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The diagnosis of small intestinal bacterial overgrowth: Two steps forward, one step backwards?

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Abstract

Small intestinal bacterial overgrowth (SIBO) was originally described decades ago as a cause of malabsorption among individuals with abnormal intestinal anatomy and/or impaired gastric acid secretion and intestinal motor functions. More recently, the concept of SIBO has been expanded to explain symptoms among a much broader patient population—a move that brings the definition of SIBO into much sharper focus. For largely logistical reasons, breath tests and, especially, those based on the excretion of hydrogen consequent on the fermentation of unabsorbed carbohydrate substrates, have almost entirely replaced jejunal aspirates in the diagnosis of SIBO. Ever bedeviled by concerns regarding their reliability, hydrogen breath tests have now come under even more critical scrutiny with the study from Sundin and colleagues in this issue suggesting that their sole function is to detect carbohydrate malabsorption and that they are incapable of defining SIBO.

1 | SIBO—AN HISTORICAL PERSPECTIVE

The role of overabundant small intestinal bacterial populations in the pathogenesis of maldigestion and malabsorption was first described in the 1970s in landmark clinical studies among subjects with surgically altered gastrointestinal anatomy or intestinal dysmotility.[1-3] The term small intestinal bacterial overgrowth (SIBO) was coined to describe this condition and relationships defined between underlying cause (hypochlorhydria, impaired motility, defective antibacterial defenses, abnormal communications between small and large intestine), and the overgrowth of bacterial species normally confined to the colon (or distal reaches of the ileum), disrupted digestion and absorption of nutrients and clinical features such as diarrhea, steatorrhea, weight loss, abdominal pain, bloating, flatulence, and malnutrition. The precise mechanisms underlying these symptoms were delineated. These included the impact of products of bacterial metabolism, the utilization, by bacteria, of nutrients, such as vitamin B_{12}, by proliferating intraluminal bacterial populations and even epithelial damage in cases of more severe overgrowth. With regard to the former, attention was focused in particular on bacterial fermentation of unabsorbed carbohydrates resulting in the production of gases and short-chain fatty acids with the latter, in turn, stimulating motility and increasing intraluminal fluid content through their osmotic effects. This was what might be referred to as classical SIBO—maldigestion and malabsorption resulting from bacterial overgrowth. For many years, the gold standard for SIBO diagnosis was the detection, on culture, of $>110^5$ colony-forming units (CFUs) of bacteria per ml of jejunal fluid obtained by direct aspiration of jejunal contents.[4] This approach was invasive, subject to contamination by oral, esophageal, gastric and duodenal contents and dependent on the accurate detection of anaerobic species—its adoption in clinical practice was, not surprisingly, limited.[5] More recent studies which enumerated bacteria using high throughput sequencing approaches have, indeed, revealed the extent to which culture-based approaches underestimate the numbers [almost 100-fold less in the paper from Sundin et al[6]] and diversity of the jejunal microbiota.

2 | THE ERA OF BREATH TESTS

An alternative approach to the diagnosis of SIBO was advanced by those who harnessed the observation that the sole source of hydrogen in the human body is derived from the fermentation of carbohydrates. Some of this hydrogen is absorbed across the intestinal mucosa and enters the systemic circulation ultimately arriving in the

lungs where it is excreted in the breath. Non-invasive tests for SIBO were soon developed based on the pattern of hydrogen excretion in the breath following a carbohydrate oral load, provided by lactulose or glucose, for example.[7,8] Hydrogen breath tests soon came to be widely adopted for the diagnosis of SIBO in clinical practice based on their non-invasive nature, low cost, and technical and logistical simplicity.[7] From the outset and despite their popularity, the limitations of hydrogen breath tests began to be appreciated. Quite early on the inferior performance of lactulose (still widely utilized) over glucose as the carbohydrate substrate was recognized[8] and the interpretation of the lactulose breath hydrogen test continues to arouse controversy. One of these diagnostic criteria, the so-called double peak with the earlier of these believed to reflect abnormal fermentation of lactulose within a small intestine affected by SIBO while the second, later, peak was thought to correspond to the arrival of the substrate in the colon, was shown to have low accuracy for the diagnosis of SIBO[5,8-10] (Figure 1). The other criterion, the "early peak" is still widely regarded as diagnostic of SIBO (Figure 1). For example, a very recent manuscript concluded that "a rise in hydrogen of ≥20 parts per million (ppm) above the baseline value by 90 minutes following substrate ingestion during a glucose or lactulose breath test for SIBO was considered positive.[11] The "early peak" has also been questioned—employing simultaneous scintigraphy and breath testing, Yu et al[12] clearly showed that the rise in breath hydrogen regardless of its timing corresponded with the arrival of the substrate in the colon suggesting that rapid transit and not SIBO was responsible for many an "early peak". That the lactulose breath hydrogen test should prove neither sensitive nor specific or reproducible should come, therefore, as no surprise.[13,14] Poor correlations between the results of lactulose breath hydrogen testing and cultures of jejunal aspirates further clouded the clinical utility of this test.[9,15]

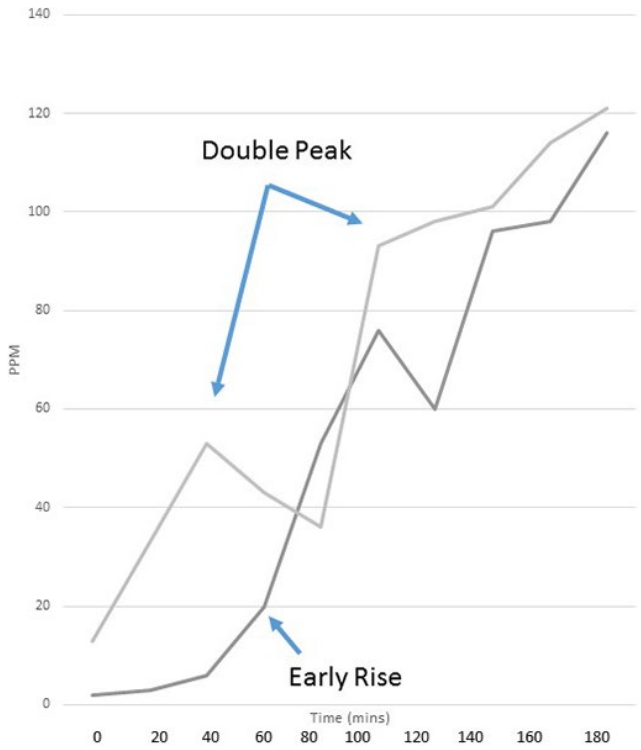

FIGURE 1 Examples of positive lactulose hydrogen breath tests. These two curves from two separate studies illustrate: *Early rise*—hydrogen concentration in exhaled breath begins to rise after 40 min and exceeds 20 ppm above baseline (time 0) by 80 min. *Double peak*—first peak at 40 min and second peak beginning at 100 min (ppm, parts per million of exhaled hydrogen)

Key Points

- SIBO has been well characterized a a cause of maldigestion and malabsorption.
- Its incrimination in the causation of other symptoms and disorders is less clearly defined and relies largely on breath testing.
- Recent studies question the accuracy of breath testing in the diagnosis of SIBO.

Oral glucose, due to its very rapid (and presumed complete) absorption in the proximal small bowel and, thus, independence from the impact of small bowel transit, appeared, at first sight, to offer a better substrate for the detection of SIBO, at least in the more proximal small intestine. It too ran into head winds. To everyone's surprise, Sellin et al[16] showed, in a group of patients with diarrhea and an intact small intestine, that this substrate was also susceptible to the influence of small intestinal transit; an observation recently confirmed by Lin et al[17] who found that a radioisotope-labeled bolus of glucose reached the cecum before the appearance of a hydrogen peak. The "early" peak may indeed be too "late" for the small intestine.

The interpretation of many earlier studies of hydrogen breath tests is confounded by a variety of methodological issues, including substantial differences in the size of the carbohydrate load administered, the osmotic and transit accelerating effects of a highly concentrated substrate solution, the duration of breath sampling, as well as the attention paid to dietary and other restrictions before and during the test.[10] Standardization, though highly desirable and promoted,[10,11] has simply not been implemented as universally as it should have been.

3 | TWO STEPS FORWARD

3.1 | Intraluminal gas sampling

There is light at the end of the tunnel. Two recent innovations promise more accurate and clinically meaningful assessments of what is really going on in the "contaminated" small intestine. In a novel approach, a system has been developed involving a capsule that one swallows and then provides real time measurements of all of the

major intraluminal gases (hydrogen, carbon dioxide, oxygen, and methane) as it transits the gastrointestinal tract.[18] Though published data to date is limited to studies in normal human volunteers, it is evident that this device is capable of detecting and quantitating concentrations of hydrogen that are orders of magnitude (4 logs higher!) higher than those detected by breath tests. This observation clearly indicates that low levels of production of intraluminal hydrogen in response to a carbohydrate substrate (in this case glucose or inulin) are not responsible for the poor performance of breath tests—much greater volumes of hydrogen are produced than were previously predicted. The profile of simultaneously measured oxygen concentrations permits the clinician to localize the capsule to the stomach, small intestine, or colon.[19] This same study again demonstrated that a glucose load of 40 g was incompletely absorbed and, as a consequence, some did undergo fermentation in the colon, resulting in a breath hydrogen peak. Of note, this dose is actually lower than the 50 g dose commonly administered to subjects undergoing testing for SIBO. This technology had a far superior signal-to-noise ratio in comparison to breath tests and, by sampling the gases at source, measured far higher concentrations than those small amounts that find their way into a breath sample. The oxygen profile can also be used to simultaneously measure transit time. By sampling at source, this technology could provide insights into how variations in the nature of the bacterial species contaminating the small bowel may affect intraluminal gas production and even its absorption; a phenomenon that will certainly impair the accuracy of hydrogen breath tests. We already know that the presence of methanogens, harbored by a not entirely negligible proportion of the general population, leads to the production of methane in preference to hydrogen.[20,21] To complicate matters further, other studies have shown that methane excretion in the breath is a poor reflection of colonic methane production.[21,22] The journey a given gas takes from its production in the intestinal lumen, across the gut wall, into the circulation and out into the breath is clearly a perilous one with all but a fraction of that originally produced surviving to appear in the breath. It is no wonder that breath tests have their problems.

The gas sampling capsule could be a "game changer"—only further studies in relevant populations will indicate its real place in the diagnostic armamentarium.

3.2 | The microbiota revolution

Indirect tests of SIBO clearly have their limitations, to say the least. The advent of high throughput sequencing, metagenomics and other omics now provides us with tools to accurately describe the complete microbial population in a given location, predict its functions via metagenomics and even measure what they actually produce using metabolomics.[23] These approaches can also bypass problems arising from the contamination of small intestinal aspirates by oral bacteria.[6] Accordingly, the next step should be to abandon indirect tests and, employing testing based on the complete characterization of the small intestinal microbiota, rewrite the textbooks relating to the prevalence, etiology and pathophysiology of SIBO. These approaches offer the potential to not only precisely delineate the composition of any contaminating bacterial populations but also to identify virulence factors, metabolic pathways, and other intrinsic bacterial properties (such as bile salt deconjugation) that may be directly responsible for the symptoms that occur in a given patient. Unfortunately, by virtue of its relative inaccessibility, our knowledge of even the normal small intestinal microbiota is limited and efforts to define its composition, using molecular techniques, in patient populations are in their infancy.[24,25] To address access and potential contamination, a novel technique that facilitates the collection of uncontaminated samples from the small intestine has been recently developed.[26]

It is unlikely that a mere increase in bacterial numbers, as measured by the culture of jejunal aspirates, may alone explain the protean clinical manifestations of SIBO. It is much more likely that these reflect the nature of the contaminating species and their unique biology. Clues to support this hypothesis are already extant. Indeed, a relationship between certain bacterial species, ammonia production, and hepatic encephalopathy was first demonstrated over 60 years ago and products of bacterial metabolism were proposed as measures of SIBO decades ago.[27-29] Our experience with empiric antibiotic therapy of SIBO also speaks to a variable impact of contaminating species on symptoms. Though the cumulative repository of high quality trials of antibiotics in SIBO remains slim, it is evident that individual antibiotics (with varying antibacterial profiles) enjoy quite variable efficacy in the clinical management of SIBO.[30-32] Further hints supporting the primacy of species and function over number comes from studies of the poorly absorbed antibiotic rifaximin which enjoys one of the better track records in terms of quality of data to support its use in SIBO.[33] In two clinical conditions where it has proven efficacy, non-constipated irritable bowel syndrome, and hepatic encephalopathy, clinical benefits do not appear to be related to major shifts in bacterial populations but rather owe their efficacy more to subtle changes in bacterial metabolism,[34-36] phenomena that will only be detected by the most detailed molecular techniques. Other data also speaks to the importance of quality over quantity when it comes to the pathogenesis of SIBO—poor correlations between bacterial numbers and symptoms,[15,37] as well as the observation that the elderly, thought to harbor a less profuse and diverse microbiota[38] are more susceptible to SIBO.[39]

Now, the study by Sundin et al[6] which involved the performance of breath testing, culture of jejunal aspirates and sequencing of these same aspirates provides firm data to support a need to shift our emphasis from the enumeration of bacteria to the definition of their function. First, they found, not surprisingly, that culture grossly underestimated bacterial numbers (by approximately 2 logs) and, second, they could not document any correlation between breath test results and bacterial numbers. Very surprisingly, they found that higher signals in the breath test correlated with lower viability of jejunal bacteria. They also agree with Berean et al[19] that glucose malabsorption represents a major contributor to positive glucose breath tests.[6] Indeed, they estimated that bacterial overgrowth at and above levels considered diagnostic of SIBO could not produce, in

the breath, hydrogen levels that are above the cutoff for commonly used diagnostic criteria. Whether or not they had factored in the concentrations of intraluminal hydrogen now reported by Berean et al[19] is not clear.

4 | ONE STEP BACKWARDS

While technology may offer real opportunities for progress in this area, we, as clinicians and clinician investigators took a step backwards when we sought to incriminate SIBO as the source of all evil. SIBO became controversial when it left its original confines as a malabsorption syndrome and came to be identified among a host of disorders which did not feature either maldigestion or malabsorption but, rather, somewhat non-specific symptoms such as bloating and altered bowel habit or no gastrointestinal symptoms at all.[40] Most contentious has been the role of SIBO in IBS.[41] Yes, SIBO is more common among those with IBS diagnosed according to current criteria but its prevalence in IBS is very dependent on what test is employed to make the diagnosis.[42] Is this really SIBO or accelerated transit?[41] Sequencing and metabolomics should help to resolve this issue and also determine whether SIBO, if truly present, is cause or consequence of a given underlying disorder.[40]

In our haste to find the "cause" for IBS, we relinquished our obligation to carefully phenotype our patient populations and to test with the rigor that was necessary. Blindly applying any test to a population as heterogeneous as IBS will only sow more confusion.

5 | SUMMARY

The application of nucleic acid amplification and other (and ever evolving) molecular techniques has revolutionized the study of the microbiota and implicated our bacterial fellow travelers in an ever-expanding spectrum of disease, ranging from diabetes to atherosclerosis and psychiatric disorders.[43-45] In animal models as well as in man, such studies have also revealed relationships between microbiota, the mucosal and systemic immune systems, the enteric neuromuscular apparatus, host metabolism and even cognitive function and human behavior that were previously considered fantastical. These same resources now need to be directed toward unraveling the conundrum that is SIBO. Does it really exist beyond the realm of its original description—maldigestion and malabsorption? Are breath tests, as these recent studies suggest,[6,19] misleading us or is there a more subtle story hiding behind a veil that has needs to be drawn back? While the studies from Berean and Sundin and their colleagues are limited in scope and great care must be taken in extrapolating their findings to clinical practice, these authors have certainly thrown down the gauntlet—it is now up to those who advocate for the use of breath tests in SIBO to respond. Only then will true consensus emerge on their real place in the diagnosis of this uncertain disorder.

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