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Small Intestinal Bacterial Overgrowth as a cause for Irritable Bowel Syndrome: Guilty or Not Guilty?

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Abstract

Purpose of Review: Small intestinal bacterial overgrowth (SIBO) has been proposed as a cause of irritable bowel syndrome (IBS). However, this relationship has been subject to controversy. This review aims to provide a current perspective on the SIBO-IBS hypothesis.

Recent Findings: Case-control studies evaluating the prevalence of SIBO in IBS and healthy subjects have shown conflicting results. Moreover, the tests available in routine clinical practice to diagnose SIBO are not valid and lack both sensitivity and specificity. Hence, interpreting the effect of interventions based on these tests is fraught with uncertainty. Furthermore, the SIBO-IBS hypothesis has paved the way to assess antibiotic therapy in non-constipated IBS, with rifaximin, a non-absorbable antibiotic, showing modest but significant clinical benefit. However, subjects were not tested for SIBO and the mechanism of action of rifaximin in IBS remains to be elucidated. Preliminary data suggests rifaximin decreases microbial richness and previous studies have noted antibacterial interventions in IBS to reduce colonic fermentation and improve symptoms. The advent of rapid culture-independent molecular techniques is a promising tool that will seek to clarify and advance our understanding of the gut microbial function.

Summary: The SIBO-IBS hypothesis lacks convincing evidence but remains under scrutiny. The mechanism resulting in symptom improvement after rifaximin treatment in some IBS subjects requires exploration. Novel molecular techniques provide an exciting and challenging opportunity to explore the host-gut microbiota interaction.

Keywords: Small intestinal bacterial overgrowth, irritable bowel syndrome, hydrogen breath tests, rifaximin

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder that manifests with symptoms of abdominal pain and altered bowel habit in the absence of abnormal findings on routine clinical tests explaining the symptoms. IBS has a prevalence of approximately 10% in adults, shows a female preponderance, and is more common in younger individuals. The burden of illness is significant with IBS having a chronic remitting-relapsing nature and is often associated with extra-intestinal somatic symptoms as well as fatigue, depression, anxiety, and diminished quality of life.[1]

The exact cause of IBS is unknown although the prevailing hypothesis is a disorder of the brain-gut axis as demonstrated by alterations in gut immunity, visceral hypersensitivity, enteric motor function disturbances and central pain processing.[1] The intestinal microbiota has also been shown to be perturbed in a subset of IBS subjects compared to healthy controls,[2] with its interactive crosstalk at the intestinal mucosal border possibly contributing towards the pathophysiology of IBS.[3] With this in regard, it has been suggested that small intestinal bacterial overgrowth (SIBO) may play a pivotal role in the aetiology of IBS, although this has been debated.[4-6] Herein, we discuss the constituents of the normal small intestinal flora, its disturbances leading to SIBO, and the methods used in clinical practise to diagnose SIBO along with their shortcomings. Finally, we discuss whether there is sufficient evidence to currently support the SIBO-IBS hypothesis and how novel technical advances in microbial analysis may help further our understanding.

The intestinal flora

The influx of gastric acid into the proximal small intestine and the propagating activity of the migrating motor complex lead to low bacterial counts ranging from 10^0-10^3 colony forming units per ml (CFU/ml) in the duodenum and jejunum, consisting predominantly of gram-positive aerobes. Distally, there is an increase in bacterial counts with the ileum having between 10^5-10^8 CFU/ml of colonic-type bacteria which comprise gram negative aerobes, obligate anaerobes, and enterococci. In contrast to the relatively sparse bacterial counts in the small intestine, the colon has between 10^{10}-10^{12} CFU/ml.[2]

Various conditions can lead to bacterial proliferation within the small intestine, thereby giving rise to SIBO.[7] These include gastric achlorhydria secondary to proton-pump inhibitor usage or atrophic gastritis, small bowel stagnation secondary to alterations in upper gastrointestinal anatomy, and disorders affecting enteric motility such as diabetes mellitus and scleroderma. The development of SIBO under such circumstances correlates with clinical symptoms of diarrhoea, bloating, abdominal pain, weight loss, anaemia and malabsorption, which improve following treatment.[7]

Tests used to diagnose SIBO and their pitfalls

The three tests currently available in routine clinical practise to make a diagnosis of SIBO are culture of small bowel aspirates, the glucose-hydrogen breath test (GHBT), and the lactulose-hydrogen breath test (LHBT). However, they all have inherent problems with regards to accuracy and interpretation, which renders their use in clinical practise questionable.[8-11]

Small bowel aspirate and culture are considered the gold standard test, with a diagnosis of SIBO made on the basis of demonstrating $>10^5$ CFU/ml of colonic-type bacteria. However, this cut-off has been challenged as being too high given that it was determined from samples following surgical diversion and not healthy controls.[12] Instead, some have proposed a level of $>10^3$ CFU/ml to denote SIBO.[12] Furthermore, the technique to obtain small bowel aspirate is cumbersome, invasive, and runs the risk of contamination from oral flora. In addition, obligate anaerobes may not be detected and aspiration of proximal small bowel contents will not necessarily address cases of SIBO affecting the distal small bowel. Hence, the sensitivity and specificity of small bowel aspirate to detect SIBO may be limited.[9-11]

In contrast, the hydrogen breath tests are simple, non-invasive, and cheaper alternatives which are widely adopted in clinical practise. They work on the premise that hydrogen production in humans occurs only as a consequence of bacterial fermentation of carbohydrates. The hydrogen diffuses into the systemic circulation and is expired by the lungs where it can be detected using gas chromatography. As glucose is a monosaccharide which undergoes rapid absorption within the proximal small intestine, thereby theoretically avoiding exposure to distal colonic-type bacteria, a hydrogen rise is not expected in subjects without SIBO. In contrast, lactulose is a non-digestible disaccharide which passes through the small bowel followed by fermentation by colonic-type bacteria, so a hydrogen-rise is expected albeit not immediately after ingestion. Hence, through biological plausibility, an early detectable rise in hydrogen following a GHBT or LHBT has been used in clinical practise to imply premature fermentation due to SIBO.[9-11]

However, the GHBT and LHBT have several shortcomings. High basal hydrogen-levels may be detected, which can be avoided by following a low-fibre diet a day before the procedure. Hydrogen-levels can increase with smoking and decrease with exercise so both events are prohibited around the time of the test. False positives can also occur through immediate fermentation by oral bacteria, although aseptic mouthwash pre-procedure aims to counteract this.[9-11] With regards to the individual tests, concerns pertaining to the GHBT are that it lacks sensitivity given that proximal absorption of glucose will miss distal cases of SIBO. In contrast, lactulose transits the whole small

bowel and would pick up all SIBO cases; consequently the LHBT has been the most widely adopted test in clinical studies.

Additional uncertainties relate to the optimal dosage of substrate for breath tests and what constitutes an abnormal and premature hydrogen-rise. The dosage of glucose and lactulose substrate has varied in studies from 50-100g and 10-25g, respectively, with a hydrogen-rise of 10 to 20 parts per million (ppm) being used to diagnose SIBO. Historically, a double-peak sign was used to identify SIBO with LHBT, with the first peak denoting small bowel fermentation (i.e. SIBO) and the second peak normal colonic fermentation. However, this lacks validity as commonly there is just one peak seen or in the case of two-peaks the first may occur when the substrate has already reached the caecum.[8-11] Subsequently, a single hydrogen-rise of 12-20ppm within 90 minutes, or in some cases 180 minutes, has been proposed to diagnose SIBO instead. However, concerns remained that this may still not be detecting SIBO as both IBS and healthy subjects have variable oro-caecal times;[9,13] in healthy subjects this is on average 90 minutes, and therefore in around 50% of cases an early hydrogen-rise will be indicative of rapid intestinal transit with ensuing colonic fermentation.[9] Finally, lactulose accelerates intestinal transit and further complicates interptretation.[14]

Indeed, a well-designed study has confirmed these suspicions.[15] Forty subjects with IBS ingested 10g of lactulose along with a radio-labelled test meal containing ^{99m}Tc. Subsequent serial LHBT and nuclear scintigraphic scanning were performed. The investigators noted that 63% had abnormal LHBT at 180 minutes and 35% at 90 minutes. The oro-caecal time based on scintigraphic scanning ranged from 10 to 220 minutes and correlated with IBS-subtype. Importantly, at the time of hydrogen-rise the accumulation of ^{99m}Tc in the caecum was >5% in 88% of cases. In summary, this study suggests that an abnormal hydrogen-rise with the LHBT in IBS patients can be explained by variations in the oro-caecal transit times and therefore does not support a diagnosis of SIBO.[15]

The specificity of the GHBT has also come under similar scrutiny.[16,17] A historical study, utilising GHBT with concurrent scintigraphy, noted 8 of 25 subjects with chronic diarrhoea (and intact gastrointestinal anatomy) to have rapid intestinal transit leading to colonic hydrogen production.[16] More recently, a retrospective study of 139 patients undergoing GHBT and concurrent scintigraphy has demonstrated high rates of false-positive results for SIBO.[17] Of the 33% (n=46) of subjects with abnormal GHBT at 90 minutes, 48% (n=22) had a hydrogen-rise after the glucose substrate had reached the caecum. Subgroup analysis demonstrated that i) for the 45 patients with a history of upper gastrointestinal surgery, 69% (n=31) had abnormal GHBT of which 65% (n=20) were false positives and ii) for the 94 patients without gastrointestinal surgery, 16% (n=15) had abnormal GHBT of which 13% (n=2) were false positives. Subjects with false-positive results had shorter mean oro-

caecal time (18 minutes) compared with true-positive (79 minutes) or negative results (86 minutes). The investigators conclude that almost half of positive GHBT results are false positives and that concurrent use of scintigraphy will be a valuable tool in discriminating between true and false-positives.[17,18]

In summary, the tests used to diagnose SIBO lack sensitivity and specificity.[9-11] An expert consensus working group initially concluded that the diagnostic accuracy of GHBT was 71.7% whereas that of LHBT was 55.1%.[19] However, in light of recent studies further emphasising the limitations of hydrogen breath tests,[15,17] these values could potentially be challenged. Future studies should aim to utilise the rapid molecular approaches that have largely replaced cultural approaches for enumeration of the dominant GI microbiota.[2] These include 16S RNA-based microbiota profiling which allows quantitative and qualitative analysis of gastrointestinal mucosal and luminal content microbiota. Moreover, "-omic" based analysis allows in depth study of microbial function.[2] This will provide important insights into the interactions between the host and gut microbiota, and the relative importance of small bowel bacteria in the pathophysiology of IBS.

The relationship between SIBO and IBS

The controversy surrounding SIBO as a cause for IBS has spanned over a decade. The main proponents for the SIBO-IBS hypothesis initially showed that a positive LHBT was seen in 78% of IBS cases and that in a subset open-label antibiotic treatment led to a negative LHBT and clinical improvement.[20] Following on, a case-control study by the same group noted a positive LHBT in 84% of IBS cases compared with 20% of healthy controls. Double-blind placebo-controlled treatment with neomycin in the LHBT-positive IBS group led to normalisation of LHBT and symptom improvement.[21] Given that neomycin can lead to bacterial resistance the investigators turned their attention to the safety and efficacy of rifaximin, a non-absorbable antibiotic, in IBS.

The TARGET 1 and 2 trials were two identical multi-centre, double-blind, placebo-controlled studies together randomising 1258 IBS subjects without constipation to either placebo or rifaximin 550mg TDS for 14 days.[22] Unfortunately, the subjects were not tested for SIBO. By week 4 there was a significant, albeit modest, improvement in global IBS symptoms in the rifaximin group compared to the placebo group (40.7% vs. 31.7%, Δ=9%, $p<0.001$), translating to a number needed to treat (NNT) of 11. Adverse events were similar between the groups and there were no cases of *Clostridium difficile* infection. In addition, a greater percentage of rifaximin- than placebo-treated subjects reported durable improvement in IBS symptoms during the 10-week follow-up period. However, the study was not designed to determine the persistence of this treatment effect beyond this time point.

This recently published TARGET 3 was a key study designed to assess the safety and efficacy of repeated courses of rifaximin in subjects with diarrhoea-predominant IBS.[23] Of 2579 IBS patients receiving rifaximin, 1074 (41.6%) showed clinical improvement within 4 weeks and were followed up for 18 weeks. Of the initial responders, 636 (59.2%) relapsed and were randomised in a double-blind manner to receive either a 2-week course of rifaximin 550mg or placebo TDS. A recapture response rate within 4 weeks was significantly greater with rifaximin than placebo (38.1% vs. 31.5%, Δ=7%, p=0.03). Adverse events were low and similar between the groups. These findings suggest that rifaximin is efficacious and safe to use in diarrhoea-predominant IBS. Since May 2015 rifaximin has been approved by the US Food and Drug Administration for the treatment of adult patients with diarrhoea-predominant IBS. However, rifaximin is an expensive drug (~$50 per day), and the modest efficacy, as well as the potential need for repeated antibiotic prescriptions in a relatively young patient group does raise apprehension.

The accumulating evidence suggests a relationship between the gut microbiota and IBS,[2] and that this interplay can be modulated via antibiotics.[24] However, in some instances, the data has been interpreted to promulgate the SIBO-IBS hypothesis although this assumption cannot be made for various reasons, which include:

1. Other groups evaluating the yield of a positive small bowel aspirate or hydrogen breath tests in subjects with IBS and healthy controls have shown conflicting results (Table 1).[21,25-38] A systematic review and meta-analysis in 2009 found the pooled odds ratio for any positive test was 3-5 fold times greater in IBS than controls.[39] However, this was not significant when the criteria that gave the lowest prevalence was used, but was significant when the criteria that gave the highest prevalence was used. Importantly, there was marked statistical heterogeneity between the studies and funnel plot asymmetry to suggest publication bias or other small study effects.[39] Finally, most studies did not take into consideration confounding factors such as proton pump inhibitor usage, which are commonly consumed by IBS subjects and independently associated with SIBO.[40] As a result the association between SIBO and IBS was deemed uncertain.[39]

   A few studies have now used culture-independent molecular techniques to analyse small bowel microbiota in IBS and healthy controls, with conflicting results.[41-44] In general, these studies have been of relatively small sample-size and used different techniques to obtain small bowel microbial content (aspirate/mucosal brushing/biopsies).[41-44] Large-scale uniform studies using modern culture-independent techniques are needed.

2. A fundamental issue is that the currently available tests are poor methods to diagnose and monitor SIBO.[9-11] The LHBT, which has been the most frequently used for this purpose in clinical studies, commonly measures oral-caecal time and colonic fermentation and therefore does not reliably diagnose SIBO.[15] The GHBT has also faced similar criticisms. Hence, it can be argued that studies using breath tests to diagnose SIBO and subsequently interpret the effect of antibiotics as altering the small intestinal microbiota may be misconstrued. Rather, the evidence suggests modulation of colonic microbiota. This would be supported by a recent case-control study using wireless motility capsule to show that intestinal intraluminal pH (a surrogate marker for fermentation) remains similar in the small bowel of IBS and healthy controls, but significantly drops in the colon for those with IBS compared with healthy controls.[45] Furthermore, previous studies have demonstrated dietary or antibacterial interventions to reduce colonic fermentation and improve IBS symptoms.[46,47] With regards to the pathophysiological mechanism of rifaximin in IBS, preliminary data has shown a decrease in gut microbial richness although large-scale studies are needed.[48]

3. Whereas the biopsychosocial model for IBS is accepted, the SIBO-IBS hypothesis has been questioned from an epidemiological and evolutionary perspective.[6] For example, the SIBO-IBS hypothesis suggests that infection causes IBS and that alternate competing hypothesis are a secondary epiphenomenon. However, antibiotics have a NNT of 11 which is less favourable compared to some of the treatment modalities used to address the brain-gut axis and not SIBO. For example, the NNT with antidepressants, antispasmodics, peppermint oil and placebo without deception has been shown to range from 2.5 to 5.[6] However, no head-to-head trials exist.

   Furthermore, whereas a dose-symptom correlation relationship has been established for psychosocial trauma and IBS, this has not been shown for SIBO and IBS.[6] A prospective dual-centre US study evaluating subjects with unexplained gastrointestinal symptoms (seemingly characteristic of IBS) found there to be no difference in overall symptom scores between those testing positive against those testing negative for SIBO, when using either small bowel aspirate or GHBT. Moreover, there was no difference in any symptom scores between subjects with small bowel aspirate of $>10^3$ CFU/ml vs. $>10^5$ CFU/ml.[49] Hence, even if SIBO is present in IBS, its relevance for symptoms is unclear.

Conclusion

There is undoubtedly great interest in underpinning the role of intestinal microbiota in IBS. The evidence suggests that this interaction appears to be with regards to the colonic microbiota and its crosstalk with the host. In contrast, the evidence for SIBO and IBS is shrouded with controversy, predominantly due to the fact that the tests used in clinical practise to diagnose SIBO are not valid. With this is mind, SIBO cannot be currently found guilty for causing IBS. Nevertheless, it remains a suspect under surveillance and with the advent of culture-independent molecular techniques future studies should aim to advance our understanding of the gut microbiome for symptom generation in patients with IBS.

Key points

- Small intestinal bacterial overgrowth (SIBO) has been proposed as a cause of IBS.
- However, the tests used in routine clinical practise to diagnose SIBO lack standardisation and validity, which leaves the SIBO-IBS hypothesis open to debate when evaluating the effects of interventions.
- Rifaximin has recently been shown to be of benefit in non-constipated IBS subjects but with no information about the presence of SIBO.
- Preliminary data suggests rifaximin to reduce microbial richness, although large-scale studies are needed to see how this relates to symptomatology.
- Future large-scale studies should utilise novel culture-independent molecular approaches to investigate the host-gut microbial interactions to determine the role of small bowel bacteria in IBS pathophysiology.

Table 1: Case-control studies using small bowel aspirate or glucose/lactulose-hydrogen breath (GHBT, LHBT respectively) tests to investigate the association between SIBO and IBS in adults

First author	Year	Country	Number of IBS cases vs. healthy controls	Test used to diagnose SIBO	SIBO prevalence: IBS vs. healthy controls
Pimentel[21]	2003	US	111 vs. 15	LHBT	84% vs. 20%,*
Walters[25]	2005	Canada	39 vs. 20	LHBT: double peak LHBT: >20 ppm H2-rise within 90mins LHBT: >20 ppm H2-rise within 180 mins	10% vs. 10% 28% vs. 30% 69% vs. 75%
Lupascu[26]	2005	Italy	65 vs. 102	GHBT	31% vs. 4%,*
Posserud[27]	2006	Sweden	162 vs. 26 54 vs. 20 46 vs. 21	Jejunal aspirate >10^5 CFU/ml (colonic bacteria) Jejunal aspirate ≥5 x 10^3 CFU/ml (any bacteria) Jejunal aspirate ≥5 x 10^3 CFU/ml (colonic bacteria) GHBT LHBT: double peak LHBT: >20 ppm H2-rise within 90mins LHBT: >20 ppm H2-rise within 180 mins	4% vs. 4% 43% vs. 12%,* 11% vs. 4% 2% vs. 0% 15% vs.20% 35% vs.45% 78% vs. 70%
Bratten[28]	2008	US	180 vs. 34	LHBT	74% vs. 85%
Rana[29]	2008	India	225 vs. 100	GHBT	11% vs. 1%,*
Parodi[30]	2009	Italy	130 vs. 70	GHBT	16% vs. 4.3%,*
Ghoshal[31]	2010	India	192 vs. 51	GHBT	8.5% vs. 2%
Lombardo[32]	2010	Italy	200 vs. 50	GHBT	24.5% vs. 6%
Rana[33]	2012	India	175 vs. 150	LHBT GHBT	34% vs. 30% 6.2% vs. 0.7%,*
Park[34]	2010	Korea	76 vs. 40	LHBT	44.7% vs.40%
Sachdeva[35]	2011	India	59 vs. 37	GHBT	23.7% vs. 2.7%,*
Moraru[36]	2014	Romania	331 vs. 105	GHBT	31.6% vs. 6.6%,*
Abbasi[37]	2015	Iran	107 vs. 107	GHBT	37.4% vs. 12.1%,*
Chu[38]	2016	China	89 vs. 13	LHBT: double peak LHBT: >20 ppm H2-rise within 90mins LHBT: >20 ppm H2-rise within 180 mins	44% vs. 38% 31% vs. 30% 75% vs. 70%

The * denotes significant results $p<0.05$

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