A Single Fasting Exhaled Methane Level Correlates With Fecal Methanogen Load, Clinical Symptoms and Accurately Detects Intestinal Methanogen Overgrowth

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INTRODUCTION: **A 2-hour breath test is the gold standard for diagnosing intestinal methanogen overgrowth (IMO). This method can be cumbersome especially if used repetitively to monitor treatment response. Therefore, we aimed to assess the reliability of a fasting single methane measurement (SMM) in diagnosing IMO and its utility as a biomarker to monitor treatment response in subjects with IMO.**

METHODS: **First, we calculated the test characteristics of SMM compared with lactulose and glucose breath test in 2 large-scale retrospective cohorts. Second, the symptomology associated with SMM using various cutoffs was analyzed. Third, in a double-blind randomized control trial, the temporal stability of SMM levels in subjects taking placebo was analyzed. Fourth, stool *Methanobrevibacter smithii* loads were quantified using quantitative polymerase chain reaction and compared with SMM levels. Last, the change in SMM over time during antibiotic therapy was analyzed.**

RESULTS: **Using the cutoff of SMM ≥10 ppm, SMM had a sensitivity of 86.4% and specificity of 100% for diagnosing IMO on the glucose and lactulose breath tests and was associated with constipation (5.65 ± 3.47 vs 4.32 ± 3.62, $P = 0.008$). SMM remained stable for 14 weeks without treatment ($P = 0.45$), and antibiotics lead to a decrease in SMM after 2 days ($P < 0.0001$). SMM was positively associate with stool *M. smithii* load ($R = 0.65$, $P < 0.0001$).**

DISCUSSION: **Fasting SMM ≥10 ppm seems to accurately diagnose IMO, is associated with constipation, and correlates with stool *M. smithii*. SMM seems to be stable without treatment and decreases after antibiotics. SMM may be a useful test to diagnose IMO and monitor treatment response.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/AJG/C375

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INTRODUCTION

Intestinal methanogens such as *Methanobrevibacter smithii* produce methane (CH_4) as a byproduct of their metabolism (1–3). CH_4 has been shown to slow intestinal transit (4,5) and to decrease ileal peristaltic velocity (6) by acting as a gasotransmitter affecting the cholinergic nervous system (7). Intestinal methanogen overgrowth (IMO), where excessive amounts of methanogens reside in the intestines, has been associated with bloating, gas, abdominal discomfort, and constipation (8). Reduction of CH_4 levels in patients with IMO correlates with improvement of symptoms (9). Treatment usually consists of a 10 to 14-day course of antibiotics such as rifaximin and neomycin (9,10).

Currently, IMO is diagnosed using a 2-hour breath test where patients are asked to ingest a sugar substrate (e.g., glucose or lactulose) and have breath samples collected every 15 minutes (8). These samples are analyzed by gas chromatography to measure the concentration of CH_4 where a $CH_4 \geq 10$ ppm at any time-point is diagnostic of IMO (8,11).

Unlike hydrogen-predominant small intestinal bacterial overgrowth where H_2 decreases significantly during fasting and increases steadily over time as bacteria ferment sugar substrates, patients with IMO have been observed to have elevated fasting CH_4 levels with less incremental changes in breath CH_4 after ingesting sugar (8,12–15). As such, a 2-hour breath test may not

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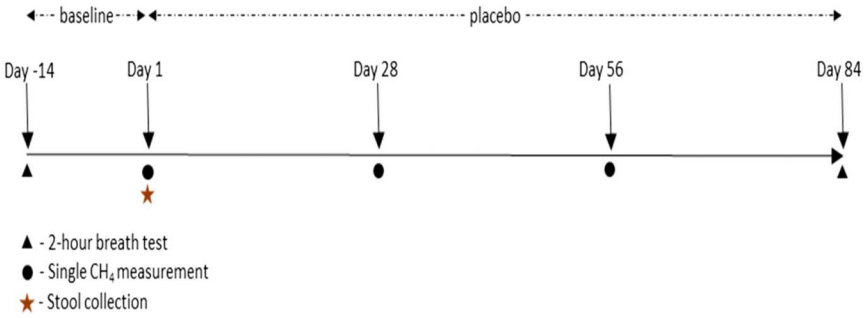

Figure 1. Evaluation of the Efficacy and Safety of Single, Daily Oral Doses clinical study design for subjects in the placebo arm. Baseline weekly CSBM and SBM were recorded for 2 weeks before initiation of placebo. Subjects received placebo from day 1 to day 84 (12-week period) and continued to record their CSBM and SBM. CH_4 measurements were taken every 4 weeks. CH_4, methane; CSBM, complete spontaneous bowel movement; SBM, spontaneous bowel movement.

be needed for the diagnosis of IMO, which could significantly improve patient access and costs. Moreover, a single fasting CH_4 level can potentially serve as a practical biomarker for monitoring treatment success/failure and disease recurrence. Therefore, using 2 large independent breath test databases, we determined the diagnostic accuracy and optimal cutoff of a fasting single CH_4 measurement (SMM) to diagnose IMO as compared to a 2-hour lactulose or glucose breath test. Second, we assessed the symptoms associated with elevated SMM. Third, we assessed the temporal stability and accuracy of SMM as part of a prospective clinical trial. Fourth, we investigated the correlation of SMM levels with fecal loads for *M. smithii*, the predominant archaeon responsible for CH_4 production in humans (3,16,17). Last, we conducted a pilot study to assess the utility of daily SMM as an on-treatment monitoring biomarker in subjects undergoing antibiotic therapy for IMO.

METHODS

Patient population and study design

To assess test characteristics of SMM, 2 separate databases were analyzed: (i) Consecutive lactulose breath tests performed from November 2005 to October 2013 at Cedars-Sinai, Los Angeles, CA, and (ii) glucose breath tests performed from January 2007 to December 2015 at the Medical College of Georgia, Augusta, GA. Repeat studies on the same subject were excluded. The sensitivity, specificity, positive predictive value, and negative predictive value of diagnosing IMO based on the various SMM cutoffs (3–10 ppm) as compared to all measurements during the 2-hour test were calculated. The margin of error for the gas chromatography in measuring CH_4 was ± 2 ppm; thus test characteristics for 1 and 2 ppm were not calculated. The test characteristics of the lactulose breath test and glucose breath tests were compared.

The glucose breath test database included baseline symptoms profiles for each subject. Subjects were given a previously validated questionnaire (18–21) assessing 10 common gastrointestinal symptoms. At establishment, the questionnaire consisted of 9 symptoms excluding constipation. Data acquisition regarding constipation was further added after an initial cohort was recruited. Subjects reported the severity of their symptoms by grading the frequency, intensity, and duration on a scale of 0–3. The scores were added resulting in a maximum score of 9 and a minimum score of 0.

To evaluate the accuracy and temporal stability of SMM, and to assess its correlation with stool *M. smithii* loads, we analyzed data from a randomized double-blind placebo-controlled trial: Evaluation of the Efficacy and Safety of Single, Daily Oral Doses of SYN-010 Compared to Placebo in Adult Patients With IBS-C (EASE-DO, NCT03763175). Trial details are summarized in the Supplementary appendix, http://links.lww.com/AJG/C375 and Figure 1. Subjects were recruited from December 2018 to October 2020. SMM measured on day 1 was compared with a 2-hour breath test performed on day 14 of the trial (i.e., prior to any interventions). Specifically, we calculated the sensitivity of SMM on day 1 for diagnosing IMO as compared to that of a 2-hour breath test on day 14.

Next, to investigate the stability of SMM over an extended period, we analyzed SMM collected over 14 weeks in subjects who received placebo. SMM were assessed on days 1, 28, and 56, and compared with baseline breath samples (before lactulose administration) from 2-hour breath tests performed on days 14 and 84. In addition, stool collected on day 1 was analyzed for *M. smithii* load and compared with SMM levels from the same day.

For the final aim of this study, adult subjects with an IMO diagnosis based on a 2-hour breath test and undergoing antibiotic therapy were recruited. They were recruited from January 2015 to December 2017. On initiation of antibiotics, SMM was collected every morning for 10 days and were analyzed.

All studies were approved by the corresponding institutional review boards.

Breath testing

For the full 2-hour breath tests, subjects were asked to consume a low-fermentable diet starting 24-hour before the breath test, then fast for the last 12 hours. On arriving to the laboratory, a breath sample was collected in a single-patient breath collection. Then, subjects were asked to ingest 10 g of lactulose or 75 g of glucose and breath samples were collected every 15-minute for 120 minutes. CH_4, H_2 and CO_2 concentrations were measured using gas chromatography (Gemelli Biotech, Los Angeles, CA, for subjects in EASE-DO trial and Quintron, Milwaukee, WI, for all other subjects). CH_4 and H_2 concentrations were adjusted to alveolar CO_2 concentration of 5.5%. Patients undergoing daily breath sampling collected their fasting breath samples at home every morning by blowing into a test tube (Extainer, Labco, Ceredigion, UK) (13).

Stool sample collection and DNA extraction

Patients collected their stool at home within 48 hours of their appointment using a Fisherbrand Commode Specimen Collection System (Thermo Fisher Scientific, Waltham, MA). After samples were received at the laboratory, they were immediately

Table 1. Test performance for SMM compared with the 2-hour lactulose breath test

SMM (ppm)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	+LR	−LR	EASE-DO sensitivity (95% CI)
≥10	86.4[a] (84.8–87.9)	100	100 (99.8–100)	97.6 (97.3–97.8)	N/A	0.14	84.8[a] (75.6–93.9)
≥9	88.8 (87.3–90.2)	100 (99.9–100)	99.9 (99.6–100)	98 (97.7–98.2)	4,569	0.11	84.8 (75.6–93.9)
≥8	90.7 (89.3–92)	99.9 (99.9–100)	99.7 (99.2–99.9)	98.3 (98.1–98.6)	1,557	0.09	84.8 (75.6–93.9)
≥7	93.0 (91.8–94.1)	99.9 (99.8–99.9)	99.3 (98.7–99.6)	98.7 (98.5–98.9)	736	0.07	84.8 (75.6–93.9)
≥6	94.6 (93.4–95.5)	99.7 (99.6–99.8)	99.1 (98.5–99.5)	99 (98.8–99.2)	572	0.05	86.4 (77.7–95.2)
≥5	96.1 (95.1–96.9)	99.7 (99.6–99.8)	98.5 (97.8–99.0)	99.3 (99.1–99.4)	353	0.04	86.4 (77.7–95.2)
≥4	97.3 (96.4–97.9)	99.6 (99.4–99.7)	97.7 (96.9–98.3)	99.5 (99.3–99.6)	227	0.03	89.8 (82.1–97.5)
≥3	98.8 (98.2–99.3)	99.3 (99.1–99.4)	96 (95.1–96.9)	99.8 (99.7–99.9)	132	0.01	91.5 (84.4–98.6)

Test characteristics of SMM (initial fasting CH_4 before lactulose) diagnosing IMO based on the 2-hour breath test (n = 12,183). EASE-DO sensitivity represents SMM taken on day 1 compared with IMO diagnosed on day 14 on a 2-hour breath test.
[a]Clinically similar between the lactulose breath test and the EASE-DO cohort based on a difference of <3% set as a priori.
CH_4, methane; EASE-DO, Evaluation of the Efficacy and Safety of Single, Daily Oral Doses; IMO, intestinal methanogen overgrowth; SMM, single CH_4 measurements.

transferred to OMNIgene•GUT tubes (DNA Genotek, Ottawa, ON, Canada). DNA extraction was carried out using the Mag-Attract PowerSoil DNA KF Kit (Qiagen, cat. No. 27000-4-KF) with some modifications. DNA quality and concentration were determined using a NanoDrop One spectrophotometer (Thermo Fisher Scientific). DNA was also extracted from an *M. smithii* stock culture following the same steps.

Quantitative polymerase chain reaction assay

Stool DNA samples were diluted to 25 ng/μL with EB buffer (Qiagen). *M. smithii* DNA loads were determined by quantitative polymerase chain reaction (qPCR) using the gene encoding the beta subunit of RNA polymerase (rpoB) as the target gene. The specific primers and probe used for *M. smithii* were described by Dridi et al. (16). Primers and probe were optimized by Applied Biosystems (Custom TaqMan Gene Expression Assays). qPCR was performed on a QuantStudio 6 Flex System (Thermo Fisher Scientific). DNA extracted from an *M. smithii* stock culture was measured at 11.25 ng/μL. This was further diluted to 1.13×10^{-7} ng/μL after a series of tenfold dilutions and used to establish a standard curve. The limit of detection was found to be at 1.13×10^{-4} ng/μL. *M. smithii* load was expressed as ng of *M. smithii* per mg of stool.

Statistical analysis

Continuous variables were summarized as mean ± SD, and categorical variables were summarized as count (%). Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of every cutoff value from 3 to 10 ppm for SMM was compared with the gold standard $CH_4 \geq 10$ ppm on a 2-hour breath test, using a 2×2 contingency table. An *a priori* difference of <3% was assumed to be clinically similar when comparing the test characteristics of SMM. Symptomology was analyzed using Wilcoxon-Rank Sum Test. Change in SMM, complete spontaneous bowel movement (CSBM), and spontaneous bowel movement (SBM) over time was analyzed using the repeated analysis of variance test. To see if there were any interaction between the change in SMM and bowel movement, we performed a 2-step analysis where we first modeled the respective variables over time using linear regression. Then, the slopes of these lines were compared using Pearson correlation. For statistical purposes, when comparing SMM to *M. smithii*, any undetectable load of *M. smithii* on qPCR was replaced by the lowest detectable *M. smithii* level divided by 2. *M. smithii* loads were normalized using natural log transformation before analysis and compared with SMM using Pearson correlation. All analyses were performed using SAS 9.4, and a 2-tailed alpha of <0.05 was used to define significance.

RESULTS

SMM accurately diagnoses IMO on the 2-hour lactulose breath test

Of 14,847 lactulose breath tests, 2,664 repeat breath tests were excluded, leaving 12,183 unique subjects. Thousand eight hundred ninety-one (15.5%) were deemed to have IMO based on the 2-hour test. To diagnose IMO, various cutoffs for SMM (3–10 ppm) were assessed, where the sensitivity ranged from 86.4% to 98.8% and specificity ranged from 99.3% to 100% (Table 1).

SMM accurately diagnoses IMO on the 2-hour glucose breath test

To assess whether the SMM is accurate for diagnosing IMO on the glucose breath test, a database of 733 subjects was analyzed. Of 733 subjects, 147 (20.1%) had IMO. The sensitivity rates of various SMM cutoffs (3–10 ppm) ranged from 86.4% to 98.8%, and specificity ranged from 81.0% to 100% (Table 2). When SMM test characteristics during glucose breath testing were compared with the lactulose breath tests, sensitivity and specificity were clinically similar for the cutoffs 3–10 ppm and 8–10 ppm, respectively.

SMM performed 2 weeks after a 2-hour lactulose breath test accurately diagnoses IMO

To assess the accuracy of SMM to diagnose IMO based on a 2-hour breath test performed on a separate day, SMM test characteristics from subjects in the EASE-DO trial on day 1 were compared with the 2-hour breath test on day 14 (n = 59). Subjects received no treatment during the screening phase of the trial between day 14 and day 1. The sensitivity rates of SMM cutoffs from 3 to 10 ppm were 84.8%–91.5% (Table 1). The sensitivity of SMM ≥10 ppm for diagnosing IMO in this cohort was similar to the sensitivities from both the lactulose and glucose breath test databases using the same cutoff. Specificity could not be assessed for various cutoffs, given that all subjects in the EASE-DO cohort had IMO.

Table 2. Test performance for SMM compared with the 2-hour glucose breath test

SMM (ppm)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	+LR	−LR
≥10	86.4[a,b] (80.9–91.9)	100[b]	100 (100–100)	97.0 (95.7–98.3)	N/A	0.14
≥9	88.4[b] (83.3–93.6)	99.4[b] (98.8–100)	97.0 (94.1–99.9)	97.4 (96.2–98.6)	147.3	0.12
≥8	92.5[b] (88.3–96.8)	98.5[b] (97.5–99.4)	93.2 (89.1–97.3)	98.3 (97.3–99.3)	61.7	0.08
≥7	93.9[b] (90.0–97.8)	96.6 (95.2–98.0)	86.3 (80.9–91.6)	98.6 (97.7–99.5)	27.6	0.06
≥6	94.6[b] (90.9–98.2)	94.2 (92.5–96.1)	79.0 (73.0–85.0)	98.7 (97.8–99.6)	16.3	0.06
≥5	95.2[b] (91.8–98.7)	91.0 (88.8–93.2)	70.7 (64.4–77.1)	98.8 (98.0–99.7)	10.6	0.05
≥4	97.3[b] (94.7–99.9)	87.8 (85.2–90.3)	64.4 (58.1–70.7)	99.3 (98.6–100)	8.0	0.03
≥3	99.3[b] (98.0–100)	81.0 (77.9–84.0)	54.3 (48.3–60.2)	99.8 (99.4–100)	5.2	0.009

Test characteristics of SMM (initial fasting CH_4 before glucose) diagnosing IMO based on the 2-hour breath test (n = 733).
[a]Clinically similar between the glucose breath test cohort and the Evaluation of the Efficacy and Safety of Single, Daily Oral Doses cohort based on a difference of 3% set as *a priori*.
[b]Clinically similar between the glucose breath test cohort and the lactulose breath test cohort based on a difference of 3% set as *a priori*.
CH_4, methane; IMO, intestinal methanogen overgrowth; SMM, single CH_4 measurements.

SMM ≥10 ppm is associated with constipation, gas, and less diarrhea

In the glucose breath test database, 732 subjects reported on all symptoms except constipation, for which data were available for 338 subjects. Subjects with SMM ≥10 ppm reported significantly higher scores for constipation (5.65 ± 3.47 vs 4.32 ± 3.62, P = 0.008) and gas (6.27 ± 2.77 vs 5.41 ± 2.98, P = 0.003), while reporting a lower score for diarrhea (3.68 ± 3.49 vs 4.38 ± 3.46, P = 0.04) (Figure 2). Using the cutoffs 10, 9, and 8 ppm, there were statistically significant differences in the reported constipation

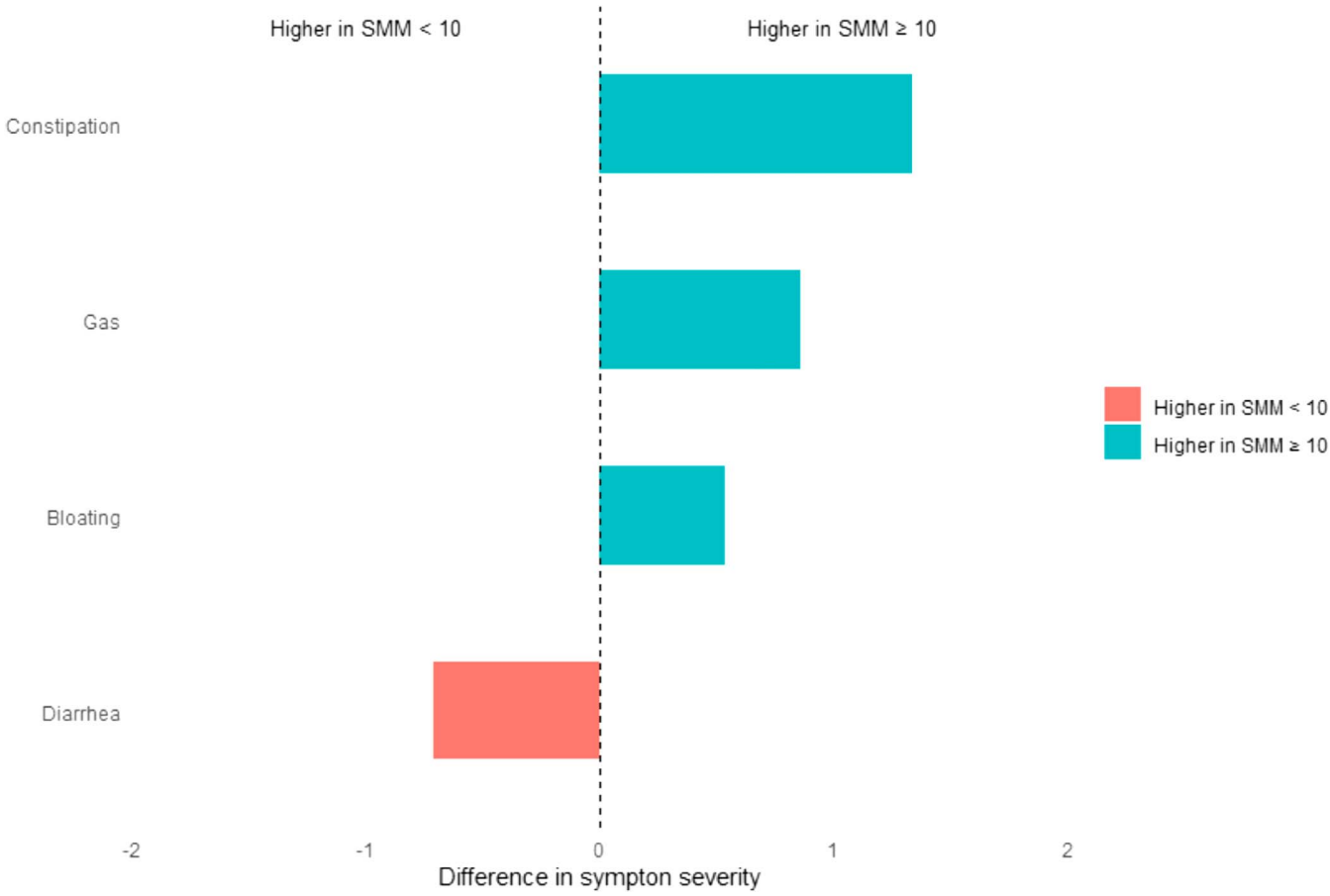

Figure 2. Difference in symptom severity for those with SMM ≥10 ppm vs those with SMM <10 ppm graded on a scale of 0–9. There was a significant difference in severity for constipation (5.65 ± 3.47 vs 4.32 ± 3.62, P = 0.008), gas (6.27 ± 2.77 vs 5.41 ± 2.98, P = 0.003), and diarrhea (3.68 ± 3.49 vs 4.38 ± 3.46, P = 0.04), whereas bloating was numerically higher for SMM ≥10 ppm (6.24 ± 3.29 vs 5.71 ± 3.36, P = 0.059).

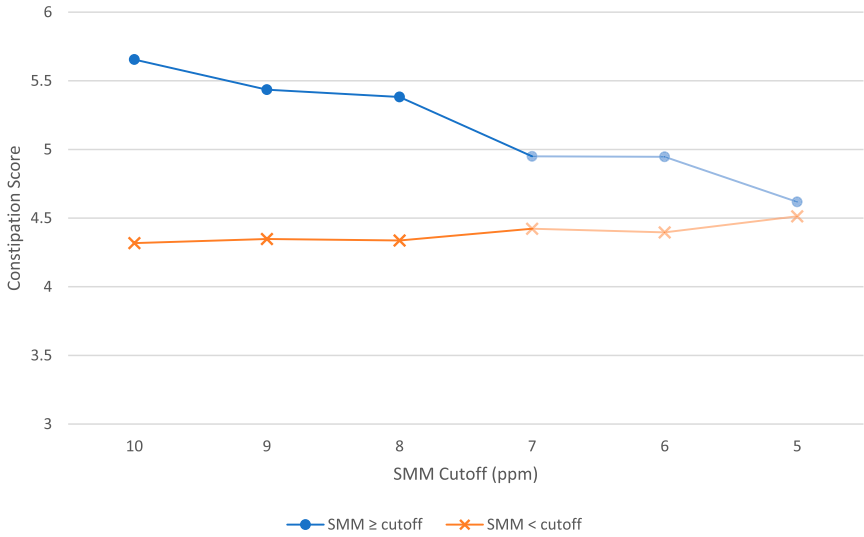

Figure 3. SMM cutoff of 10 ppm provided the largest difference in constipation score (graded from 0 to 9). There was no significant difference for cutoff values ≤7 ppm. SMM, single CH_4 measurement.

scores, with the higher SMM values having a worse constipation severity (Figure 3). At SMM of 7 ppm, the difference in constipation severity between the 2 groups was not significant (4.95 ± 3.66 vs 4.42 ± 3.62, $P = 0.23$). Similarly, at SMM <7 ppm, the difference in constipation severity was not significant. There was a trend toward higher bloating severity in the SMM ≥10 ppm group, which did not reach statistical significance (6.24 ± 3.29 vs 5.71 ± 3.36, $P = 0.059$). Other symptoms were statistically similar (see Supplementary Table 1, http://links.lww.com/AJG/C375). The SMM cutoff of 10 ppm provided the largest difference in symptomology.

Subjects with IMO who do not receive active treatment have stable SMM over time

To measure the stability of SMM over time, we analyzed SMM over time in the placebo arm of the EASE-DO cohort (n = 20). The cohort had a mean age of 48.1 ± 9.3 years, and 14 subjects (70%) were female. Baseline SMM was 50.1 ± 33.0 ppm. There was no significant decrease in SMM over time ($P = 0.45$, Figure 4). During the study, no change in SBM was seen in the placebo group ($P = 0.45$), whereas CSBM changed over time ($P = 0.0005$, see Supplementary Figure 1, http://links.lww.com/AJG/C375). There was no correlation between CSBM and SMM (R = 0.23, $P = 0.33$). This may be because of the subjective components of "completeness" when assessing CSBM, which may be more prone to a placebo response.

SMM correlates with stool *M. smithii* DNA load

SMM and stool samples were available from 28 subjects on day 1 from the EASE-DO trial. Average SMM was 49.7 ± 37.5 ppm, and the average *M. smithii* DNA concentration was 0.80 ± 1.14 ng per mg of stool. There was a significant positive correlation between

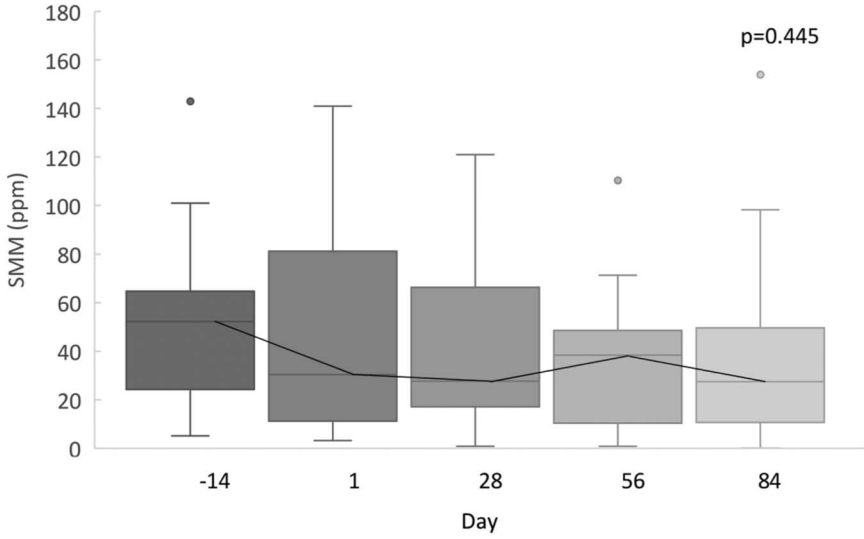

Figure 4. SMM taken during the EASE-DO trial. For subjects participating in EASE-DO trial who received placebo, SMM did not change over time (n = 20). EASE-DO, Evaluation of the Efficacy and Safety of Single, Daily Oral Doses; SMM, single CH_4 measurement.

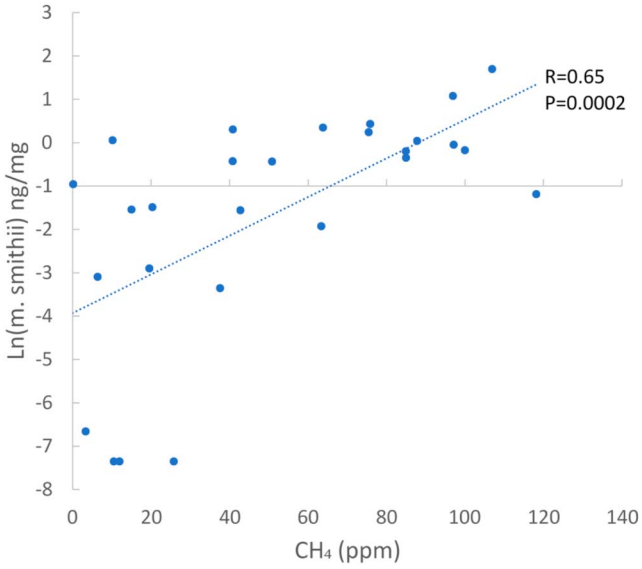

Figure 5. Fecal *Methanobrevibacter smithii* load is positively associated with SMM. SMM, single CH_4 measurement.

SMM and stool *M. smithii* DNA load (R = 0.65, $P = 0.0002$, Figure 5).

Daily SMM decreases during antibiotic therapy

Last, 11 subjects with IMO (45.5 ± 14.9 years and 7 (63.6%) women) were recruited to measure the change in SMM after antibiotic therapy. Nine (81.8%) subjects received rifaximin and neomycin, whereas 1 (9.1%) received neomycin alone and another received neomycin and metronidazole. Mean baseline CH_4 was 69.9 ± 35.2 ppm, which showed significant decrease on daily SMM while receiving antibiotics ($P < 0.0001$) (Figure 6). By day 3, SMM was significantly different compared with baseline ($P < 0,001$) (see Supplementary Table 2, http://links.lww.com/AJG/C375).

DISCUSSION

Our data suggest that a single fasting exhaled CH_4 measurement can accurately diagnose IMO. Using the same cutoff as the North American Consensus, i.e., $CH_4 \geq 10$ ppm, SMM has a sensitivity of 86.4% and a specificity of 100% on both the lactulose and glucose breath test. In addition, SMM seems to be stable over time in subjects who do not receive treatment ($P = 0.45$). Furthermore, SMM correlates with stool *M. smithii* load (R = 0.65 $P < 0.0001$) and SMM ≥10 ppm is positively associated with the severity of constipation (5.65 ± 3.47 vs 4.32 ± 3.62, $P = 0.008$). Given that SMM ≥10 ppm shares similar test characteristics between glucose and lactulose breath test and has the largest difference in the severity of constipation, 10 ppm seem to be the most clinically meaningful cutoff for SMM.

Similar to a study by Gottlieb et al. (13), we show high sensitivity and specificity for SMM in diagnosing IMO on the lactulose breath test. In this study, we further show that SMM can accurately diagnose IMO based on the glucose breath test and that SMM measured 2 weeks apart from a 2-hour breath test has a sensitivity of 84.8% (95% CI: 75.6–93.9) when using a cutoff of ≥10 ppm. Notably, SMM had similar sensitivity and specificity between the cutoffs between 10 and 8 ppm for both lactulose and glucose breath tests, whereas SMM cutoffs ≤7 ppm had higher sensitivities for the lactulose breath test. In addition, SMM can accurately diagnose IMO when performed independently of the 2-hour breath test on a separate date.

Subjects with SMM ≥10 ppm reported higher scores on constipation and gas and a lower score on diarrhea. This finding corroborates the physiological role of CH_4 in slowing intestinal transit. Pimentel et al. (4) showed that infusion of CH_4 slows canine small bowel transit and breath CH_4 has been shown to be associated with decreased small bowel and colonic transit in humans (5,22). Bloating was another symptom that was numerically worse in subjects with elevated SMM, which has been shown to improve with antibiotic therapy in subjects with CH_4 and IBS-C (9). Of note, we saw an increase in the severity of constipation as SMM increased, which is suggestive of a biological gradient. According to the Bradford-hill criteria, such a relationship supports a causal relationship, providing further evidence that CH_4 is causing constipation in a subset of patients with IBS-C (23). However, constipation is a multifactorial disease state and expectedly the correlation is not perfect. In other words, not all patients with IMO are constipated and not all constipated patients have IMO.

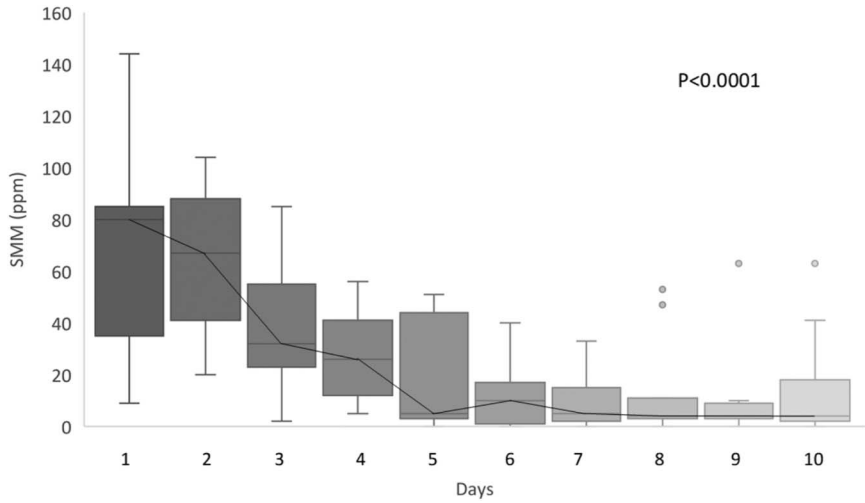

Figure 6. Daily SMM levels rapidly and significantly drop during antibiotic therapy. SMM, single CH_4 measurement.

Currently, IMO cannot be diagnosed via duodenal aspiration because of limitation of culturing archaea in clinical microbiology laboratories. In addition, a recent study by Cangemi et al. (24) has shown contamination in one-fifth of duodenal aspirates obtained using standard techniques, making the breath test a more clinically relevant test for diagnosis of IMO. SMM gives a simple, noninvasive method of performing association studies to measure intestinal CH_4, which may indirectly measure *M. smithii* loads (Figure 5).

Although SMM remained stable when subjects received placebo over 12 weeks, it reliably decreased with antibiotic therapy in patients with IMO. SMM can be a simple and inexpensive tool to monitor treatment response in patients with IMO. Although a larger trial comparing symptomatic response to SMM levels is needed, our data suggest that SMM may be useful in confirming eradication or lack of eradication of IMO, which can be useful in tailoring management of IMO. This is akin to the recommendation for confirming *H. pylori* eradication for patients with dyspepsia (25).

In the clinical setting, a clinician may measure SMM initially to diagnose IMO without the need for a full breath test. If SMM is negative (SMM $<$10 ppm) and the clinician has a high suspicion of IMO, then a full breath test can be considered. This would potentially cut down the cost, time, and discomfort experienced with the ingestion of lactulose or glucose at centers where gas chromatography is readily available. After a positive SMM, a clinician may opt to treat with antibiotics for IMO, which would negate the need for a 2-hour breath test. In addition, SMM may be useful in monitoring CH_4 levels after antibiotics. If SMM remains high during or after treatment and the patient remains symptomatic, the clinician may opt to treat with an alternative antibiotic. If the SMM levels are low and the patient remains symptomatic, one would consider treating constipation via a different pathway. In cases where the goal of breath testing is to diagnose hydrogen-predominant small intestinal bacterial overgrowth, a full 2-hour breath test is required.

The strength of our study is the multilevel and comprehensive methodology in assessing SMM as both a diagnostic test and a biomarker for treatment response. Our data were collected form 3 different cohorts at 2 different centers to account for discrepancies in location and methodology. Our results showed remarkable similarity across cohorts using the SMM cutoff of 10 ppm for diagnosing IMO. In addition, we show that SMM is associated with constipation and stool *M. smithii* loads. Furthermore, SMM was stable over time and decreased during antibiotic therapy, which suggests its role as a diagnostic and monitoring biomarker for IMO.

There are several limitations. Our study did not include a validation cohort, and the glucose and lactulose breath tests were done at 2 respective institutions, which may have led to subtle differences in methodologies. A validation cohort would be needed to verify our results. Next, to measure the *M. smithii* load, we used qPCR, which may not only account for live organisms but remnant DNA from nonviable organisms. Further studies focusing on transcriptomics and metabolomic are needed to help elucidate this relationship. Furthermore, although we show that SMM is stable over time without treatment, and decreases with antibiotics, our findings are based on a relatively small sample size. A study with a greater sample size is needed. Last, we did not assess symptomology in the pilot trial because our primary goal was to determine whether SMM reliably decreases with treatment. This was done given that patients with IBS have a placebo response rate of roughly 37.5% (26) and with a sample size of 11, report of symptomatic response is likely to be inaccurate. A large double-blind randomized controlled trial to assess the reduction of daily CH_4 levels and symptoms with antibiotic therapy is needed. SMM levels and symptoms should be followed after treatment to assess the average duration of CH_4 suppression and rate of recurrence of IMO. Last, SMM should be collected in the morning while the patient is fasting in concordance with our study design.

In summary, SMM with a cutoff of 10 ppm seems to accurately diagnose IMO, is associated with constipation, and positively correlates with stool *M. smithii*. Although further validation studies are needed, SMM shows promise as an inexpensive, noninvasive biomarker for intestinal methanogen load.

CONFLICTS OF INTEREST

Guarantor of the article: Ali Rezaie, MD, MSc.
Specific author contributions: W.T., M.P., S.R., R.M., G.L., A.R.: planning and/or conducting the study, collecting and/or interpreting data, and/or drafting the manuscript. M.J.V.: collecting and/or interpreting data and/or drafting the manuscript. C.C., W.M., M.,S., J.,T., M.,R., A.,H., J.,W., E.,K.: collecting and/or interpreting data.
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Potential competing interests: M.P. is a consultant for Synthetic Biologics. M.P.: is also a consultant for and received grant support from Bausch Health. S.R. has received grant support from Progenity and Bausch Health and is on the advisory boards for Progenity and Bausch Health. A.R. reports serving as a consultant/speaker for and receiving research grants from Bausch Health. In addition, Cedars-Sinai Medical Center has a licensing agreement with Gemelli Biotech. A.R., M.P., and R.M. have equity in Gemelli Biotech. All other authors report no conflict of interest.

Study Highlights

WHAT IS KNOWN

- ✓ Intestinal methanogen overgrowth (IMO) is diagnosed on a 2-hour breath test.
- ✓ IMO is associated with constipation.
- ✓ Methane is produced by *Methanobrevibacter smithii*.
- ✓ IMO can be treated with antibiotics.

WHAT IS NEW HERE

- ✓ Single methane measurement (SMM) can accurately diagnose IMO as compared to a 2-hour breath test.
- ✓ SMM is associated with constipation, gas, and less diarrhea.
- ✓ In the absence of a directed therapy, SMM remains stable.
- ✓ Higher SMM is associated with higher stool *Methanobrevibacter smithii* load.
- ✓ Daily SMM decreases with directed antibiotic therapy.

REFERENCES

1. Ghoshal U, Shukla R, Srivastava D, et al. Irritable bowel syndrome, particularly the constipation-predominant form, involves an increase in Methanobrevibacter smithii, which is associated with higher methane production. Gut Liver 2016;10(6):932–8.
2. Takakura W, Pimentel M. Small intestinal bacterial overgrowth and irritable bowel syndrome–an update. Front Psychiatry 2020;11:664.
3. Miller TL, Wolin MJ, Conway de Macario E, et al. Isolation of Methanobrevibacter smithii from human feces. Appl Environ Microbiol 1982;43(1):227–32.
4. Pimentel M, Lin HC, Enayati P, et al. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. Am J Physiol Gastrointest Liver Physiol 2006;290(6):G1089–95.
5. Suri J, Kataria R, Malik Z, et al. Elevated methane levels in small intestinal bacterial overgrowth suggests delayed small bowel and colonic transit. Medicine (Baltimore) 2018;97(21):e10554.
6. Jahng J, Jung IS, Choi EJ, et al. The effects of methane and hydrogen gases produced by enteric bacteria on ileal motility and colonic transit time. Neurogastroenterol Motil 2012;24(2):185–90. e92.
7. Park YM, Lee YJ, Hussain Z, et al. The effects and mechanism of action of methane on ileal motor function. Neurogastroenterol Motil 2017;29(9).
8. Pimentel M, Saad RJ, Long MD, et al. ACG clinical guideline: Small intestinal bacterial overgrowth. Am J Gastroenterol 2020;115(2):165–78.
9. Pimentel M, Chang C, Chua KS, et al. Antibiotic treatment of constipation-predominant irritable bowel syndrome. Dig Dis Sci 2014;59(6):1278–85.
10. Low K, Hwang L, Hua J, et al. A combination of rifaximin and neomycin is most effective in treating irritable bowel syndrome patients with methane on lactulose breath test. J Clin Gastroenterol 2010;44(8):547–50.
11. Rezaie A, Buresi M, Lembo A, et al. Hydrogen and methane-based breath testing in gastrointestinal disorders: The North American Consensus. Am J Gastroenterol 2017;112(5):775–84.
12. Bond JH, Engel RR, Levitt MD. Factors influencing pulmonary methane excretion in man. An indirect method of studying the in situ metabolism of the methane-producing colonic bacteria. J Exp Med 1971;133(3):572–88.
13. Gottlieb K, Le C, Wacher V, et al. Selection of a cut-off for high- and low-methane producers using a spot-methane breath test: Results from a large north American dataset of hydrogen, methane and carbon dioxide measurements in breath. Gastroenterol Rep (Oxf) 2017;5(3):193–9.
14. Rezaie A, Chang B, Chua KS, et al. Accurate identification of excessive methane gas producers by a single fasting measurement of exhaled methane: A large-scale database analysis ACG category award: 1787. J Am Coll Gastroenterol 2015;110:S759–S60.
15. Baker JR, Chey WD, Watts L, et al. How the North American Consensus protocol affects the performance of glucose breath testing for bacterial overgrowth versus a traditional method. Am J Gastroenterol 2021;116(4):780–7.
16. Dridi B, Henry M, El Khechine A, et al. High prevalence of Methanobrevibacter smithii and Methanosphaera stadtmanae detected in the human gut using an improved DNA detection protocol. PLoS One 2009;4(9):e7063.
17. Kim G, Deepinder F, Morales W, et al. Methanobrevibacter smithii is the predominant methanogen in patients with constipation-predominant IBS and methane on breath. Dig Dis Sci 2012;57(12):3213–8.
18. Erdogan A, Rao SS, Gulley D, et al. Small intestinal bacterial overgrowth: Duodenal aspiration vs glucose breath test. Neurogastroenterol Moti 2015;27(4):481–9.
19. Jacobs C, Coss Adame E, Attaluri A, et al. Dysmotility and proton pump inhibitor use are independent risk factors for small intestinal bacterial and/or fungal overgrowth. Aliment Pharmacol Ther 2013;37(11):1103–11.
20. Choi YK, Kraft N, Zimmerman B, et al. Fructose intolerance in IBS and utility of fructose-restricted diet. J Clin Gastroenterol 2008;42(3):233–8.
21. Rao SSC, Rehman A, Yu S, et al. Brain fogginess, gas and bloating: A link between SIBO, probiotics and metabolic acidosis. Clin Transl Gastroenterol 2018;9(6):162.
22. Attaluri A, Jackson M, Valestin J, et al. Methanogenic flora is associated with altered colonic transit but not stool characteristics in constipation without IBS. Am J Gastroenterol 2010;105(6):1407–11.
23. Schunemann H, Hill S, Guyatt G, et al. The GRADE approach and Bradford Hill's criteria for causation. J Epidemiol Community Health 2011;65(5):392–5.
24. Cangemi DJ, Lacy BE, Wise J. Diagnosing small intestinal bacterial overgrowth: A comparison of lactulose breath tests to small bowel aspirates. Dig Dis Sci 2021;66(6):2042–50.
25. Moayyedi P, Lacy BE, Andrews CN, et al. ACG and CAG clinical guideline: Management of dyspepsia. Am J Gastroenterol 2017;112(7):988–1013.
26. Ford AC, Moayyedi P. Meta-analysis: Factors affecting placebo response rate in the irritable bowel syndrome. Aliment Pharmacol Ther 2010;32(2):144–58.