

INTESTINAL MICROBIOTA, GAS AND NOVEL DIAGNOSTIC APPROACHES

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Abstract

The intestinal microflora in humans (microbiota) plays a key role in health and disease due to the close interaction with the host. The development of the intestinal microbiota occurs primarily during infancy. Microbiota constitutes an ecologically dynamic community and, in the adult, over 500 different bacterial species can be found in the gastrointestinal tract, with both types and concentrations of microorganisms different across the intestine (max concentration in the colon up to 1012 CFU/mL). The microbiota is heavily involved in protective, trophic and metabolic processes in health but also can be the cause or be associated with various diseases. Among all possibilities, two frequent conditions will be treated in the present review, i.e. gas-related syndrome and small intestinal bacterial overgrowth (SIBO). Both of them can cause bothering and vague symptoms which need to be carefully assessed to rule out more harmful conditions. Since microbiota is able to induce considerable amounts of gases, i.e. H₂ and CH₄, such volatile compounds can be measured in expired air by using breath testing as accurate and noninvasive diagnostic tool. This review will focus on the most recent work in this area and will report about the recently published guidelines from the Rome Consensus on the use of breath testing in Gastrointestinal diseases.

Keywords: breath test, intestinal gas, small intestinal bacterial overgrowth.

MICROBII INTESTINALI, GAZUL ȘI NOI ABORDĂRI DIAGNOSTICE

Rezumat

Microflora intestinală joacă un rol cheie în starea de sănătate și boală, datorită relațiilor strânse cu gazda. Dezvoltarea microflorei intestinale se produce mai ales în prima copilărie. Microbii intestinali reprezintă o comunitate ecologică dinamică și la adult se găsesc peste 500 de specii diferite în tubul digestiv, cu modificări depinzând de situarea în intestin (maxim în colon 1012 CFU/ml). Microbii au rol protectiv, trofic și metabolic la subiecții sănătoși și pot fi asociați cu diferite boli. Referatul de față discută relația problematică a gazului intestinal și sindromul de suprapopulare bacteriană intestinală. Ambele condiții cauzează simptome vagi, care trebuie evaluate cu atenție, spre a exclude suferințe mai nocive. Întrucât microbii pot induce cantități mari de gaz, de ex. CH₄ și H₂, astfel de compuși volatili pot fi măsurați în aerul expirat prin teste respiratorii, care sunt neinvazive și acurate. Lucrarea de față prezintă cele mai noi contribuții în acest domeniu, bazate și pe consensul Roma despre testele respiratorii.

Cuvinte cheie: teste respiratorii, gaz intestinal, floră microbiană intestinală.

The Intestinal Microbiota

In health, enteric bacteria colonize the alimentary tract immediately after birth, but after the first year of life the composition of the intestinal microflora remains relatively constant throughout life. At least 500 different bacterial

species are located in the human gastrointestinal tract and constitutes a complex ecosystem named microbiota [1,2].

Types and concentrations of microorganisms differ along the alimentary tract [3], since the health human stomach, and the proximal small bowel contain only a few species of bacteria (usually lactobacilli, enterococci, oral streptococci and other gram-positive aerobic or rare facultative anaerobes derived from oropharynx, in concentrations of 10^4 CFU/mL of jejunal content). Coliforms do not exceed a concentration of 10^3 CFU/mL and bacteroides are rarely found.

The terminal ileum is a transitional zone between the proximal small bowel aerobic microflora and the dense population of anaerobic bacteria populating the colon. In the terminal ileum enterobacteria and other coliforms reach a concentration of 10^9 CFU/mL [4]. The colon by contrast hosts a complex and variegated microbiota (up to 10^{12} CFU/mL of several species, mainly anaerobes such as bifidobacteria, lactobacilli, bacteroides, and clostridium). Proper protective, trophic and metabolic processes are guaranteed by the intestinal microbiota [5]. Positive effects have been documented on the growth of intestinal villi, crypts, enterocytes, and Peyer's patches.

Additional and important salutary physiologic functions by intestinal microbiota include **fermentation of non-digestible dietary residues** by intestinal bacteria with production of fatty acids absorbed by the colonic mucosa and used as an energy source, **production of nutrients** (i.e. deconjugated bile salts) and vitamins (folate and vitamin K). Also, **in the case of non-pathogen, commensal strains**, a protective function develops: locally-produced chemicals or bacteriocins are able to kill or inhibit the multiplication of other surrounding pathogens: in this respect bacterial-enterocyte crosstalk plays a key role via binding sites and toll-like receptors [6,7]. **Bacterial metabolism of some medications** within the intestinal lumen (i.e. splitting of sulfasalazine into 5-aminosalicylic).

Different conditions exist with respect to substrate availability in the human colon. About 20-60 g of carbohydrates and 5-20 g of proteins per day are available at the level of caecum and right colon. This condition results in very intense fermentation, production of short chain

fatty acids from saccharolysis, an acidic pH equal to 5-6 and rapid bacterial growth. In the distal colon, by contrast, the putrefactive processes are more intense with increased proteolysis, and neutral pH because of the less availability of the substrates and more static luminal bacteria [4]. Several mechanisms prevent the excessive colonization of the intestine by bacteria, including appropriate intestinal motility, gastrointestinal secretions, mucus layer and preserved anatomical conditions, and immune system (Table 1).

Intestinal gas and small intestinal bacterial overgrowth (SIBO)

Gas in the intestine derive from mainly from three events: air swallowing, intraluminal production, and diffusion from blood. The interaction between gut microflora and substrate is essential in this respect [8]. The amount of intestinal gas is around 200 ml per day, both in the fasting and postprandial states [9]. About 500-1500 ml/day of gas is excreted per rectum [10]. More than 99% of expelled intestinal gas are represented by nitrogen (N_2), oxygen (O_2) in very low concentrations, and variable concentrations of carbon dioxide (CO_2), hydrogen (H_2), and methane (CH_4) [9]. There are anatomical-related changes of intestinal gas, in that stomach gas contains high concentrations of N_2 and O_2 similar to the atmosphere, while flatus contains less O_2 and more CH_4 [9]. Minor constituents include sulfur-containing compounds such as dimethylsulfide, methanethiol, hydrogen sulfide, short-chain fatty acids, skatoles, indoles, volatile amines, and ammonia, all odour-producing compounds of flatus [11,12].

Overproduction of gastrointestinal gas can be responsible of the so-called "gas related syndrome", a series of gas disorders producing non specific gastrointestinal symptoms (eructation, flatulence, abdominal bloating and distension) [13,14]. Such symptoms can be found in about 16-30% of the general population and in about 90% of the patients with irritable bowel syndrome (IBS) [15]. Although most of the underlying conditions for the gas-related syndromes can be benign, differential diagnoses can be needed, especially in third-referral centers

Table 1. Conditions and Mechanisms keeping appropriate bacterial distribution in the intestine and therefore protecting against bacterial overgrowth.

Condition	Mechanisms
Antegrade peristalsis	prevents attachment of ingested microorganisms
Gastric acid and bile	destroy many microorganisms before they leave the stomach
Digestion by proteolytic enzymes	destroy bacteria in the small intestine
The intestinal mucus layer	traps bacteria
Intact ileocecal valve	inhibits retrograde translocation of bacteria from the colon to the small bowel
Immune system	prevents bacterial overgrowth
Immunoglobulins (IgA) secreted in the gastrointestinal tract	aids in preventing bacterial proliferation

Table 2. Differential diagnosis of excess intestinal gas.

Obstruction
Adhesions
Gastric outlet narrowing (Gastrointestinal cancer)
Motility disorder (Diabetes mellitus, Scleroderma, Thyroid disorder, Intestinal pseudoobstruction, Medications)
Psychiatric illness (Anxiety/hyperventilation, Depression, Somatization disorder)
Irritable bowel syndrome
Malabsorptive disorder (Lactose intolerance, fructose intolerance, Pancreatic insufficiency, Inflammatory bowel disease, Celiac sprue)
Infection (Bacterial overgrowth, Giardia lamblia, Helicobacter pylori)

Adapted from Abraczinskas and Goldfinger, Uptodate 2009 v. 17.2.

(Table 2). Although the ultimate mechanisms underlying symptom generation and perception in gas-related syndrome are not fully understood, factors as visceral hypersensitivity, stress, anxiety and psychological factors, and gas production might play a role.

Any condition leading to the perturbation of the equilibrium between enteric flora and the surrounding system is a predisposing factor for small intestinal bacterial overgrowth (SIBO) (Table 1). SIBO is characterized by nutrient malabsorption associated with an increased number and/or type of bacteria in the upper gastrointestinal tract. SIBO patients may be asymptomatic or have one or more symptoms including bloating, abdominal discomfort, watery diarrhea, dyspepsia, and weight loss [16,17]. There are several causes of SIBO, as depicted in Table 3. SIBO has recently been considered as an aggravating factor in IBS [18,19], has been associated with rosacea [20] and

can be present in a subgroup of patients with nonalcoholic steatohepatitis [21]. Above all, SIBO should be included in the differential diagnosis of patients who present with abdominal pain, borborygmi, diarrhea, weight loss, macrocytic anemia, abdominal distension with succussion splash, or other stigmata of malabsorption.

Novel diagnostic approaches: the H₂-breath tests (H2BT)

The intestinal microbiota can be involved in a series of pathological conditions some of which have been mentioned earlier. Two of such conditions are the gas-related syndromes and the SIBO. Due to the large variations in gas production and the enormous number of bacterial strains in the intestine, no ideal diagnostic tool is currently available for the diagnosis of such conditions. This is quite disturbing since both the gas-related syndrome and

Table 3. Disorders associated with bacterial overgrowth.

<i>Small intestinal stasis</i>	<ul style="list-style-type: none"> • Anatomic abnormalities • Small intestinal diverticulosis • Surgically created blind loops (end-to-side anastomosis) • Strictures (Crohn's disease, radiation, surgery)
<i>Abnormal small intestinal motility</i>	<ul style="list-style-type: none"> • Diabetes mellitus • Scleroderma • Idiopathic intestinal pseudoobstruction • Radiation enteritis • Crohn's disease
<i>Abnormal communication between the proximal and distal gastrointestinal tract</i>	<ul style="list-style-type: none"> • Gastrocolic or jejunocolic fistula • Resection of the ileocecal valve
<i>Associations usually with multifactorial causes</i>	<ul style="list-style-type: none"> • Hypochlorhydria due to atrophic gastritis or medications. These are usually not clinically significant unless there coexist concomitant motility disturbances of the small bowel • Immunodeficiency states (common variable immunodeficiency, AIDS, severe malnutrition) • Chronic pancreatitis • Cirrhosis, nonalcoholic steatohepatitis • Alcoholism • End stage renal disease • Advanced age • Rosacea

Adapted from Vaderhoof and Young, Uptodate 2009 v. 17.2.

the SIBO can produce bothering symptoms or aggravate an underlying disease. An advancement in this field has been provided by the recent publication in the 2009 issue of *Alimentary Pharmacology and Therapeutics* of the Rome Consensus Conference on Methodology and indications of H₂-breath testing in gastrointestinal diseases. Experts were selected on the basis of a proven knowledge and expertise in H₂-breath testing and divided into Working Groups (methodology; sugar malabsorption; small intestine bacterial overgrowth; oro-coecal transit time and other gas-related syndromes). Over a two-years time span, systematic review of the literature were performed, and then statements were formulated on the basis of the best scientific evidences available, which were debated and voted by a multidisciplinary jury. Recommendations were then modified on the basis of the decisions of the Jury by the members of the Expert Group. Lastly, the final statements were graded according to the level of evidence and strength of recommendation were presented for the use of H₂-breath testing in the clinical practice [8] (Table 4).

The principle of H₂BT is based on the administration of a carbohydrate per os which is fermented by the intestinal microflora with production of two gas: hydrogen (H₂) and methane (CH₄). Rise of such gas in expired air are is therefore a marker of bacteria-substrate metabolic contact and interaction; H₂ is the most widely measured gas by means of mass spectrometers and currently small portable, less expensive easy-to-use equipments have been marketed [22,23]. Current applications of H₂BTs are depicted in Table 5. Certain general recommendations should be considered to improve the test performance: certain carbohydrate-containing foods (bread, pasta, fibers, milk) need to be avoided prior to testing because they

cause prolonged hydrogen secretion; cigarette smoking or physical exercise sufficient to produce hyperventilation needs to be avoided for two hours prior to testing; since oral bacteria may lead to an early hydrogen peak, pretest mouth-washing with an antiseptic should be performed. Breath hydrogen testing is safe, easy to perform, and can be used in women of child-bearing age and children, but limitations include the presence of lung diseases, presence of SIBO (when other conditions need to be assessed, i.e. orocecal transit time, carbohydrate malabsorption).

H₂BT for oro-caecal transit time (OCTT)

The appearance in the expiratory breath of gases produced by colonic fermentation of an ingested organic compound may be used to measure oro-caecal transit [24]. Following the ingestion of a meal or drink containing a non-absorbable carbohydrate, a rise in hydrogen concentration in breath signals the arrival of the head of the meal or drink at the caecum giving a measure of mouth to caecum transit time. The test relies on the preferential localization of gut bacteria into the colon. A liquid meal containing 10 g of lactulose in 100 mL of water [25] or a solid meal containing backed beans as a source of non-absorbable carbohydrate (stachyose and raffinose), [26] are most often used. The transit time with the solid meal is significantly longer than with the liquid meal in the same individuals and there is no significant correlation between the two measurements [26]. The addition of lactulose to a solid meal accelerates small bowel transit [27]. The current protocol employing the liquid solution includes 10 g of lactulose in 100 mL of water, and a cut-off of hydrogen ≥ 10 ppm (based on studies with barium meal [28]) followed by at least two other subsequent increments [29]. The oro-caecal transit

Table 4. Grading of Evidence and Grading of strength of recommendation used in the process of the Rome Consensus Conference for H₂ breath testing in gastrointestinal diseases [8,54].

Grading of the evidence
Class I : Conditions with evidence or general accord that a particular procedure or treatment is useful or effective
Class II : Conditions with conflicting evidence or discordant opinions that a particular procedure or treatment is useful or effective
IIa The weight of evidence/opinion is in favour of utility/efficacy
IIb Utility/efficacy is less well defined by evidence/opinions
Class III: Conditions with evidence or general accord that a particular procedure or treatment is not useful or effective while sometimes it can be dangerous
Grading of the strength of recommendations
A. Data derived from multiple large and intermediate size RCT
B. Data derived from a few, small-size RCT, from a careful analysis of nonrandomized studied or observational registers
C. Recommendations based on Experts' consensus

Table 5. Current diagnostic applications of hydrogen breath testing.

<ul style="list-style-type: none"> • Small intestine bacterial overgrowth (SIBO) • Gas-related syndromes • Oro-coecal transit time • Sugar malabsorption (lactose, fructose, sorbitol)
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time in healthy subjects ranges between 40 and 170 min for the lactulose meal [28,30-37] and between 192 and 232 min for a solid meal [26]. Transit time shortens with increasing doses of lactulose [30]. Recently inulin, a naturally occurring polysaccharide, has been proposed as an ideal substrate to be added to a solid meal for hydrogen breath test and transit time assessment [38,39]. Inulin is less osmotically active. At variance with lactulose, inulin does not shorten oro-caecal transit time that ranged between 420 and 570 min after ingestion of 5 or 10 g inulin with the solid meal; the advantages of this solid meal in comparison to those previously used remain to be established.

The Rome consensus group has concluded that H₂ breath test to assess oro-caecal transit time has no definite clinical indications (level of evidence: I; force of recommendation: C) [8,29], due to a wide variation of results in healthy people [26,28,30-37]. Moreover, the test reproducibility, in particular with the liquid meal, is rather poor. About 5-27% of normal subjects fail to produce an increment of hydrogen breath concentration after the meal due to the absence of a hydrogen producing flora in the colon [40,41]. By contrast, given its excellent safety, the test has been used to demonstrate the drug effects on oro-caecal transit. The transit was accelerated by misoprostol [42], erythromycin [43], metoclopramide [44], and paroxetine [45], and it was delayed by loperamide [46,47], ritodrine [48], codeine, dopamine [49,50], peppermint oil [51], n-butylscopolamine [51], and imipramine [45]. The Consensus group concluded that H₂ breath test is useful to assess oro-caecal transit time in clinical pharmacology (level of evidence: I, force of recommendation: B).

H₂BT for SIBO

The gold standard for the diagnosis of SIBO relies on the demonstration of excessive bacterial concentrations (i.e. above 10⁵ CFU/mL, normal values are ≤10⁴ CFU/mL) in a jejunal aspirate performed during endoscopy or by fluoroscopy with jejunal intubation [52]. Several bacterial strains can be detected with culture but this procedure is seldomly feasible in clinical practice. Lactulose or better glucose breath hydrogen testing are the most recommended tests for SIBO as a diagnostic tests due to their wide availability and easily available low cost equipments [22,23]. Overgrowth of the small intestine with anaerobic colonic bacteria will result in an early and large increase in breath hydrogen concentration occurring well before the meal reaches the colon and hindering the assessment of oro-caecal transit time. In SIBO an early rise in hydrogen production followed by the later colonic peak suggests bacterial overgrowth in more proximal segments of the gastrointestinal tract (Figure 1). Glucose (50 to 75 g per os for adults) is also used as a substrate since, in the presence of bacterial overgrowth, it may be metabolized to hydrogen in the small bowel prior to absorption (Figure 2) [53].

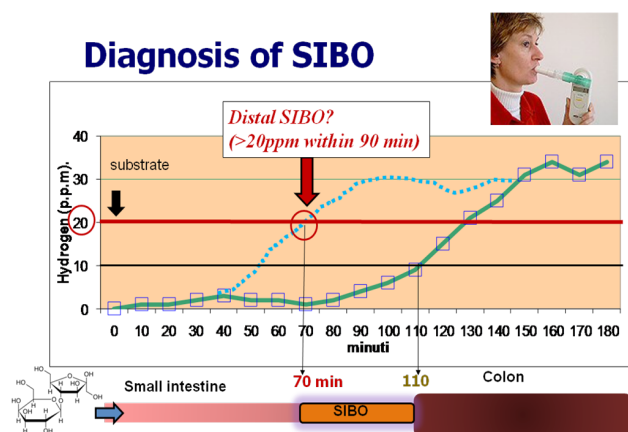


Figure 1. Representative cartoon depicting the current methodology for the diagnosis of SIBO in the distal tract of the small intestine by H₂BT. In this case the substrate used is lactulose (10g in 250 mL water per os). In the healthy subject the oro-caecal transit time is 110 min, while in the SIBO patient a consistent increase in H₂ is seen at 70 min and is above the cut-off limit of 20 ppm (circle) [53] (P. Portincasa, 2009).

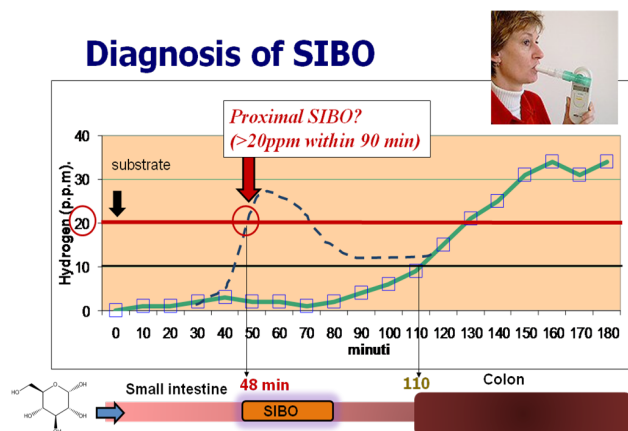


Figure 2. Representative cartoon depicting the current methodology for the diagnosis of SIBO in the proximal tract of the small intestine by H₂BT. In this case the substrate used is glucose (50g in 250 mL water per os). In the healthy subject the oro-caecal transit time is 110 min, while in the SIBO patients a consistent single-peak increase in H₂ is seen at 48 min and is above the cut-off limit of 20 ppm (circle) [53] (P. Portincasa, 2009).

Literature analysis suggests that glucose H₂BT is better than lactulose H₂BT with respect to sensitivity (63% vs. 52%), positive predictive value (80% vs. 62%), negative predictive value (66% vs. 54%), and diagnostic accuracy (72% vs. 55%) [8]. The consensus concluded that the jejunal aspirate culture is traditionally considered the gold standard diagnostic test for SIBO, despite some serious methodological limitations and lack of accessibility to clinical practice and that glucose BT is the most accurate H₂BT for non-invasive diagnosis of SIBO. Methodological indications include the use of a dose of 50g glucose in 250 mL water, 120 min duration, sampling intervals of 15 min

and a cut-off value of 12 part per million (ppm) compared to baseline of hydrogen in breath to suspect SIBO. Another statement included that GBT is indicated in symptomatic patients with predisposing conditions to SIBO but not in IBS patients, at least in the everyday clinical practice (level of evidence IIA, strength of recommendation B) [8].

H2BT for gas-related syndrome

The role of intraluminal gas in the pathophysiology of functional symptoms is still highly unclear, and partly due to lack of sensitive and consistent methods to measure intraluminal gas. At present no test, and no substrate, proved effective for measuring intraluminal gas content. The Rome consensus concluded that available data do not clearly prove that gas production and intestinal gas content in patients with 'gas-related symptoms' differ from controls (level of evidence IIA, strength of recommendation B), and that H2BT do not provide clear evidence that increased gas production/excretion is present in patients with gas-related symptoms (level of evidence II B, strength of recommendation B). Moreover, the prevalence of sugar malabsorption (lactose, fructose) in IBS patients and gas-related symptoms is not higher than in the general population (level of evidence I, strength of recommendation A) [8]. In these subjects, therefore, no H2BT is routinely recommended, unless clinical evaluation suggests the coexistence of motility defects, or SIBO, or sugar malabsorption.

Conclusions and future perspectives

The gastrointestinal microbiota plays an important role in the homeostasis of the gastrointestinal tract in the healthy subject. Bacterial activity is involved in key processes that ensure beneficial health. One or more symptoms as eructation, flatulence, abdominal bloating and distension, watery diarrhea, and weight loss may be a sentinel event in which gastrointestinal disease occurs according to a change in the balance host-microbiota. Two frequent conditions with bothering symptoms are the gas-related syndrome and the small intestinal bacterial overgrowth. Breath testing, a novel diagnostic approach, represents a new noninvasive strategy for investigation of microbiota-dependent gas production from the intestine, as a marker of interaction host-microflora. By using appropriate guidelines, the methodology of breath testing is therefore likely to underpin future disease prevention strategies, personalized health care regimens, and further development of novel therapeutic interventions.

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