Pros and Cons of Breath Testing for Small Intestinal Bacterial Overgrowth and Intestinal Methanogen Overgrowth

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Abstract: Breath testing is the most widely utilized modality to diagnose small intestinal bacterial overgrowth (SIBO) and/or intestinal methanogen overgrowth (IMO). Although SIBO can be diagnosed with small bowel aspiration and breath testing, IMO can only be diagnosed with breath testing in clinical practice. Breath testing can tailor antibiotic therapy and predict response to treatment; however, the test is limited by its indirect method of measurement and concerns about the variability of orocecal transit time. Like any clinical test, breath testing has inherent strengths and limitations, and results must be interpreted with consideration of the clinical context and influencing factors. Recent studies have demonstrated the expanding clinical utility of breath testing in the diagnosis, management, and prediction of treatment response in SIBO and particularly in IMO along with the identification of distinct breath test patterns such as flat-line and high baseline hydrogen. This article reviews the strengths and limitations of breath testing in diagnosing SIBO and IMO as well as its expanding utility in clinical practice.

Keywords
Lactulose breath test, glucose breath test, small intestinal bacterial overgrowth, intestinal methanogen overgrowth

Breath testing is the most widely utilized diagnostic modality for small intestinal bacterial overgrowth (SIBO) and intestinal methanogen overgrowth (IMO).[1,2] SIBO, a condition in which excessive amounts of coliform bacteria typically found in the colon are seen in the small intestine, results in various gastrointestinal (GI) symptoms, including bloating, abdominal pain, flatulence, nausea, dyspepsia, diarrhea, and constipation. IMO, an independent entity from SIBO, in which excessive methanogens reside in the small and/or large intestine, results in bloating, gas, abdominal discomfort, and constipation.[3,4] Both SIBO and IMO have been increasingly recognized as causes of common GI symptoms.[5] Similarly, the number of conditions linked to SIBO is increasing, and research has suggested an association between the microbiome and irritable bowel syndrome (IBS).[6-8] Although SIBO, IMO, and IBS are separate disease entities, these conditions can overlap, and antibiotic therapy has been found to reduce symptoms in IBS patients with abnormal breath testing.[9-11] Currently in clinical practice, IMO is only diagnosed with breath testing. SIBO can be diagnosed with breath

Table. Strengths and Limitations of Breath Testing

Strengths
Exhaled breath hydrogen and methane are exclusive biomarkers of metabolically active gut microbes
Safe, simple, and noninvasive
Widely accessible and inexpensive with home testing option
Antibiotic therapy can be tailored based on breath test pattern[a]
• Breath testing is the only diagnostic test for IMO • IMO test results are not affected by OCTT
Spot methane measurement is a rapid point-of-care method to diagnosis IMO and assess treatment response
Lactulose breath test can help identify patients with diarrhea-predominant irritable bowel syndrome who are more likely to be rifaximin responders
Limitations
Indirectly measures microbial overgrowth
Accuracy of result relies on patient compliance to protocol: • Oral care • Avoid exercise or smoking on day of test • Avoid fermentable foods on day prior to test • No antibiotics 4 weeks before test • Discontinuation of promotility agents or laxatives 1 week before test • Avoid colonoscopy bowel preparation at least 2 weeks before test
Various commercial home tests: • May use thresholds for breath hydrogen and methane that are different from cutoffs outlined in clinical guidelines • May include a combined criteria of hydrogen and methane, which are not supported by data and can lead to false-positive results
Low breath hydrogen can occur when excessive methanogens and hydrogenotrophic bacteria are present
SIBO test results can be affected by variations in OCTT: • Rapid OCTT can result in false-positive result • Slow OCTT can result in false-negative result
Conditions that impair delivery of the carbohydrate substrate to the small intestine can result in false-negative results (ie, gastroparesis, gastric outlet obstruction, achalasia, and enterocutaneous fistula)

[a]Hydrogen-predominant SIBO treatment: rifaximin; IMO treatment: rifaximin/neomycin, rifaximin/flagyl, or ciprofloxacin/flagyl.

IMO, intestinal methanogen overgrowth; OCTT, orocecal transit time; SIBO, small intestinal bacterial overgrowth.

testing or small bowel aspiration, although the latter is not readily available in most clinical settings and is limited by a high contamination rate. Breath testing has been subject to criticism owing to its indirect manner of measuring microbial overgrowth and concerns about false-positive results. Glucose and lactulose are the 2 main substrates used for breath testing for SIBO and IMO, and each test type has distinct advantages and disadvantages. Like many diagnostic tests, breath tests have inherent strengths and limitations, and results can be affected by multiple factors (Table). This article summarizes the current literature on the pros and cons of breath testing and its utility in clinical practice for the diagnosis and management of SIBO and IMO.

Small Intestinal Bacterial Overgrowth

The symptoms of SIBO are well known to be nonspecific and nonpredictive; hence, symptoms alone cannot be used to diagnose SIBO. A retrospective study by Baker and colleagues found that symptomatic patients with GI symptoms referred for breath testing had similar rates of heartburn, regurgitation, chest pain, nausea, abdominal pain, bloating, gas, diarrhea, and constipation irrespective of glucose breath test results.[12] Additionally, response to empiric antibiotic therapy as a means to rule in SIBO or IMO is not recommended because of the cost, risk of *Clostridioides difficile* colitis, and development of drug resistance.[13] Therefore, objective testing is recommended in patients with both symptoms and predisposing conditions to prevent overdiagnosis.

There is no true gold standard test for SIBO diagnosis; however, 2 primary methods are currently used. The most direct method of evaluating for SIBO is an upper endoscopy with small intestinal fluid aspiration.[1] A threshold of greater than 10^3 colony-forming units of coliforms/mL is needed for SIBO diagnosis.[1] This method is costly, invasive, and not widely available in most clinical settings. Small bowel aspiration also has a risk of contamination by oral flora and sampling error, particularly if bacteria in the mid or distal small bowel are not sampled, which further limits its clinical utility.[14,15]

A recent study by Cangemi and colleagues found that use of single lumen catheters to aspirate the small bowel is associated with a 19.6% contamination rate, underlining the lack of a true gold standard test for the diagnosis of SIBO.[15] Furthermore, deep sequencing studies indicate that not all hydrogenogenic bacteria are culturable,[16] and as commercial microbiology laboratories are not able to culture methanogenic archaea, intestinal aspiration cannot be used to diagnose IMO. Consequently, the more widely used alternative to assess for SIBO is breath testing. The breath test detects microbial overgrowth by quantify-

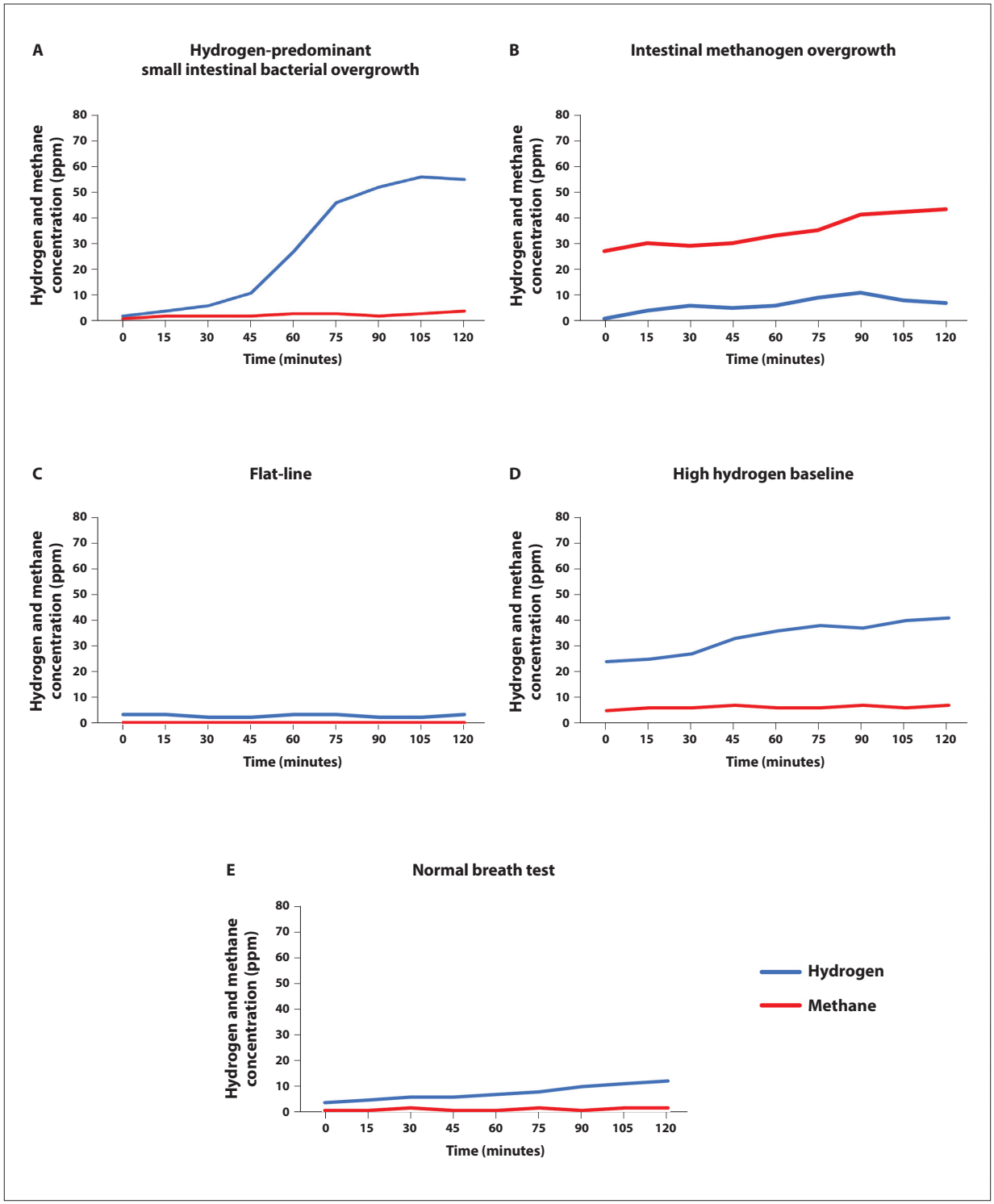

Figure. Diagnostic criteria and breath test patterns according to the North American Consensus statement are shown. Diagnosis of hydrogen-predominant small intestinal bacterial overgrowth can be made via small bowel aspiration showing greater than 10^3 cfu/mL or with breath testing that shows an early rise in hydrogen (blue line) of 20 parts per million (ppm) or more above baseline by 90 minutes (**A**). Intestinal methanogen overgrowth is defined as methane (red line) of 10 ppm or more at any point during the test (**B**). Flat-line pattern is defined as no methane and low fixed hydrogen production (low fixed hydrogen ≤3 ppm and no rise >1 ppm above baseline) (**C**). High baseline hydrogen is defined as baseline hydrogen greater than 20 ppm with no excessive methane (**D**). Normal breath test result (**E**).

ing hydrogen gas, which is produced when a carbohydrate substrate is fermented by bacteria, and methane, which is produced by archaea.[17,18] The gases then diffuse into the bloodstream and are eventually expired by the lungs.[17] Per the North American Consensus statement, an early rise in hydrogen of 20 parts per million (ppm) or more above baseline by 90 minutes is considered a positive glucose or lactulose breath test result for SIBO (Figure A).[1] A recent comparative study by Baker and colleagues proposed that a cutoff of greater than 12 ppm is more sensitive for SIBO compared with the criteria set by the North American Consensus in the case of the glucose breath test.[19]

Pros of Breath Testing

Breath testing is overall the preferred diagnostic method for SIBO. Breath testing is safe, simple, and noninvasive. The test is also widely available and can be administered at home, which is helpful for patients who are unable to travel or who live in remote areas.[2,20] Although breath testing is an indirect assessment of microbial overgrowth, recent duodenal microbiome data using 16S ribosomal RNA sequencing suggest that breath hydrogen is positively correlated with a relative abundance of specific classes of bacteria. Additionally, patients with SIBO have upregulation of metabolic pathways that lead to hydrogen production.[16] Another advantage of breath testing is that it can distinguish distinct gas patterns of SIBO from those of IMO or overgrowth of hydrogenotrophic bacteria, and therefore the results can help tailor antibiotic therapy. This differentiation is important because while hydrogen-predominant SIBO responds well to rifaximin, archaea associated with IMO are resistant to most antibiotics and respond better to combination therapy (eg, rifaximin/neomycin) compared with a single antibiotic (eg, rifaximin alone).[11,20]

Both lactulose and glucose substrates have their unique advantages and disadvantages, and there is no consensus on which is the preferred substrate. Lactulose, a synthetic disaccharide that is nondigestible and nonabsorbable, has the theoretical advantage of being able to sample the entire small intestine and can potentially identify distal SIBO. Glucose, a monosaccharide that is rapidly absorbed in the proximal small intestine, is considered a more specific test because it is less likely to result in false-positives from colonic fermentation. A recent meta-analysis cited a sensitivity of 42% and specificity of 70.6% for the lactulose breath test compared with a sensitivity of 54.5% and specificity of 83.2% for the glucose breath test.[21] As the current techniques of small bowel aspiration have significant contamination rates, calculation of the exact sensitivity and specificity rates of breath testing is not possible because the presumed gold standard (ie, small bowel aspiration) accuracy is suboptimal. Lactulose may be the preferred substrate for diabetic patients because it does not carry any risk of hyperglycemia. The lactulose breath test also has clinical utility in patients with diarrhea-predominant IBS. One study found that of patients with diarrhea-predominant IBS who underwent a lactulose breath test, those with a positive result were more likely to have improved symptom response to rifaximin compared with those who had a negative result.[9] Glucose, which is rapidly absorbed by the small bowel, is unlikely to result in colonic fermentation and may be a better substrate for patients with known or suspected rapid orocecal transit times (OCTTs).[22] Currently, both substrates are acceptable options to diagnose SIBO.

Cons of Breath Testing

One of the limitations of breath testing for SIBO is the variability of OCTT. Hence, the major criticism of glucose and lactulose breath testing is that the early rise in breath hydrogen reflects colonic fermentation rather than SIBO. A study by Yu and colleagues combined lactulose breath testing with scintigraphy and found that 88% of individuals (22/25) with a positive result (defined as rise in breath hydrogen of 20 ppm from baseline by 180 minutes) had 5% or more of the technetium 99m (equivalent to 0.5 g of lactulose) in the cecum on scintigraphy before the test result became positive.[23] The problem with this conclusion is that 5% of the test meal, which is equivalent to 0.5 g of lactulose, is unable to cause an instantaneous and significant rise in breath hydrogen. It is expected that it would take time for the substrate to ferment and produce gas, and then for the gas to diffuse into the bloodstream and be expired by the lungs for quantification. In fact, Read and colleagues observed that when lactulose was infused directly into the cecum at a rate of 0.15 g/min, it took approximately 40 minutes for the breath hydrogen to rise by 20 ppm.[24] At a slower rate of 0.02 g/min, it took several hours to increase the breath hydrogen by 20 ppm. Additionally, when 5% or more of the substrate is in the cecum, the majority of the substrate is still within the small intestine and likely the main contributor to the early rise in breath hydrogen.

There has also been criticism that the glucose breath test is prone to false-positive results in patients with a history of upper GI surgery. Lin and Massey found that of 46 patients with abnormal breath test results who underwent glucose breath testing concurrently with scintigraphy, 22 had false-positive results caused by colon fermentation, and of the 22 patients, 20 had a history of upper GI surgery, whereas only 2 patients had no prior surgery.[22] The authors concluded that scintigraphy should be performed with glucose breath testing in those with a history of foregut surgery, but the feasibility of this clinical practice is unclear. Overall, the clinician

performing the breath test assumes that the OCTT is within normal range. Scintigraphy has demonstrated that normal OCTT is approximately 83 minutes. Still, the uncertainty of OCTT is a major drawback when interpreting breath test results. For patients who are at risk for rapid small bowel transit (eg, those with a history of upper GI surgery), clinicians could consider concurrent scintigraphy or evaluating for SIBO with an upper endoscopy and small bowel aspiration.

Another downside of breath tests is the concern regarding the potential for false-negative results. A false-negative result can occur when an underlying process prevents the carbohydrate substrate from reaching the small intestine. Some examples include achalasia, gastroparesis, gastric outlet obstruction, and an enterocutaneous fistula. A false-negative test can also occur when there is delayed OCTT, and the substrate does not reach the region of small bowel affected by bacterial overgrowth. Additionally, breath hydrogen can be reduced when a predominance of methane-producing organisms are consuming hydrogen to generate methane.[6,25-28] Lastly, when patients have symptoms suggestive of SIBO but are not responding to antibiotic treatment, the clinician should consider the presence of another process such as small intestinal fungal overgrowth, which can be diagnosed using small bowel aspiration with fungal cultures.[29] One challenge in breath testing is the recognition of new breath test patterns. Test results that were previously thought to be normal, such as the flat-line and elevated baseline hydrogen patterns, may actually represent presence of excess hydrogenogenic or hydrogenotrophic bacteria that can benefit from treatment (as discussed in the "Emerging Breath Test Patterns" section).

Intestinal Methanogen Overgrowth

A major strength of breath testing is its ability to diagnose IMO. Breath testing is currently the only available way to identify IMO in clinical practice. Because IMO is attributed to overgrowth of methanogenic archaea (anaerobic organisms from the domain Archaea), rather than bacteria, it is a separate clinical entity from SIBO.[20] Per the North American Consensus, a methane level of 10 ppm or more at any point during breath testing is considered diagnostic for IMO (Figure B).[1] Unlike breath testing for SIBO, IMO is not affected by OCTT. Methane gas slows intestinal transit, and consequently, IMO is associated with constipation and constipation-predominant IBS.[4,30,31] Methane levels are not prone to fluctuations and directly correlate to the severity of constipation.[32] A large retrospective study (N=1461) further demonstrated how IMO is a distinct condition from SIBO.[33] In the study, methane producers were more likely to be older and had a lower frequency of vitamin B_{12} deficiency or diarrhea compared with those with hydrogen-predominant SIBO. Additionally, many classic risk factors for SIBO such as Roux-en-Y gastric bypass, diabetes, and prior cholecystectomy were not associated with IMO.[33]

Identifying IMO also has important therapeutic implications because species of archaea are resistant to most antibiotics.[34] In a retrospective study by Low and colleagues, IMO responded better to combination therapy with rifaximin and neomycin rather than rifaximin alone.[11] According to the study, 85% of patients treated with rifaximin and neomycin attained clinical response compared with 56% of patients in the rifaximin-only group. Additionally, 87% of patients treated with rifaximin and neomycin were able to eradicate methane on breath testing compared with only 29% of patients in the rifaximin-only group. Furthermore, in a small randomized controlled study (N=31) of patients with constipation-predominant IBS with a breath methane level greater than 3 ppm, the combination of neomycin and rifaximin reduced constipation, straining, and bloating more than rifaximin alone.[35] Breath testing can provide a reliable assessment of treatment response. In the same study, patients who had reduced methane levels to less than 3 ppm after combination therapy reported lower constipation severity compared with patients who did not have reduced methane levels after treatment.

Lastly, a recent study by Takakura and colleagues found that a fasting single methane measurement (SMM) was able to accurately diagnose IMO and monitor response to treatment.[36] A fasting SMM of 10 ppm or more had a sensitivity of 86.4% and specificity of 100% for diagnosing IMO on the glucose and lactulose breath test. Moreover, IMO was associated with a higher rate of constipation and bloating and, importantly, directly correlated with stool methanogen load. After antibiotic therapy, there was a significant decrease in SMM starting at 2 days. Therefore, a spot methane level provides a reliable and rapid point-of-care method to diagnose IMO and monitor treatment response.

Emerging Breath Test Patterns

Our understanding of breath test patterns continues to expand. Recently, research has identified 2 distinct clinically important patterns: flat-line and high baseline hydrogen. These patterns are important to recognize because they may indicate overgrowth of specific strains of bacteria. Studies have shown that patients with either pattern may respond to antibiotic therapy, and the response may be different depending on the pattern. Further studies are needed to better characterize the clinical significance of these unique breath patterns.

Flat-Line Pattern

The flat-line pattern, defined as no methane and low fixed hydrogen (≤3 ppm and no rise >1 ppm above baseline) production (Figure C),[37] is uncommon and more frequently seen in patients with inflammatory bowel disease.[38] The flat-line pattern may be clinically important because a significant proportion of patients with this pattern respond to antibiotics. Of patients with the flat-line pattern treated with antibiotics, 56% responded to any antibiotics; of those, 58% responded to rifaximin, and 47% responded to a combination of rifaximin and neomycin.[39] It has been proposed that the flat-line pattern may be attributed to excess hydrogenotrophic bacteria consuming hydrogen to produce hydrogen sulfide. Alternatively, the flat-line pattern could be seen in the setting of gastroparesis, when the substrate is not able to reach the small bowel, or recent antibiotic use. If an excess of hydrogenotrophic bacteria is found to contribute to the flat-line pattern, measurement of exhaled hydrogen sulfide may be useful. Currently, measurement of hydrogen sulfide gas is not widely available, although it is offered in certain home breath tests.[2]

High Baseline Hydrogen Pattern

High baseline hydrogen greater than 20 ppm with no excessive methane is another uncommon but potentially important pattern on breath testing (Figure D). In a large-scale study of lactulose breath testing (N=14,847), 107 (0.7%) patients were found to have an elevated baseline hydrogen level (>20 ppm).[40] These patients confirmed strict compliance to a low fermentation diet on the day prior to the test and fasted 8 hours overnight. Of the 107 patients with high baseline hydrogen treated with antibiotics, 52% responded to any antibiotics. The response rate to rifaximin was higher at 73%, whereas the response rate to rifaximin and neomycin was low at 25%. This breath test pattern is important to identify because patients with the pattern may benefit from antibiotic therapy with rifaximin. Further studies with small bowel aspirate culture data and deep sequencing are needed to identify which strains of bacteria can produce hydrogen in a fasted state.

Conclusion

Glucose and lactulose breath testing offers a rapid, safe, and noninvasive method for diagnosing SIBO and IMO. Like all clinical tests, breath testing has certain limitations, and the results must be interpreted thoughtfully, keeping the clinical context in mind. Breath testing carries many advantages, including its ability to identify distinct breath test patterns that can help predict treatment response and tailor antibiotic therapy and may be useful in monitoring treatment response. Newer technologies in the pipeline, such as intraluminal gas sampling and next-generation sequencing, may provide greater diagnostic accuracy and direct-targeted treatment in the future.

Disclosures

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