

Altered immunological parameters

The immune system plays a role as evidenced by the high prevalence of SIBO in patients who have immunodeficiency.[35, 65] Duodenal and jejunal immunoglobulin A immunocytes have been shown to be significantly increased in the mucosa of patients with SIBO.[66, 67]

SMALL INTESTINAL BACTERIAL OVERGROWTH AND IBS

In many conditions, it can be difficult to assess whether SIBO is a cause for the GI symptoms and/or malabsorption or whether these occur as a result of a primary disease and SIBO is just an epiphenomenon.[19, 68] In this regard, SIBO as the aetiology or as a bystander in IBS has received the most attention. There is a definite overlap between the symptoms that define IBS[69] and those which are typical of SIBO (e.g. abdominal pain, bloating, flatulence, diarrhoea and/or constipation).

A systematic review and meta-analysis of studies investigating the frequency of SIBO in IBS found that the prevalence of SIBO in subjects meeting diagnostic criteria for IBS was between 4% and 64%.[70] Variation in prevalence rates depended on the type of test used and the criteria used to define a positive test result. From the 12 studies reviewed, there was found to be a three to fivefold increase in the odds of a positive test result in individuals with IBS. However, this failed to reach statistical significance when the criteria that gave the lowest prevalence of a positive test were used.[6, 11] Also, there was shown to be significant heterogeneity between studies, small study effects and publication bias leading to a likely overestimation of the prevalence of a positive test for SIBO. The authors concluded that there is insufficient evidence to justify the routine exclusion of SIBO in people with IBS. This reiterates the findings of an earlier Rome Consensus Report.[71]

In another recent systematic review and meta-analysis of case–control studies in IBS patients with abnormal breath tests, the authors came to a different conclusion – 'This meta-analysis demonstrates that the breath test is a valid and important catalyst in the development of the bacterial hypothesis for IBS'.[72] The weight of their argument (odds ratio of 9.64 for abnormal breath test in IBS vs. controls) was based on three studies that utilised age- and sex-matched controls. However, two of these studies used paediatric subjects. Also, significant between-study

heterogeneity was demonstrated and there was a large imbalance between the size of case and control groups in the studies reviewed.

The continued controversy surrounding the implication of SIBO in the pathogenesis of IBS is due to a lack of confidence in the validation of breath testing.[73] It will remain a problem until robust definitions of what constitutes significant SIBO are reached. This will not happen until objective diagnostic measures are defined.

Table 3 | Limitations associated with common diagnostic techniques

Small intestinal aspiration and culture technique
Invasive and cumbersome
Time-consuming and expensive
Technical difficulties with transport and culture of the aspirate
No consensus on sample handling and microbiological techniques
Appropriate use of anaerobic techniques is necessary
Representation of the sample is unknown
Oral bacteria may contaminate the aspirate
No consensus for defining SIBO in quantitative terms
SIBO occurring more distally in the small intestine may be missed
False-negative results may occur where overgrowth is caused by obligate anaerobes
Location of sampling and the amount of fluid recovered can be variable
Breath testing technique
Low fibre diet required for 24 h before the test
Smoking, sleep and exercise can affect test accuracy
Antibiotics and laxatives need to be avoided before the test
No consensus on a definition for a positive test (regardless of the substrate used)
Breath sampling frequency is highly variable
Both H_2 and CH_4 gases should be measured
Luminal pH differences affects carbohydrate metabolism
Carbohydrate malabsorption may lead to false-positive result
Consideration of oropharyngeal bacteria
Rapid transit time can give a false-positive result
Delayed gastric emptying/slow transit can give a false-negative result
Inconsistencies in the definition of an "early peak" in lactulose breath testing
Therapeutic trial approach
Follow-up may be difficult
No standardised approach towards the type, dose or duration of the antibiotic regimen
No consensus on the meaning of a clinical response to antibiotics
Over-prescribing of antibiotics
Risk of serious side effects from antibiotic treatment
Difficulty in identifying patients without SIBO vs. those with SIBO caused by an antibiotic-resistant organism

H_2, hydrogen; CH_4, methane; SIBO, small intestinal bacterial overgrowth.

DIAGNOSTIC CHALLENGES IN SIBO

Traditionally, many authors have regarded the direct aspiration and culture of duodenal fluid as the 'gold standard' approach for diagnosing SIBO.[74, 75] However, having applied the criteria of Reid *et al.* for the development and application of a diagnostic test to the currently available approaches for diagnosing SIBO[76] in their systematic review, Khoshini *et al.* concluded that no gold standard diagnostic test for SIBO exists.[1] All of the commonly used methods of diagnosing SIBO have inherent limitations; clinicians should be cautious when interpreting the results of such tests (Table 3).

In terms of microbiological quantification, there is a lack of clarity on the cut-offs that define a positive culture and technical difficulties associated with transporting and culturing the aspirate. Aspiration-based approaches also suffer from being invasive, costly and potentially risky to the patient. Furthermore, culturing reveals only a fraction (estimated at 20%) of microbiota compared with genomic methods.[77]

In recent years, owing to the invasive nature of the direct aspiration and culture technique, indirect tests have been developed and are now commonly used alternatives. Breath tests have advantages over the direct culture method, in that they are simple to use, cheap and non-invasive. However, there is no breath test specifically validated for the diagnosis of SIBO. Hydrogen-based breath tests are currently the most popular and work on the assumption that the only source of hydrogen (H_2) production in the body is from fermentation of carbohydrates by GI microbiota.[73]

The most commonly used substrates in breath tests are glucose and lactulose, with the former having a greater diagnostic accuracy than the latter. Compared with the direct aspiration method, the glucose-H_2 breath test has a sensitivity of 62.5% and a specificity of 81.7%.[71] For this test, it is considered positive if there is a clearly recognisable H_2 peak, exceeding 10–20 parts per million.[74] The lactulose-H_2 breath test has a sensitivity of 52.4% and a specificity of 85.7% when compared with the direct aspiration method.[71]

The original definition of a positive lactulose-H_2 breath test in detecting SIBO [78] was later revised, so as to minimise false-positive results. The modified criteria for a positive test are as follows: an increase in breath H_2 of >10 parts per million (resulting from small intestinal bacterial fermentation) above basal that occurs >15 min before the

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prolonged peak (resulting from colonic fermentation) and also within 20 min of ingestion of the lactulose.[79]

However, 8–27% of humans do not have detectable H_2 production from their GI microbiota, but instead produce methane (CH_4) gas.[80, 81] For example, *Staphylococcus aureus*, *Streptococcus viridans*, Enterococci, Serratia and Pseudomonas species do not produce H_2. Therefore, if H_2 is analysed in isolation, the test may miss overgrowth of non-H_2-producing bacteria, leading to false-negative tests.

Again, there is a lack of consensus how to define an abnormal breath test. There is neither agreement on the optimal duration of the breath tests nor on the cut-off levels that define a positive result. Ultimately, there are theoretical and practical problems underlying the use of breath tests that limit their potential for substantial improvement in diagnosing SIBO.

The third approach for diagnosing SIBO is to treat it when symptoms and/or non-invasive surrogate markers are clinically suggestive of SIBO (Table 4) and to use the clinical response to antibiotics as an affirmation of SIBO as the cause of the patient's complaints – the so-called 'therapeutic trial'.[30] With the problems associated with culture and breath testing methods, it is unsurprising that Khoshini *et al.* found that almost one third of studies used this therapeutic trial approach for diagnostic purposes. There is, however, no standardised approach towards the type, dose or duration of the antibiotics and reported clinical response rates range from 35% to 100%.[1]

A therapeutic trial can also be used in association with other diagnostic tests, i.e. all of the following could be taken into consideration, so as to confirm the presence of SIBO: abnormal GI symptoms/non-invasive surrogate markers, abnormal test(s) (e.g. breath test and/or aspirate and culture) and clinical response to antibiotics. Measuring response necessitates assessing symptom change systematically, as well as the resolution of abnormal parameters such as low serum vitamin B12 concentrations or improved body weight.

As SIBO is often a manifestation of other GI disorders, there is as yet no typical patient. Part of the difficulty in establishing a confident diagnosis of SIBO in patients with common GI symptoms is the lack of a standardised investigative tool.

TREATMENT FOR SIBO

The primary goal of therapy in SIBO should be the treatment of any underlying disease or structural defect, although for many conditions, this cannot be achieved. Management should include correction of any nutritional deficiencies, where present. This may involve nutritional support and/or supplemental fat-soluble vitamins, vitamin B12 and minerals. The use of prokinetic agents may be considered for patients with gastroparesis or intestinal dysmotility. However, the efficacy of these agents has not yet been proven.[87–90]

Treatment for SIBO aims to modify the GI microbiota, usually with antibiotics, in a way that will result in symptomatic improvement. Due to the limitations associated with qualitative and quantitative bacteriological studies and because the contaminating bacterial populations are quite numerous, choice of antibiotic remains primarily empiric. Effective treatment generally includes one or more drugs with activity against both aerobic and anaerobic enterobacteria.

Many different antibiotic regimens have been advocated for use in SIBO, including ciprofloxacin, metronidazole, neomycin, norfloxacin and doxycycline. There exists no consensus on the most efficacious dose or duration of treatment.[8, 41, 89, 91] In one study, 70% of patients with SIBO showed a good response to ciprofloxacin, while a regimen of amoxicillin–clavulanic acid and cefoxitin eradicated more than 90% of strains isolated from SIBO patients.[41, 92]

There has been a growing interest in the use of rifaximin (a non-absorbed rifamycin analogue) in SIBO management, especially in patients with IBS.[40, 93–97] A systematic review demonstrated the efficacy and short--term safety of rifaximin for IBS patients.[98] Although, the exact mechanisms by which rifaximin improves IBS symptoms remain incompletely defined, rifaximin's benefits in IBS patients are likely, at least in part, due to alteration of the quantity, location and/or quality of the host's GI microbiota.

A systematic review of the use of rifaximin in patients testing positive for SIBO has not yet been published. Although the published data on its use in this setting does point towards the benefit of the drug in the global improvement of symptoms associated with SIBO,[99, 100] further evidence in favour of rifaximin needs to be elucidated.

Given the high prevalence of primary and acquired bacterial resistance, cost of treatment, likely placebo effect and potential side effects of treatment, decisions on antibiotic management should be tailored to the individual as much as possible.

Probiotics are another potential treatment for SIBO; however, there are only pilot studies addressing their use. One open-labelled pilot study assessed the effect of *Lactobacillus casei* Shirota on SIBO patients, where SIBO

Table 4 | Non-invasive surrogate markers of small intestinal bacterial overgrowth

Reference	Patient group	Sample size	Marker	Data in favour of marker	Data not in favour of marker
16	Systemic sclerosis patients	$n = 51$	Hb	Patients with SIBO had significantly lower median levels of Hb (12.25 vs. 13.9 g/dL), $P = 0.002$	–
16	Systemic sclerosis patients	$n = 51$	Vitamin B12	–	No significance difference between patients with and without SIBO, $P = 0.133$
82	NASH	$n = 45$	Intestinal permeability	–	No significant difference between the intestinal permeability of NASH patients with SIBO compared with those without, measured by the lactulose-rhamnose intestinal permeability test
7	Patients with SIBO and controls	$n = 33$	Serum folate	–	No significant difference in serum folate levels between the two groups
47	Total gastrectomy patients	$n = 43$	Hb	Blood Hb concentration tended to be lower in the patients with a maximum hydrogen concentration in the glucose breath test, $P = 0.056$	–
47	Total gastrectomy patients	$n = 43$	Weight	In the glucose breath test, a negative correlation was observed between the maximum hydrogen concentration and weight loss/gain, $P = 0.034$	–
62	Elderly hypochlorhydric subjects	$n = 17$	Intestinal permeability	–	No significant difference between subjects with SIBO and controls for permeability as measured by lactulose and mannitol excretion
62	Elderly hypochlorhydric subjects	$n = 17$	Serum folate	–	Normal values for the parameter in all subjects with SIBO
62	Elderly hypochlorhydric subjects	$n = 17$	Vitamin B12	Two subjects had values below the normal range for vitamin B12, two subjects were already receiving vitamin B12 injections	–
83	Elderly subjects	$n = 16$	Hb	10 of the 16 patients with SIBO had a Hb level <12 g/dL	–
83	Elderly subjects	$n = 16$	Vitamin B12	4 of the 16 patients with SIBO had subnormal serum vitamin B12 levels	–
84	Patients with SIBO and healthy controls	$n = 22$	Serum unconjugated bile acid concentration	Of the nine subjects with SIBO, 2-h postprandial serum unconjugated bile acid concentrations were elevated in $n = 7$	–

Table 4 | (Continued)

Reference	Patient group	Sample size	Marker	Data in favour of marker	Data not in favour of marker
85	Patient with a 'stagnant loop'	$n = 1$	Serum unconjugated bile acid concentration	Subject had grossly elevated serum unconjugated bile acid concentrations throughout the day	–
86	Patients with GI diseases	$n = 35$	Vitamin B12	–	No significance difference in vitamin B12 levels between patients with and without SIBO

Hb, haemoglobin; g/dL, grams per decilitre; GI, gastrointestinal; NASH, non-alcoholic steatohepatitis; SIBO, small intestinal bacterial overgrowth.

was demonstrated by an early rise in breath H_2 after lactulose.[101] Following the 6-week intervention (65 billion bacteria/day), 64% of patients no longer had a positive breath test, but there was no significant improvement in abdominal symptoms.

In another pilot study, patients were randomised to receive either a probiotic or metronidazole as treatment for SIBO.[102] The probiotic contained *Lactobacillus casei*, *Lactobacillus plantarum*, *Streptococcus faecalis* and *Bifidobacterium brevis*. A statistically significant difference in symptomatic response favoured the use of the probiotic over the antibiotic ($P = 0.036$). Probiotics may have a beneficial effect in this setting, but double-blind, randomised, placebo-controlled trials are essential to demonstrate their dose effects and clinical relevance.

POTENTIAL FOR NEW DIAGNOSTIC TESTS FOR SIBO

The changes or dysbiosis of GI microbiota in SIBO are difficult to characterise in clinical practice. Although advances in genomic technology allow for phylogenetic analysis and typing in the research setting, such methods are laborious, expensive and not suitable for routine clinical application. More accessible means of gaining insight into the dysbiosis associated with SIBO include metabolic profiling of biofluids using metabonomics technology, the use of an electronic nose and/or field asymmetric ion mobility spectrometry (FAIMS) to detect volatile organic compounds (VOCs) from gases of luminal origin.

Metabonomics using proton nuclear magnetic resonance spectroscopy

Characterisation of the microbial content of the intestine is a concept that may prove useful in the identification of a new diagnostic method for SIBO. The GI microbiota have co-evolved with humans and metabonomics technology, when based on proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy, can exploit this co-evolution. It has the potential to identify biomarkers and prognostic factors and therefore might enhance the clinical diagnosis of SIBO. Each subject has their own 'metabolic fingerprint', which changes in response to disease, environmental or genetic perturbations. This concept can be applied to SIBO, by evaluating and comparing the metabolic fingerprints of healthy and diseased subjects.

Proton nuclear magnetic resonance spectroscopy is based on the application of a radiofrequency pulse to the nuclear ensemble placed in a magnetic field and observing the response after the duration of the pulse.[103, 104] Parallel application of other analytical platforms, for example, gas chromatography mass spectrometry allows the comprehensive study of the metabolome (the quantitative complement of all the low molecular weight molecules present in a biological sample) (Figure 1).[105]

It provides a robust, efficient, reproducible and relatively cheap approach for high-throughput metabolic screening of biofluids such as blood, urine, small intestinal fluid and faecal water. By combining ^{1}H-NMR spectroscopy with multivariate analysis methodologies, there is growing evidence to suggest that the metabolic profile of biofluids shows clustering of specific components in diseased individuals.[107, 108]

In a metabonomics study investigating the content of aspirates from the upper small bowel in patients with malabsorption syndrome, those with the syndrome had significantly higher median quantities of bile acids/cholesterol, acetate, lactate and formate than controls.[107] In those with malabsorption syndrome and SIBO, significantly greater quantities of acetate, lactate, formate and unconjugated bile acids were found compared with controls ($P < 0.01$

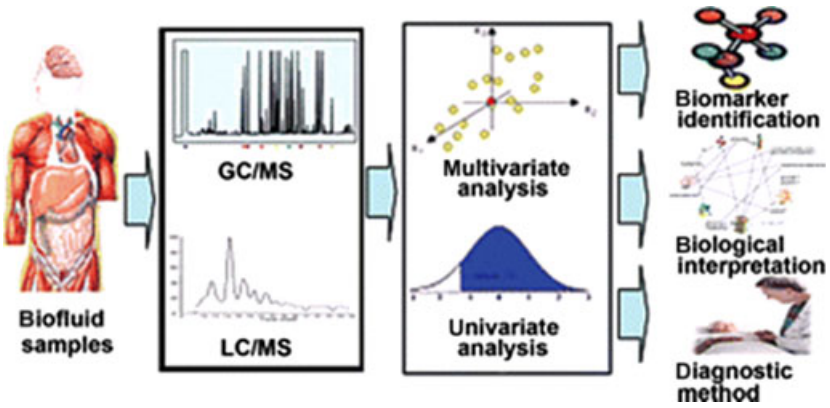

Figure 1 | Mass spectrometry-based metabonomics. GC/MS, gas chromatography mass spectrometry; LC/MS, liquid chromatography mass spectrometry. Reproduced from[106] with permission from The Royal Society of Chemistry.

for all), implying that SIBO itself might elicit a specific, potentially diagnostic metabonomic signature.

In another study using faecal samples from patients with inflammatory bowel disease (IBD) (n = 10 Crohn's disease, CD and n = 10 ulcerative colitis, UC) and healthy controls (n = 13), a metabonomics approach was employed to aid with diagnosis.[109] The researchers reported that the faecal samples obtained from the patients with CD and UC manifested similar global differences in metabolic profiles compared with the healthy subjects. A depletion of short-chain fatty acids, including acetate and butyrate, was a prominent feature of CD patients when compared with healthy subjects. In addition, a high concentration of glycerol was found in the faeces of CD patients in comparison to UC patients. Higher concentrations of amino acids were also found in the faeces of patients with both CD and UC as compared with the healthy controls. This could be a consequence of a malabsorption caused by the inflammation.

Chronic inflammatory cells in the lamina propria have been reported in rats with SIBO secondary to an experimental blind loop.[110] Also, data in humans indicate that local lamina propria immunoglobulin A plasma cell and intraepithelial lymphocyte counts are increased in SIBO.[67, 111, 112] As the condition has been shown to result in microscopic mucosal inflammation, it is plausible to consider that overall differences in the metabolic profiles of SIBO patients and controls are likely to be found.[113] Also, as it is thought that a dysbiosis of the GI microbiota is involved in IBD, either in initiating it or in maintaining it, and as SIBO is also related to dysbiosis, it may be that following the successful application of metabonomics in IBD, it will also prove relevant in SIBO.[109, 114]

Occasionally, a single marker molecule will provide an adequate measure of a disease. However, in reality, most human diseases are polygenic in origin and are conditionally linked to environmental influences.[105] Thus, it is more likely that multiple marker molecules will be needed. Studies have shown that inherent factors such as gender, age, circadian rhythms and external factors such as diet, physical activity, stress and drugs can modulate metabonomic profiles.[115, 116] Sample collection, storage and preparation also need to be considered as sources of variation in the profiles.

Electronic nose and field asymmetric ion mobility spectrometry

The concept of using volatile molecule detection as a means to diagnose SIBO is not a new one. Gas chromatography of jejunal fluid has previously been used to detect and identify volatile fatty acids (short-chain fatty acids) resulting from the fermentation of organic material by nonsporing anaerobic microorganisms. In one study, an increase in the concentrations of the fatty acids, acetate and propionate, was shown in the jejunal contents of patients with stagnant loop syndrome.[117]

In another series of patients thought to have SIBO, a complete microbiological analysis of jejunal aspirates was performed.[75] The results from this were then compared with other testing methods including gas chromatographic detection of the volatile fatty acids in the aspirates. The gas chromatography method was found to have a sensitivity of 56% and a specificity of 100%. Factors to explain the low sensitivity of this method include the preponderance of facultative Gram-negative over anaerobic bacteria in the study group and the required 12-h fast pre-intubation, which would have resulted in a lack of fermentable substrate available to the bacteria.

Gas chromatography, along with mass spectrometry is still considered the gold standard of sample analysis. These traditional approaches provide information on the individual chemical components within a sample, unlike the electronic nose, which analyses the sample as a whole to produce a 'chemical fingerprint'.

The electronic nose was first developed in the 1980s as a way of mimicking the biological olfactory system and used to detect VOCs from human samples, e.g. breath, sweat, blood, urine or faecal samples.[118] VOCs are an important component of the metabolome and include alcohols, aldehydes, furans, ketones, pyrroles, terpenes and others.[119] The electronic nose is not attempting to measure one specific compound, but is able to measure a collection of multiple marker molecules. Colonic fermentation creates several chemicals including VOCs, which may prove important for GI homeostasis. The electronic nose comprises an array of metal gas sensors, whose resistance is modulated in the presence of a target gas/vapour such as VOCs. Each sensor is different in some way (generally broadly tuned to a chemical group) and so the interaction between the sample and each sensor is unique.

The 'chemical fingerprint' produced by this method has been shown to be disparate in different disease groups due to a relative change in the proportions of the VOCs emitted in diseased individuals. The investigation of faecal VOCs may be a promising way of diagnosing SIBO because human faecal samples represent dietary end-products resulting from digestive and excretory processes and intestinal bacterial metabolism. In SIBO, the presence of anaerobic bacteria in the small bowel effectively leads to fermentation occurring in the small bowel in addition to the colon, i.e. an altered intestinal bacterial metabolism. This altered metabolism has the potential to be identified using the electronic nose method.

The electronic nose has not yet been piloted in patients with suspected SIBO. It has, however, proven successful in a range of GI, metabolic and infectious diseases.[120] A pilot study identified a distinct pattern of VOCs in the faeces of patients with UC, *Clostridium difficile* and *Campylobacter jejuni*, which strongly suggests that specific changes occur in the pattern of VOCs in GI disease.[121] In another study by this group, the analysis of VOCs from the faeces of Bangladeshi patients affected by cholera showed that fewer VOCs were detected in cholera samples in contrast to healthy controls.[122]

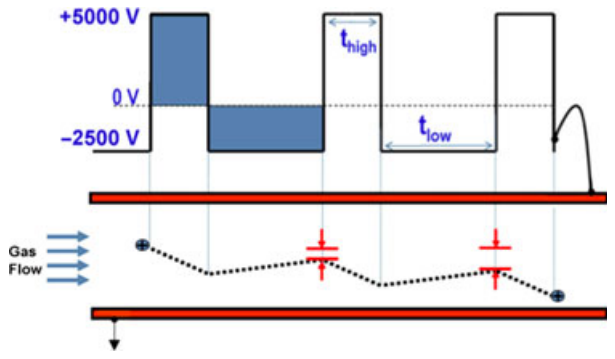

Figure 2 | Illustration of the FAIMS effect (parallel plate example), showing ion drift. V, volts; *t*, time. Source: Thermo Fisher Scientific, 2013.

The electronic nose has recently been combined with a newer technology, FAIMS. This is a technique that is able to detect VOCs that emanate from biological material in real-time. It functions by introducing ionised samples (composed of ions of varying shapes, charges and sizes) between two metal plates. An asynchronous high-voltage waveform is applied between the plates and produces conditions whereby some ions drift and hit the plates, while others remain between the plates. Using different voltages, a complex mixture of gases can be separated by differences in mobility across the plates (Figure 2).

A pilot study used both an electronic nose and FAIMS technology together to investigate if they could identify differences in faecal gas emissions between patients who developed high toxicity and low toxicity during pelvic radiotherapy.[123] The faecal samples from 23 patients were analysed (11 in the low-toxicity group and 12 in the high-toxicity group). Principle component analysis was applied to the electronic nose data and Fisher discriminant analysis to the FAIMS data. This showed that, perhaps unsurprisingly, it was possible to separate patients after treatment by their toxicity levels. However, distinct differences in the two groups were also identified in their pre-treatment samples, suggesting that severity of side effects can be predicted.

A combination of the electronic nose and FAIMS technologies were used to test their potential usefulness in differentiating between IBD subjects and controls using urine samples.[124] Secondly, the ability of the techniques to distinguish between active IBD compared with those in clinical remission was assessed. Of the 62 adults included, there were three groups: 24 patients with UC, 24 patients with CD and 14 controls. The first two groups were divided further into those with a relapse or in remission.

When the electronic nose samples were analysed using discriminant function analysis, there was a clear separation of groups. Classifications purely according to disease groups or control led to an accuracy of 88%. This distinction was confirmed by repeating the analysis with the FAIMS technology, which gave accuracy in excess of 75% ($P < 0.001$) as compared to random classification.

The results also demonstrated that there is a fundamental difference in the VOCs emitted from the urine samples between groups in remission and those with relapse.

As with the pelvic radiotherapy and IBD patients, it is plausible to think that distinct differences in VOC patterns would also be observed between SIBO patients and healthy controls. The possibility of using an electronic nose for the detection of SIBO is particularly attractive due to its non-invasive nature, its portability and the potential to use this technology in the out-patient setting. It can be operated at room temperature and pressure. It is cheap, less time-consuming and complex than gas chromatography mass spectrometry. Many of the advantages of the electronic nose also apply to FAIMS and combining the two instruments may prove a powerful technique for the future development of a diagnostic tool for SIBO.

CONCLUSION

Small intestinal bacterial overgrowth is a significant clinical problem, which is difficult to diagnose accurately and for which the optimal therapeutic options are not defined. The concept of discovering novel biomarkers in biofluids or VOCs from biological samples to aid with SIBO diagnosis is attractive. The advantages of these techniques are manifold: rapid, non-invasive and requiring minimal preanalysis sample preparation. Of course, there is one major obstacle in both cases – the lack of a 'gold standard' diagnostic test for SIBO. This will make the interpretation of data challenging.

To validate the possible usefulness of any of these techniques in SIBO, diagnosis would necessitate the use of carefully defined groups of patients. There would then be potential to define a highly sensitive and specific test, which could revolutionise the diagnosis, and therefore management, of SIBO. In addition, the techniques described could identify novel biomarkers, potentially allowing patient stratification to facilitate a more personalised approach to tailoring antibiotic treatment to the individual. Thus, there would be a reduced alteration of the symbiotic GI microbiota caused by unnecessary antibiotic administration.

As the techniques are designed to detect all of the biochemical metabolites present simultaneously in a single measurement, it is likely that one or more will be shown to be superior to the currently available diagnostic methods for SIBO. Proving this hypothesis could open a new avenue in gastroenterology practice and research.

AUTHORSHIP

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