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ORIGINAL ARTICLE

Pattern of methane levels with lactulose breath testing; can we shorten the test duration?

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Abstract

Background and Aim: Methane levels in methane-positive lactulose breath tests are frequently elevated at time zero. We hypothesized that baseline methane level is sufficient to detect excessive methane production and thereby avoid extended testing. Our aim was to determine if baseline methane levels were sufficient to identify methane-positive individuals as defined by current guidelines.
Methods: A retrospective study of lactulose breath tests was conducted at an open access motility lab. A methane-positive study was defined as a methane level ≥10 ppm at any time. Small intestinal bacterial overgrowth (SIBO) was defined as a ≥20 ppm rise in hydrogen from baseline by 90 min. Dual-positive SIBO and methane studies were identified. Demographics, symptoms, and indications were recorded.
Results: Of 745 tests, 33.1%, 15.0%, and 3.1% were SIBO, methane, and dual-positive, respectively. Precisely 96.4% of methane-positive studies had methane levels ≥10 ppm within 90 min and 75.9% had levels ≥10 ppm at time 0. An additional elevation of ≥20 ppm over baseline within 90 min was observed in 32.1%. Of 22 methane-positive patients with constipation, methane levels were ≥10 ppm at baseline in 81.8% and were ≥10 ppm within 90 min in all cases.
Conclusions: Nearly 25% of methane-positive studies were not identified by a fasting methane level, but 96% were identified within 90 min. Most methane-positive studies did not have a rise of 20 ppm above baseline. Our findings suggest the lactulose breath test for hydrogen and methane can be complete at 90 min.

Introduction

Standard breath tests used in the diagnosis of small bowel bacterial overgrowth (SIBO) measure intestinal hydrogen (H_2) and methane (CH_4) gases produced via microbial fermentation and which have diffused into the circulation and expired. According to current guidelines, a breath test is positive for SIBO when there is a rise in H_2 of ≥20 ppm from baseline by 90 min. On the other hand, a breath test is considered positive for methane when there is a CH_4 level of ≥10 ppm at any time during the study.[1,2] Currently, CH_4 is measured concomitantly with H_2 at all time points during the test. Much is unknown about the significance of elevated CH_4 levels at different time points and/or whether measuring CH_4 levels at all time points of the breath test adds value to the test.

During performance of a standard fasting 150 min lactulose breath test, we have observed that in studies that meet the definition of a methane-positive study, a high methane level is frequently observed at time zero. We hypothesized that when methane status is the primary indication for the study, the methane level at baseline may be sufficient to detect excessive methane gas production and thereby preclude the need for extended testing. To elucidate if baseline methane levels were sufficient to identify methane-positive individuals as defined by current guidelines, we conducted a retrospective cohort study of lactulose breath tests from an open access motility lab to determine what percentage of methane-positive tests (≥10 ppm at any point of the 90 min study) were identified by a ≥10 ppm CH_4 level at time zero.

Methods

Study design. We retrospectively reviewed the results of fasting lactulose breath test performed at an open access motility lab at a tertiary care institution between 2013 and 2019. Indications that were reported in the study were recorded and correlated with available information per chart review. Age and gender were recorded for studies that showed methane-elevation. Lactulose breath test: On the day before the test, patients were asked to consume a low carbohydrate diet without vegetables and fiber and to avoid dairy products. The test was performed after an overnight fast, and for the duration of the test patients were asked not to smoke or engage in any exercise. Antibiotics were stopped 2 weeks prior to the procedure and if tolerated by the patients, laxatives stopped 1 week prior to breath testing.

After a baseline breath sample, 10 g of lactulose were consumed, followed by a cup of water, and samples were obtained every 30 min for a period of 150 min. Breath samples were collected in a bag (QuinTron Instrument Company, Inc.) and alveolar gas was analyzed for both H_2 and CH_4 levels by chromatography (QuinTron Micro Analyzer, QuinTron Instrument Company, Inc.).

Data collection. We recorded hydrogen and methane levels at times 0, 30, 60, 90, 120, and 150 min reported in breath test reports. Repeat studies were excluded. Per consensus guidelines, a methane-positive test was defined as a methane level ≥10 ppm detected at any given time point of the study. A small intestinal bacterial overgrowth (SIBO) positive test was defined as a rise of ≥20 ppm in hydrogen from baseline by 90 min.[1,2] Methane-positive tests were further characterized as methane-positive alone or dual-positive for methane and SIBO. Symptoms listed on the referral and/or after review of the electronic medical record were recorded.

Data analysis. Demographic characteristics and clinical symptoms were shown as median values, ranges, and percentages. This study was approved by the Institutional Review Board. For this type of study, formal consent is not required.

Results

Between 2013 and 2017, 745 lactulose breath tests were performed. Of these, 247 (33.1%) were SIBO positive and 112 (15%) were methane-positive. Eighty-nine tests (11.9%) were methane-positive alone, and 23 (3.1%) were dual-positive for methane and SIBO.

Methane-positive tests. Eighty-six females and 26 males, aged 19–92 years, had methane-positive breath tests (Table 1). Of these methane-positive tests (n = 112), 108 (96.4%) had CH_4 levels ≥10 ppm within 90 min, regardless of baseline levels (Table 2). Eighty-five tests (75.9%) had CH_4 levels ≥10 ppm at time zero; 36 tests (32.1%) had an additional elevation in CH_4 of ≥20 ppm over baseline within 90 min following lactulose ingestion. Seven of these 36 tests (19.4%) were also positive for SIBO (dual-positive, see below). Tests with CH_4 levels higher or lower than 10 ppm at baseline had similar patterns of methane elevation over time (Table 3).

Methane-positive alone tests. Seventy-one female and 18 males, aged 19–92 years, had breath tests that were methane-positive only (Table 1). Of these tests (n = 89), 86 (96.6%) had CH_4 levels ≥10 ppm within 90 min, regardless of baseline levels (Table 2). In 29 (32.6%) tests, an additional elevation in CH_4 of ≥20 ppm over baseline was observed within 90 min (Table 4). Seventy-four tests (83.1%) out of 89 had CH_4 levels ≥10 ppm at time zero. Similar patterns of CH_4 levels for patients with methane levels higher or lower than 10 ppm at baseline are reported in Table 4.

Dual-positive tests (SIBO-positive and methane-positive). Fifteen females and 8 males, aged 19–80 years, had a dual SIBO-positive and methane-positive tests (Table 1). Of these tests, 22 (95.7%) had CH_4 levels ≥10 ppm within 90 min, regardless of baseline levels (Table 2). Eleven tests (47.8%) had CH_4 ≥10 ppm at time zero; seven (30.4%) had an additional elevation in CH_4 levels of ≥20 ppm over baseline within 90 min (Table 5).

Indications and symptoms. Bloating, abdominal pain, constipation, and diarrhea were the most frequent indications for lactulose breath testing (Table 1). In individuals with SIBO alone, 225 out of the 247 had symptoms recorded (91.1%). Bloating was recorded in 201 (89.3%), abdominal pain in 160 (71.1%), constipation in 9 (4.0%), and diarrhea in 20 (8.9%). In methane-positive alone tests, 87 out of 89 individuals had symptoms recorded (97.8%). Bloating was recorded in 61 (70.1%), abdominal pain in 25 (28.7%), constipation in 22 (25.3%), and diarrhea in 17 (19.5%). In dual-positive tests, 21 out of 23 individuals had symptoms recorded (91.3%). Bloating was recorded in 16 (76.2%), abdominal pain in 6 (28.6%), constipation in 0 (0%), and diarrhea in 5 (23.8%). Of 22 methane-positive patients with constipation, methane levels

Table 1 Demographics and indications for breath-testing

Symptoms	Small intestinal bacterial overgrowth-positive (n = 247)	Methane-positive (n = 89)	Dual-positive (n = 23)
Age—Median (range)	43 (19–92)	49 (19–92)	50 (19–80)
Gender—n (%)			
Male	82 (33.2)	18 (20.2)	8 (34.8)
Female	165 (66.8)	71 (79.8)	15 (65.2)
Symptoms recorded	n = 225 (91.1)	n = 87 (97.8)	n = 21 (91.3)
Symptoms—n (%)			
Bloating	201 (89.3)	61 (70.1)	16 (76.2)
Abdominal pain	160 (71.1)	25 (28.7)	6 (28.6)
Constipation	9 (4.0)	22 (25.3)	0
Diarrhea	20 (8.9)	17 (19.5)	5 (23.8)
Halitosis	3 (1.3)	1 (1.1)	0
Excess belching	0	1 (1.1)	0

Multiple symptoms/indications possible for each study.

Table 2 Methane positive diagnosis at each time point of lactulose breath test and identification of methane-positive alone and dual-positive tests

	0 min	30 min	60 min	90 min	≤90 min
Category	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Methane positive (*n* = 112)	85 (75.9)	8 (7.1)	9 (8.0)	6 (5.4)	108 (96.4)
Methane positive alone (*n* = 89)	74 (83.1)	6 (67.4)	5 (9.0)	1 (1.1)	86 (96.6)
Dual small intestinal bacterial overgrowth and methane positive (*n* = 23)	11 (47.8)	2 (8.7)	4 (17.4)	5 (21.7)	22 (95.7)

Table 3 Methane-positive tests with an increase in CH_4 ≥20 ppm above baseline levels

	30 min	60 min	90 min	≤90 min
≥20 ppm CH_4	*n*	*n*	*n*	*n* (%)
Methane-positive (*n* = 112)	13	15	8	36 (32.1)
CH_4 <10 ppm baseline (*n* = 27)	2	4	0	6 (22.2)
CH_4 ≥10 ppm baseline (*n* = 85)	11	11	8	30 (35.3)

were ≥10 ppm at baseline in 18 (81.8%). Methane levels were ≥10 ppm within 90 min in all cases.

Discussion

In this retrospective cohort study, we evaluated all studies that met the definition of methane-positive tests per ACG guidelines.[1] We then categorized these tests as methane-positive alone or dual-positive for SIBO and methane. While most tests with methane level ≥10 ppm at baseline during a lactulose breath test can be classified as a methane-positive test, nearly 25% of methane-positive tests were not identified by the fasting baseline CH_4 level. Therefore, using the currently accepted definition of a methane-positive test (≥10 ppm at any point during the test), baseline testing alone is not sufficient to diagnose a methane-positive test. In addition, although this was not an aim of our study, we also observed, importantly, that over 95% of cases of methane-positive tests are identified within 90 min, whether they occur with SIBO or not. Finally, most cases of methane-positive tests did not have a rise of 20 ppm above baseline, regardless of baseline levels.

Various cut-offs for CH_4 and time points have been used to define high methane producers in healthy adults and those with symptoms.[3–6] We apply the more stringent cut-off, as outlined in the most recent guidelines.[1] Our work suggests that when considering both SIBO and methanogenic overgrowth via a lactulose breath test, it is sufficient to limit the test to 90 min, even when using a methane cut-off of ≥10 ppm. The cost-effectiveness of avoiding unnecessarily extending the study beyond 90 min should be addressed in future studies. We show that a test duration of 90 min is sufficient to detect SIBO and will detect 96.4% of all methane positive patients. Our work also shows that if the purpose of the test is to detect intestinal methanogenic overgrowth, then a fasting baseline reading is not sufficient, as it misses about 25% of cases of methane-positive patients in our cohort. A prior study in abstract form reported that a fasting CH_4 level of ≥10 ppm predicts excessive methane production with specificity of 100% and sensitivity of 86.4%.[7] In our cohort, the sensitivity of using CH4 ≥10 ppm at time zero to identify methane-positive tests was 75.9%. Within 90 min, however, greater than 95% of methane-positive tests were detected. In support of our findings, another study that utilized a different method of breath sample collection and multiple instruments for gas analysis reported a sensitivity of 81% with a 10 ppm cut-off, which rose to 94.5% when the cut-off was decreased to 4 ppm.[8]

An increased population of CH_4 in the small bowel might theoretically be reflected by an increase of 20 ppm in CH_4 above baseline, akin to SIBO as defined by a similar rise in hydrogen level.[1] However, unlike H_2, the concept of small intestinal methanogen overgrowth is not clearly established. We therefore determined whether there was a ≥20 ppm increase in CH_4 levels above baseline in methane-positive tests. Interestingly, 32.1% of tests in our cohort that were methane-positive had an additional elevation in methane levels of ≥20 ppm above baseline within 90 min. If we apply the H_2 concept to CH_4, the rise ≥20 ppm above baseline within 90 min suggests a small bowel population of methanogens. This observation suggests that an increased

Table 4 Methane-positive alone tests with an increase in CH_4 ≥20 ppm above baseline levels

	30 min	60 min	90 min	≤90 min
≥20 ppm CH_4	*n*	*n*	*n*	*n* (%)
Methane positive alone (*n* = 89)	12	9	8	29 (32.6)
Methane positive alone, CH_4 <10 ppm baseline (*n* = 15)	1	1	0	2 (13.3)
Methane positive alone, CH_4 ≥10 ppm baseline (*n* = 74)	11	8	8	27 (36.5)

Table 5 Dual small intestinal bacterial overgrowth-positive and methane-positive tests with an increase in CH_4 ≥20 ppm above baseline levels

	30 min	60 min	90 min	≤90 min
≥20 ppm CH_4	*n*	*n*	*n*	*n* (%)
Dual positive (*n* = 23)	1	6	0	7 (30.4%)
Dual positive, CH_4 <10 ppm baseline (*n* = 12)	1	3	0	4 (33.3)
Dual positive, CH_4 ≥10 ppm baseline (*n* = 11)	0	3	0	3 (27.3)

colonic methanogen population may not preclude an increased small bowel population of methanogens.

Methane is produced almost exclusively by three types of anaerobic methanogenic archaea, the most common of which is *Methanobrevibacter smithii*.[9,10] Production of one molecule of CH_4 requires availability of four H_2 molecules (endogenous or dietary substrates). Most of the methane-positive tests in our cohort were methane-positive alone (79.5%). Only 20.5% of tests were dual-positive for SIBO and methane-positive. Furthermore, most of the tests that had an additional elevation in methane of ≥20 ppm (hence possibly suggesting small bowel population of methanogens) were methane-positive alone and were not dual positive for methane and SIBO. The significance of a high basal level of methane remains unclear.

Previous studies have shown that breath hydrogen concentrations after lactulose ingestion tend to be lower in methanogenic patients,[11] likely because of the four H_2 atoms required for each carbon in the methane molecule. On the other hand, a study in abstract form reported that detection of an early rise in H_2 production decreases in high methane producers.[12] Dual-positive studies in our cohort were notable for CH_4 levels that were frequently ≤10 ppm at baseline (95.6%) unlike methane-positive alone studies in which 83.1% had CH_4 levels ≥10 ppm at baseline. These observations suggest that an over-population of H_2 producing bacteria in the gut may interfere with methanogens.

Bloating and abdominal pain were the two most predominant symptoms in methane-predominant bacterial overgrowth, consistent with prior reports.[13] Constipation was more prevalent in methane-positive tests than in SIBO tests in our cohort, similar to prior studies.[14,15] We found that in methane-positive tests in which constipation was the reported indication, methane levels were ≥10 ppm at baseline. This observation is consistent with prior reports that the degree of breath methane production correlates with constipation.[5,16] Interestingly, constipation was not reported as an indication in any of the dual-positive tests, although the numbers were too small to draw meaningful conclusions.

Limitations of our study included the lack of use of surveys and dependence on chart review correlated with indication recorded by the technician at the time of the study. In addition, the relationship between CH_4 and H_2 production in lactulose breath tests merits further investigation. Overall, our findings suggest that while baseline testing alone is not sufficient to diagnose a methane-positive test, the standard lactulose breath test for hydrogen and methane can be complete at 90 min. The shorter duration simplifies the test and has the potential to cut costs, with negligible effect on the results.

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