Clinical Investigation

Hydrogen- and Methane-Based Breath Testing and Outcomes in Patients With Heart Failure

ANNA MOLLAR, PhD,[1] MARIA PILAR VILLANUEVA,[2] EDUARDO NÚÑEZ, MD, MPH,[1] ARTURO CARRATALÁ, MD,[2] FRANCISCO MORA, MD, PhD,[4] ANTONI BAYÉS-GENÍS, MD, PhD,[3,5] MIGUEL MÍNGUEZ, MD PhD,[4] VANNINA G. MARRACHELLI, PhD,[6] DANIEL MONLEON, PhD,[6,7] DAVID NAVARRO, MD, PhD,[8] JUAN SANCHIS, MD, PhD,[1,3] AND JULIO NÚÑEZ, MD, PhD[1,3]

Valencia, Madrid, and Badalona, Spain

ABSTRACT

Background: Recent evidence endorses gut microbiota dysregulation in the pathophysiology of heart failure (HF). Small intestinal bacterial overgrowth (SIBO) might be present in HF and associated with poor clinical outcomes. Lactulose breath testing is a simple noninvasive test that has been advocated as a reliable indicator of SIBO. In patients with HF, we aimed to evaluate the association with clinical outcomes of the exhaled hydrogen (H_2) and methane (CH_4) concentrations through the lactulose breath test.

Methods and Results: We included 102 patients with HF in which lactulose SIBO breath tests were assessed. Cumulative gas was quantified by the area under the receiver operating characteristic curve of CH_4 (AUC-CH_4) and H_2 (AUC-H_2). Clinical end points included the composite of all-cause death with either all-cause or HF hospitalizations, recurrent all-cause hospitalizations, and recurrent HF hospitalizations. Medians (interquartile ranges) of AUC-H_2 and AUC-CH_4 were 1290 U (520-2430) and 985 U (450-2120), respectively. In multivariable analysis, AUC-H_2 (per 1000 U) was associated with all-cause death/all-cause hospitalization (hazard ratio [HR] 1.21, 95% CI 1.04−1.40; $P = .012$), all-cause death/HF hospitalization (HR 1.20, 95% CI 1.03−1.40; $P = .021$), and an increase in the rate of recurrent all-cause (incidence rate ratio [IRR] 1.31, 95% CI 1.14−1.51; $P < .001$) and HF (IRR 1.41, 95% CI 1.15−1.72; $P = .001$) hospitalizations. AUC-CH_4 was not associated with any of these end points.

Conclusions: AUC-H_2, a safe and noninvasive method for SIBO estimation, is associated with higher risk of long-term adverse clinical events in patients with HF. In contrast, AUC-CH_4 did not show any prognostic value. (*J Cardiac Fail 2019;25:319−327*)

Key Words: Gut, heart failure, small intestinal bacterial overgrowth, breath tests, prognosis.

Relatively few bacterial populations live within the small intestine.[1] Small intestinal bacterial overgrowth (SIBO) is a disease caused by an excessive amount of bacteria in the upper alimentary tract.[2] This phenomenon is mainly due to the entrance of colonic species into the small intestine. Different conditions are causally linked with SIBO development, among them structural abnormalities of the gastrointestinal (GI) tract and GI disorders such as

From the [1]Cardiology Department, Hospital Clínico Universitario, INCLIVA, Universitat de València, Valencia, Spain; [2]Servicio de Bioquímica Clínica, Hospital Clínico Universitario, Universitat de Valencia, INCLIVA, Valencia, Spain; [3]CIBER Cardiovascular, Madrid, Spain; [4]Digestive Department, Hospital Clínico Universitario, INCLIVA, Universitat de València, Valencia, Spain; [5]Cardiology Service and Heart Failure Unit, Hospital Universitari Germans Trias i Pujol, Badalona, Spain; and Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain; [6]Metabolomic and Molecular Image Lab, Health Research Institute, INCLIVA, Valencia, Spain; [7]Pathology Department, Universitat de València, Valencia, Spain and [8]Microbiology Department, Hospital Clínico Universitario, INCLIVA, Universitat de València, Valencia, Spain.

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Reprint requests: Julio Núñez, MD, PhD, FHFA, Cardiology Department, Hospital Clínico Universitario, Avda Blasco Ibáñez 17, 46010 Valencia, Spain. Tel: +34652856689; Fax: +34963862658. E-mail: yulnunez@gmail.com

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decreased gastric acidity, reduced peristaltic activity, and mucosal damage. Hemodynamic disturbances have also been reported.[3]

In recent years, the link between the gut and the heart has increased the attention of the medical community.[4] The gut hypothesis suggests that a decreased cardiac output and/or splanchnic venous congestion might disrupt the gut barrier and increase its permeability. Consequently, the entrance of bacteria and bacteria toxins into the circulatory system has been advocated as a potential factor that might promote heart failure (HF) progression.[5] In support of this hypothesis, some authors have reported a link between higher levels of circulating bacterial products with morphologic/functional intestinal disturbances, heightened inflammatory activity, and severity of HF.[6–9] Moreover, in patients with HF, some authors have reported higher concentrations of adherent bacteria within the colonic mucus as assessed by biopsies taken during sigmoidoscopy.[7]

Despite the growing evidence supporting the gut-heart connection, there are no data—to our knowledge—about the prevalence and the role of SIBO in HF. In daily clinical practice, breath tests have emerged as readily available, safe, inexpensive, and noninvasive methods for SIBO estimation.[2] A breath test consists of administering a carbohydrate load, such as lactulose or glucose, and measuring the exhaled gas concentrations of hydrogen (H_2) and methane (CH_4) (exclusively produced by bacterial fermentation) over a period of time.[10] In the present sample of patients with HF, we aimed to evaluate (1) the prevalence of SIBO identified by lactulose breath testing, (2) the prognostic value of the exhaled concentration of H_2 and CH_4 from the lactulose breath test, and (3) the association of SIBO with long-term adverse clinical events.

Methods

Study Population

This was a prospective single-center study that enrolled 107 ambulatory patients recruited from March 15, 2015, to February 2, 2016. The inclusion criteria were: (1) HF diagnosis according to the current clinical practice recommendations,[11] (2) at least 1 previous episode of acute decompensated HF, (3) signs of fluid overload, and (4) New York Heart Association functional class II–IV. Exclusion criteria were: (1) treatment with corticosteroids, antibiotics, or fiber supplements at screening, (2) previous history of acute coronary syndrome in the past 3 months, and (3) any GI, neoplastic, or inflammatory active disease. Two hundred twenty-two patients were initially preselected for participation. One hundred seven agreed to participate and signed the informed consent form. Of these, 4 patients were excluded owing to concomitant antibiotic treatment and another 1 owing to an acute coronary syndrome episode within the past 3 months. Finally, 102 patients were included in this work, as shown in Fig. 1.

This study conformed to the principles outlined in the Declaration of Helsinki and was approved by an Institutional Review Committee. All subjects accepted their participation by signing the informed consent form.

Study Protocol

Physical examination, electrocardiography, blood laboratory tests, 2-dimensional echocardiography (Agilent Sonos 5500 [Philips] and ie33 [Philips]), and SIBO breath tests were performed in all participants. Information related to

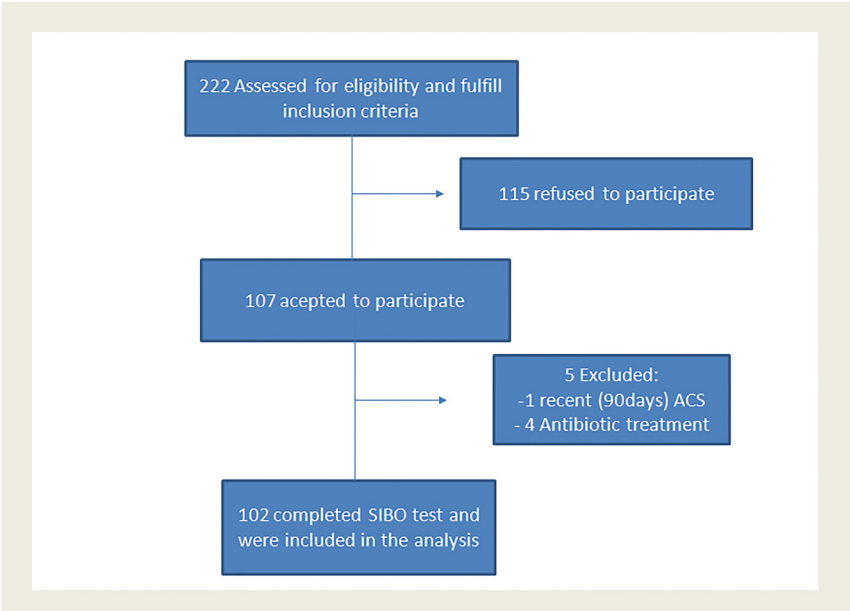

Fig. 1. Flow chart. ACS, acute coronary syndrome.

demography and medical history was obtained from the electronic medical records system.

Blood Laboratory Tests

Blood variables were measured in peripheral venous samples after fasting for 12 hours overnight. Blood cell counts, plasma creatinine, sodium, potassium, and N-terminal pro−B-type natriuretic peptide (NT-proBNP) were measured in all patients after routine clinical practice. Interleukin (IL) 1β, IL-6, IL-10, and tumor necrosis factor (TNF) α were measured from a frozen sample with the use of a commercially available assay (HSCYTMAG-60SK Milliplex High-Sensitivity Human Cytokine Magnetic Panel).

Lactulose Breath Test

Patients were told to follow a strict low-residue diet the day before the test and were asked not to eat, drink, or smoke for 12 hours before the test. After obtaining an initial basal sample of breath, the patients ingested 10 g lactulose dissolved in 250 mL water, followed by measuring of breath H_2 and CH_4 samples every 20 minutes for 2 hours. CH_4, H_2, and carbon dioxide levels were measured by means of gas chromatography. H_2 and CH_4 concentration were corrected for carbon dioxide levels. Cumulative H_2 or CH_4 measurements over time were integrated into a single measurement by the area under the concentration receiver operating characteristic curve (AUC), CH_4 (AUC-CH_4) and H_2 (AUC-H_2), with the use of the trapezoid method.[12−14]

Outcomes and Follow-Up

Patient follow-up was censored if death or valve replacement ensued. Clinical end points evaluated included all-cause death/all-cause hospitalization, all-cause death/HF hospitalization, and 2 recurrent end points: all-cause hospitalizations and HF hospitalizations. Only unplanned hospitalizations were included. Clinical end points were verified through the patients' clinical charts and adjudicated by an investigator blinded to the patients' clinical characteristics and to the results of the breath tests.

Exposures of Interest

The following exposures were tested: (1) AUC-H_2, (2) AUC-CH_4, (3) SIBO-H_2, defined as a rise of $\geq$20 ppm by 90 minutes after baseline or baseline levels $>$20 ppm, and (4) SIBO-CH_4, defined as a rise of $\geq$12 ppm by 90 minutes after baseline or baseline levels $>$12 ppm.

Statistical Analysis

Continuous variables were expressed as mean $\pm$ SD or median (interquartile range [IQR]) when appropriate. Discrete variables were summarized as percentages. Baseline characteristics among the quartiles of the exposures were compared by means of analysis of variance, Kruskall-Wallis, or chi-square tests as appropriate. We selected explanatory variables for the multivariable regression models with subject-matter knowledge as the main criterion. The small size of the cohort was also considered for limiting the number of covariates. As such, all regression models included a common set of clinical relevant covariates, independent from their level of significance: age (years), sex (0/1), systolic blood pressure (mm Hg), left ventricular ejection fraction (LVEF; %), NT-proBNP (pg/mL), estimated glomerular filtration rate (mL·min^{-1}·1.73 m^{-2}), anemia (0/1), and sodium (mmol/L). The linearity assumption for continuous covariates was tested by means of multivariable fractional polynomial.[15] Because all continuous covariates met the linearity assumption, they were included in the models untransformed. Regression estimates for all final models are presented in Supplemental Tables 1 and 2.

Time-to-Event Methods. The proportional hazards assumption was tested for each exposure by means of the scaled Schoenfeld residuals and the "log-log" plots. The 2 binary exposures, SIBO-H_2 and SIBO-CH_4, had a significant departure from the proportionality assumption, and therefore their prognostic values were tested with the use of a covariate-adjusted restricted mean survival time (RMST) method.[16] The association between the 2 continuous exposures (AUC-H_2 and AUC-CH_4) with both time-to-event outcomes was tested with the use of a Cox proportional model .

Recurrent Event Methods. Multivariable regression methods for both recurrent events (repeated all-cause hospitalizations and HF hospitalizations) were carried out with bivariate negative binomial regression.[17] Risk estimates from this method are presented as incidence rate ratios (IRRs) and 95% confidence intervals [CIs].

We set a 2-sided P value of $<$.05 as the threshold for statistical significance. All analyses were performed with the use of Stata 15.1 (Stata Statistical Software, release 15 [2017]; Statacorp, College Station, Texas).

Results

Baseline Characteristics for the Entire Population

The median (IQR) age of the study population was 75 (70−78) years and 71.6% were male. The proportions of patients with reduced ($<$40%), preserved ($\geq$50%), and mid-range (41%−49%) LVEF were 44.2%, 42.1%, and 13.7%, respectively. Medians (IQRs) of AUC-H_2 and AUC-CH_4 were 1290 (520-2430) U and 985 (450-2120) U, respectively. Ischemia was the most frequent etiology (42.2%), and 71.6% of patients were recently discharged (within previous 30 days) from an acute decompensated HF episode. All characteristics of the study population are summarized in Table 1.

Baseline Characteristics Across Quartiles of AUC-H^2 and AUC-CH^4

Recent admission for acute decompensated HF was more frequent in those patients at the upper quartiles of AUC-H_2. Overall, there was a monotonic increase in levels of inflammatory markers among AUC-H_2 quartiles (IL-1β, IL-10,

Table 1. Baseline Characteristics Across Quartiles of AUC-H_2

Characteristic	Study Population (n = 102)	AUC-H_2 Q1 (40−520) (n = 27)	AUC-H_2 Q2 (530-1280) (n = 24)	AUC-H_2 Q3 (1300−2430) (n = 26)	AUC-H_2 Q4 (2480−9010) (n = 25)	*P* Value
Demographics and medical history						
Age, y	75 (70−78)	73 (70−78)	74 (73−77)	76 (66−82)	75 (72−76)	.962
Male	73 (71.6)	21 (78)	19 (79)	18 (69)	15 (60)	.412
Previous admission for AHF (previous 30 d)	73 (71.6)	15 (56)	14 (58)	23 (88)	20 (80)	.016
Hypertension	88 (86)	23 (85)	21 (87)	23 (88)	21 (84)	.965
NYHA III−IV	39 (38)	7 (26)	8 (33)	12 (46)	12 (48)	.291
Diabetes mellitus	47 (46)	13 (48)	12 (50)	13 (50)	9 (36)	.707
Dyslipidemia	81 (79)	13 (74)	12 (87)	13 (84)	9 (72)	.430
Renal failure	43 (42)	10 (37)	8 (33)	10 (38)	15 (60)	.218
Vital signs						
Heart rate, beats/min	74 (65−90)	73 (62−90)	70 (65−86)	80 (66−90)	75 (64−90)	.893
SBP, mm Hg	130 (115−148)	129 (110−145)	127 (112−147)	135 (121−152)	125 (116−144)	.605
DBP, mm Hg	70 (62−80)	67 (61−80)	70 (64−79)	71 (63−83)	71 (61−81)	.868
Laboratory						
Hemoglobin, g/dL	12.2 ± 12.4	12.58 ± 2.08	12.23 ± 1.51	12.37 ± 2.02	12.40 ± 1.73	.961
Sodium, mmol/L	140 ± 3.4	140 ± 3	140 ± 3	139 ± 4	140 ± 3	.801
NT-proBNP, pg/mL	3276 (1284−10,009)	2638 (1193−10,909)	1770 (928-7537)	4464 (2055−15,191)	3734 (1227−10,966)	.170
Creatinine, mg/dL	1.58 ± 0.79	1.54 ± 0.76	1.45 ± 0.54	1.62 ± 0.99	1.71 ± 0.83	.700
eGFR, $mL{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$	52.8 ± 24.2	55.7 ± 28.2	55.0 ± 21.7	53.3 ± 23.2	47.0 ± 23.0	.566
Urea, mg/dL	66 (50−105)	63 (44−105	67 (48−98)	71 (59−99)	72 (48−118)	.711
CRP, mg/L	10.1 (4.4−22.8)	9.2 (3.91−24.60)	12.62 (5.70−22.30)	11.40 (3.65−25.00)	8.40 (5.00−15.70)	.872
IL-1β, pg/mL*	3.2 (3.2−3.2)	3.2 (2.16−3.20)	3.20 (3.20−3.20)	3.20 (3.20−3.20)	3.20 (3.20−4.16)	.001
IL-6, pg/mL	7 (3.2−174.2)	4.40 (3.20−12.48)	9.30 (3.51−18.97)	7.25 (3.20−28.24)	10.73 (4.20−20.09)	.564
IL-10, pg/mL	3.2 (1.5−6.1)	2.37 (1.48−3.26)	3.2 (1.18—4.81)	2.90 (1.18−7.56)	5.49 (3.25−9.19)	.020
TNF-α, pg/mL	13.2 (9.3−20.6)	11.78 (7.56−15.73)	12.11 (9.12−14.47)	13.99 (10.15−26.44)	18.89 (12.69−21.61)	.020
Echocardiography						
LVEF, %	44.8 ± 15.5	44.29 ± 14.48	41.95 ± 15.20	42.28 ± 16.12	50.90 ± 15.57	.162
Pharmacologic treatment						
Beta-blockers	74 (72.3)	18 (66.67)	20 (83.33)	18 (69.23)	18 (72.00)	.543
ACEI/ARB	67 (65.7)	19 (70.37)	17 (70.83)	15 (57.69)	16 (64)	.729
Furosemide equivalent dose, mg/d	80 (60−120)	80 (40−120)	80 (60−160)	60 (60−80)	80 (60−120)	.176

Values are presented as median (interquartile range), mean ± SD, or n (%). AUC-H_2, area under the curve of hydrogen; AHF, acute heart failure; NYHA, New York Heart Association functional class; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); NT-proBNP, N-terminal pro−B-type natriuretic peptide; CRP, C-reactive protein; IL, interleukin; TNF, tumor necrosis factor; LVEF, left ventricular ejection fraction; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

and TNF-α). The same was true among AUC-CH_4 quartiles and IL-1β. No other significant differences were found among the patients' characteristics and both exposures (Tables 1 and 2).

Prevalence of SIBO

SIBO-H_2 and SIBO-CH_4 were detected in 39 (38.2%) and 48 (47.1%) patients, respectively. The combination of these 2 criteria included 66 patients (64.7%).

Outcomes

At a median (IQR) follow-up of 1.77 (1.20−2.17) years, 32 deaths (31.4%), 64 all-cause hospitalizations (62.8%), and 34 HF hospitalizations (33.3%) were documented. Sixty-nine patients (67.65%) had a follow-up of ≥1 year. Only 1 patient was censored before 1 year for a cause different from death. The composite of death/all-cause hospitalization was registered in 68 patients (66.7%)—with a median follow-up of 0.94 years (range 0.03−3.10)—whereas the composite of death/HF hospitalization was registered in 51 (50%) patients—with a median follow-up of 1.43 years (range 0.03−3.10).

AUC-H_2 and AUC-CH_4. Distributions of the AUC-H_2 and AUC-CH_4 across the end points are shown in Supplemental Fig. 1. In the multivariate setting, AUC-H_2 (per 1000 U) was linear and independently associated with all-cause death/all-cause hospitalization (hazard ratio [HR] 1.21, 95% CI 1.04−1.40; $P = .012$) and all-cause death/HF hospitalization (HR 1.20, 95% CI 1.03−1.40; $P = .021$) as shown in Fig. 2. The addition of AUC-H_2 to the base model did not improve the C-statistics (0.671 vs 0.620, $\Delta = 0.051$ [−0.019 to 0.119]; and 0.662 vs 0.637, $\Delta = 0.025$ [−0.036 to 0.087]) for all-cause death/any hospitalization and all-

Table 2. Baseline Characteristics Across Quartiles of AUC-CH_4.

Characteristic	Study Population (n = 102)	AUC-CH_4 Q1 (120−450) (n = 27)	AUC-CH_4 Q2 (500−970) (n = 24)	AUC-CH_4 Q3 (1000−2120) (n = 26)	AUC-CH_4 Q4 (2270−7100) (n = 25)	*P* Value
Demographics and medical history						
Age, y	75 (70−78)	75 (71−78)	75 (72−79)	74 (67−83)	74 (68−77)	.938
Male	73 (71.6)	19 (70)	15 (62)	17 (65)	22 (88)	.149
Previous admission for AHF (previous 30 d)	73 (71.6)	16 (59)	19 (79)	16 (61)	21 (84)	.117
Hypertension	88 (86)	24 (89)	21 (87)	22 (85)	21 (84)	.949
NYHA III−IV	39 (38)	9 (33)	11 (46)	9 (35)	10 (40)	.793
Diabetes mellitus	47 (46)	14 (52)	8 (33)	11 (42)	14 (56)	.376
Dyslipidemia	81 (79)	21 (78)	22 (92)	18 (69)	20 (80)	.237
Renal failure	43 (42)	7 (26)	12 (50)	12 (46)	12 (48)	.241
Vital signs						
Heart rate, beats/min	74 (65−90)	80 (70−92)	66 (61−81)	74 (66−90)	80 (68−97)	.162
SBP, mm Hg	130 (115−148)	130 (116−141)	135 (116−149)	123 (110−148)	130 (119−152)	.503
DBP, mm Hg	70 (62−80)	71 (62−83)	72 (64−79)	68 (63−75)	72 (61−83)	.808
Laboratory						
Hemoglobin, g/dL	12.2 ± 12.4	11.94 ± 2.22	12.23 ± 1.42	12.90 ± 2.02	12.54 ± 1.48	.302
Sodium, mmol/L	140 ± 3.4	140 ± 2	140 ± 4	140 ± 3	139 ± 4	.678
NT-proBNP, pg/mL	3276 (1284−10,009)	3898 (1532−10,079)	3722 (1394−9165)	2935 (1193−10,966)	2596 (1265−9758)	.950
Creatinine, mg/dL	1.58 ± 0.79)	1.48 ± 0.70	1.61 ± 0.80	1.51 ± 0.70	1.73 ± 0.97	.663
eGFR, $mL{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$	52.8 ± 24.2	55.0 ± 23.9	51.2 ± 25.6	51.7 ± 19.5	53.1 ± 28.3	.945
Urea, mg/dL	66 (50−105)	61 (47−104)	75 (53−118)	68 (57−104)	65 (49−104)	.599
CRP, mg/L	10.1 (4.4−22.8)	9.88 (5.70−18.20)	16.41 (5.34−26.93)	5.56 (1.80−15.70)	12.00 (5.00−24.60)	.183
IL-1β, pg/mL*	3.2 (3.2−3.2)	3.20 (3.20−3.20)	3.20 (3.20−3.20)	3.20 (3.20−3.20)	3.20 (3.20−4.39)	.034
IL-6, pg/mL	7 (3.2−174.2)	9.12 (2.67−20.419)	6.91 (3.37−20.72)	5.26 (3.42−11.02)	18.89 (12.69−21.61)	.794
IL-10, pg/mL	3.2 (1.5−6.1)	3.20 (1.48−5.39)	3.56 (1.48 6.34)	1.19 (1.48−6.83)	5.34 (1.48−8.30)	.531
TNF-α, pg/mL	13.2 (9.3−20.6)	13.05 (8.79−18.51)	15.78 (11.04−20.85)	11.91 (8.97−20.62)	12.57 (10.95−21.61)	.694
Echocardiography						
LVEF, %	44.8 ± 15.5	39.95 ± 13.99	46.87 ± 16.31	49.93 ± 17.16	42.91 ± 13.37	.114
Pharmacologic treatment						
Beta-blockers	74 (72.3)	19 (70.37)	17 (70.83)	23 (88.46)	15 (60.00)	.119
ACEI/ARB	67 (65.7)	22 (81.48)	12 (50.00)	18 (69.23)	15 (60.00)	.109
Furosemide equivalent dose, mg/d	80 (60−120)	80 (60−100)	80 (60−120)	70 (40−120)	60 (60−120)	.935

Values are presented as median (interquartile range), mean ± SD, or n (%). Abbreviations as in Table 1.

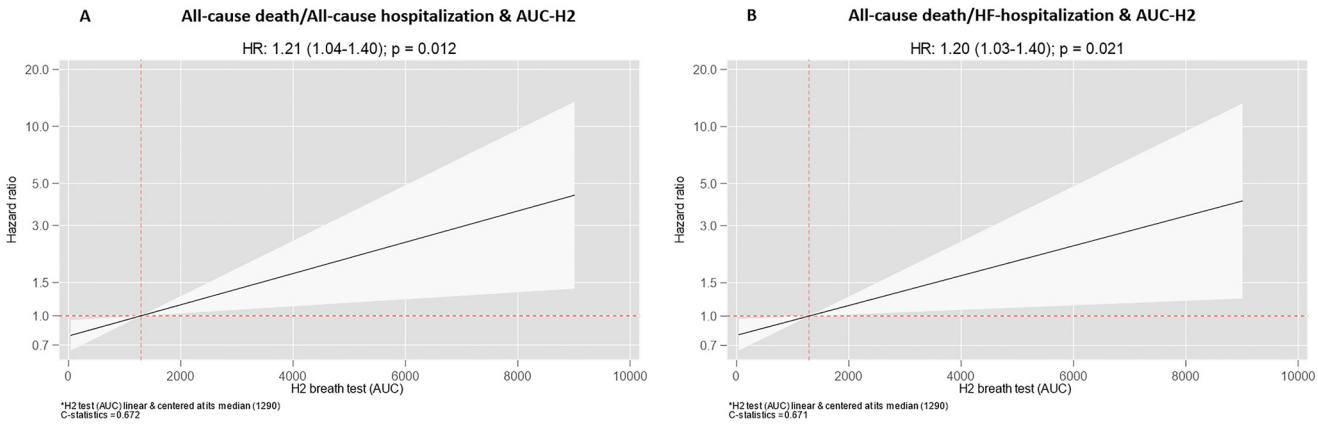

Fig. 2. Multivariate linear association between AUC-H_2 and the risk of time to first event. (A) AUC-H_2 and time to death and/or all-cause admission. (B) AUC-H_2 and time to death and/or HF admission. AUC, area under the concentration receiver operating characteristic curve; HF, heart failure; HR, hazard ratio. Estimates of risk adjusted for age (y), sex (0/1), systolic blood pressure (mm Hg), left ventricular ejection fraction (%), N-terminal pro−B-type natriuretic peptide (pg/mL), estimated glomerular filtration rate ($mL{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$), anemia (0/1), and sodium (mmol/L).

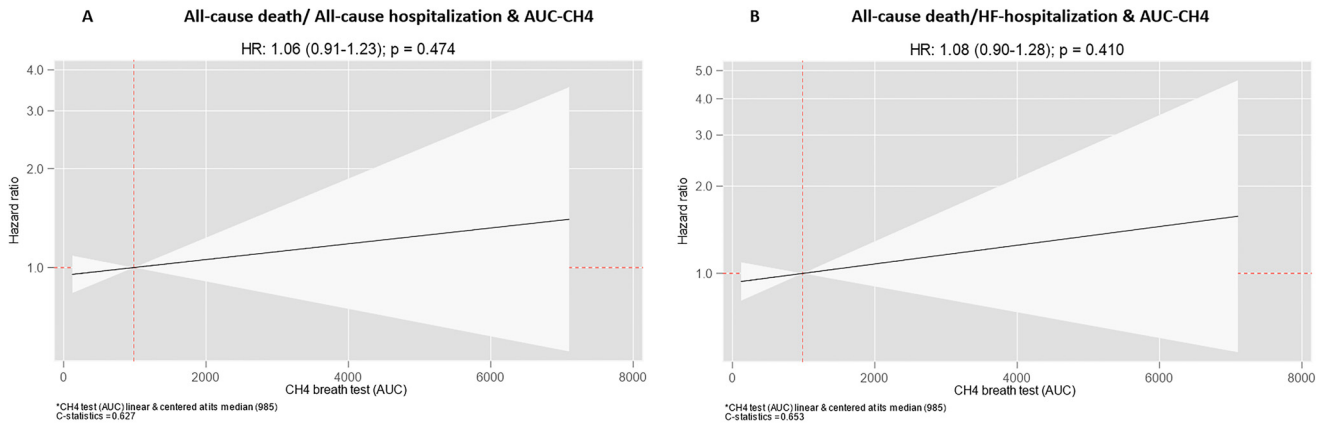

Fig. 3. Multivariate linear association between AUC-CH_4 and the risk of time to first adverse events. (A) AUC-CH_4 and time to death and/or all-cause admission. (B) AUC-CH_4 and time to death and/or HF-admission. Estimates of risk adjusted for age (y), sex (0/1), systolic blood pressure (mm Hg), left ventricular ejection fraction (%), N-terminal pro−B-type natriuretic peptide (pg/mL), estimated glomerular filtration rate (mL·min^{-1}·1.73 m^{-2}), anemia (0/1), and sodium (mmol/L). Abbreviations as in Fig. 2.

cause death/HF hospitalization, respectively. In contrast, AUC-CH_4 showed no effect on either end point: HR 1.06, 95% CI 0.91−1.23, $P = .474$ for all-cause death/all-cause hospitalization (Fig. 3A); and HR 1.08, 95% CI 0.90−1.29, $P = .410$ for all-cause death/HF hospitalization (Fig. 3B). Likewise, AUC-CH_4 did not increase the C-statistics for both end points: 0.622 vs 0.620, $\Delta = 0.002$ (−0.018 to 0.022); and 0.651 vs 0.637, $\Delta = 0.014$ (−0.018 to 0.048).

SIBO-H_2 and SIBO-CH_4. Because of significant departure from the hazard proportionality assumption, SIBO-H_2 and SIBO-CH_4 were modeled with the use of an RMST approach. In these analyses (Table 3), SIBO-H_2 was shown to be an independent predictor for the composite of death/all-cause hospitalization (ΔRMST −0.15 years; $P = .043$). This difference means that patients in the SIBO-H_2 group had, on average, 0.15 years fewer time free of events at 1-year follow-up. On the composite of death/HF hospitalization, however, SIBO-H_2 had no significant effect (ΔRMST −0.10 years; $P = .157$). The effect of SIBO-CH_4 on both end points was also not significant (Table 3).

Table 3. RMST Regression Estimates of the Association of SIBO With Both Composite End Points

Variable	RMST (y)		Δ RMST	95% CI	*P* Value
Death/any admission					
SIBO-H_2	0.76	0.61	−0.15	−0.30 to −0.05	.043
SIBO-CH_4	0.75	0.64	−0.12	−0.25 to −0.02	.097
Death/HF admission					
SIBO-H_2	0.83	0.73	−0.10	−0.23 to −0.04	.157
SIBO-CH_4	0.84	0.73	−0.11	−0.24 to −0.18	.091

Estimates of risk adjusted for age (y), sex (0/1), systolic blood pressure (mm Hg), left ventricular ejection fraction (%), N-terminal pro−B-type natriuretic peptide (pg/mL), estimated glomerular filtration rate (mL·min^{-1}·1.73 m^{-2}), anemia (0/1), and sodium (mmol/L). RMST, restricted mean survival time; CI, confidence interval; SIBO, small intestinal bacterial overgrowth; SIBO-H_2, small intestinal bacterial overgrowth defined as a rise of hydrogen ≥20 ppm by 90 minutes after baseline or baseline levels >20 ppm; SIBO-CH_4, small intestinal bacterial overgrowth defined as a rise of methane ≥12 ppm by 90 minutes after baseline or baseline levels >12 ppm.

Recurrent Events. During the follow-up, 174 all-cause hospitalizations in 64 patients (62.8%) were registered and distributed as follows: 1 hospitalization: 20 (19.6%); 2: 17 (16.7%); 3: 13 (12.8%); 4 7 (6.9%); and ≥5: 7 (6.9%). Among all hospitalization, we registered 72 HF hospitalizations in 34 patients (33.3%).

All recurrent event analyses included the modeling of all-cause mortality (as a terminal event) to account for informative dropout. The same set of covariates was used in the count and mortality submodels. Table 4 presents all of the exposures' regression estimates. AUC-H_2 was significantly related to both repeated all-cause and HF hospitalization. In contrast, there was no effect of AUC-CH_4 on any of these end points. Likewise, current definitions of SIBO (SIBO-H_2 and SIBO-CH_4) did not show a significant prognostic effect on recurrent hospitalizations.

Discussion

To the best of our knowledge, this study is the first to show that exhaled H_2 after lactulose breath testing, a surrogate of SIBO, was related to higher risk of adverse events, including the composite of death/all-cause hospitalizations and recurrent hospitalizations. In contrast, exhaled CH_4 had no effect on either of those end points. We think that these findings add indirect evidence about the role of gut microbiota, and probably SIBO, in the pathophysiology of HF.

The Gut Hypothesis in HF

There is compelling evidence that HF is accompanied by intestinal disturbances as a consequence of ischemia and/or splanchnic venous congestion.[5,7–9] It is postulated that disruption of the intestinal barrier owing to decompensated HF could lead to increased gut permeability, translocation of endotoxins, microbial components, and some derived microbial metabolites, and consequently the release of inflammatory biomarkers.[18] Different studies have shown morphologic abnormalities of the GI tract in HF compared

Table 4. Regression Estimates for Recurrent Hospitalization End Points

	All-Cause Hospitalizations			HF Hospitalization		
Variable	IRR	95% CI	*P* Value	IRR	95% CI	*P* Value
AUC-H_2, per 1000 U	1.31	1.14−1.51	<.001	1.41	1.15−1.72	.001
AUC-CH_4, per 1000 U	1.05	0.87−1.28	.924	1.00	0.75−1.34	.997
SIBO-H_2	1.27	0.70−2.31	.425	1.77	0.86−3.63	.122
SIBO-CH_4	1.04	0.56−1.94	.905	0.97	0.41−2.28	.938

Estimates of risk adjusted for age (y), sex (0/1), systolic blood pressure (mm Hg), left ventricular ejection fraction (%), N-terminal pro−B-type natriuretic peptide (pg/mL), estimated glomerular filtration rate (mL·min^{-1}·1.73 m^{-2}), anemia (0/1), and sodium (mmol/L). IRR, incidence rate ratio; other abbreviations as in Tables 1−3.

with control subjects. For example, Sandek et al and Valentova et al detected higher bowel wall thickness, suggestive of edema, and major concentrations of adherent bacteria within the mucus.[6−8] Lower cardiac performance and worse clinical outcomes were observed in patients admitted with acute decompensated HF who also had increased colonic wall thickness.[19] Moreover, intestinal functional abnormalities, such as increased in gut permeability, reduced absorption, and lower intestinal mesenteric artery blood flow, have also been documented in patients with chronic HF.[7]

Despite the evidence linking either morphologic or functional intestinal abnormalities with heightened inflammatory activity and severity of the disease, the precise causative links between these intestinal disturbances and the pathophysiology of HF progression is unclear. Dysregulation of splanchnic blood volume and microbiota-mediated deleterious effects have emerged as the most plausible mechanisms.[4,20]

SIBO in HF

Compared with the 1×10^{11}/mL organisms colonizing the colon, the number of organisms colonizing the duodenum and jejunum are low (fewer than 1×10^{5}/mL organisms). Indeed, the jejunum does not hold anaerobic bacteria, and about one third of jejunal aspirates may be sterile in healthy people.[21] The presence of 1×10^{13}/mL organisms of duodenal aspirate fluid is diagnostic for bacterial overgrowth.[22] The clinical manifestations of SIBO are nonspecific (bloating, flatulence, abdominal discomfort, diarrhea, abdominal pain) and usually overlooked, leading to its prevalence underestimation.[23] Among others, SIBO has been reported to be more prevalent in the elderly,[24] inflammatory diseases,[22] intestinal hypomotility,[3] obesity,[25,26] diabetes,[3] and congestive states such as hepatic cirrhosis[27]; incidentally, these are common conditions found in patients with HF. The clinical meaning of SIBO in the pathophysiology of gut diseases remains unclear, and the evidence supporting a potential role in cardiovascular disease is even more scarce.[28] Recent works suggest an association of SIBO with subclinical atherosclerosis and coronary artery disease.[28,29] The role of SIBO in HF has not been yet fully elucidated. SIBO has been shown to be associated with greater severity and higher risk profile in common comorbidities and congestive states mimicking advanced HF, such as end-stage renal failure and liver cirrhosis.[30−32] In HF patients, there is a growing body of research demonstrating quantitative and qualitative colonic bacterial abnormalities in HF. For example, Sandek et al found higher concentrations of adherent bacteria within mucus obtained by biopsies taken during sigmoidoscopy in chronic HF patients compared with control subjects.[6] More recently, Pasini et al found massive quantities of pathogenic bacteria and *Candida* in the feces of 60 patients of patients with stable chronic HF.[33] However, to the best of our knowledge, no previous studies linked HF, SIBO, inflammatory activity and adverse events. Therefore, our findings are not only in line with the role of gut involvement in the pathophysiology of HF, but also add new evidence about small intestine involvement, the relation to inflammatory activity, and the risk for adverse clinical end points. All of these are different aspects that must be integrated in the aim of better understanding the pathogenic role of gut microbiota in HF.

There are several issues and questions that remain to be reevaluated in larger prospective studies. For example, the accuracy of exhaled gas test for SIBO estimation in HF patients is unclear, given that there are several conditions different from SIBO that may increase H_2 and CH_4 concentrations. Also, the reason why exhaled H_2 but not CH_4 was related to adverse prognosis is not clear. There have been limited data published on the effect of H_2-predominant SIBO compared with CH_4-predominant SIBO.[34] We speculate that these divergent findings may represent a true lack of association (in other non-HF studies, exhaled CH_4 was related only to constipation), but they may also be due to a lack of power because only 30% of population has gut flora with methanogenic bacteria.[2,34] Furthermore, the role of age is another issue to consider, knowing that multiple authors have suggested advanced age as an independent risk factor for SIBO.[2,23] However, there is an incomplete knowledge about the underlying causes of the aging process, the age-related changes in intestinal motility, and the role of achlorhydria due to underlying gastric disease or the use of acid-neutralizing agents.

Several other limitations should be acknowledged. First, this was a small study performed in a single center; these 2 factors might increase the possibility of selection bias, limiting the generalization of these results to other populations of HF. Second are the known limitations inherent in its

observational nature. Third, the diagnosis of SIBO did not include the criterion standard, but there is evidence showing that lactulose breath test has an acceptable level of accuracy.[2] Fourth, given the high prevalence of SIBO in the elderly, a lack of an age-matched non-HF control group precluded analyzing the specific contribution of HF on the surrogates of SIBO evaluated here. Finally, there was no evidence that AUC-H_2 and AUC-CH_4 increased discrimination accuracy of the base model. We suspect that low statistical power plays an important role on these negative results, because they highly depend on sample size.

The strengths include the novelty of the results and the fact that lactulose breath testing is widely available, safe, inexpensive, and noninvasive,[22] advantages that make it ideal for assessing SIBO in daily clinical practice.

Clinical Perspectives

A growing body of evidence supports a pathogenic role of gut microbiota in HF. Advances in microbiology assays have contributed to better understanding of the complexity of bacterial microbiota and its role in health and disease. SIBO measurement has emerged as a readily available, nonexpensive, and noninvasive technique that offers a reliable way to document increased bacterial activity within the small intestine. We think that this technique opens new avenues for exploring the role of microbiota in the pathophysiology of HF. In addition, further studies are needed to elucidate the role of breath test for identifying and quantifying SIBO in HF patients.

Translational Outlook

Breath tests are easy methods for identifying SIBO. These tools emerge as a potential method for quantifying the contribution of small intestinal microbiota in the pathophysiology of HF and, perhaps, identifying a subgroup that would benefit from specific treatments such as modification of microbiota composition, modulation of the immune response, or even intensifying HF drug therapy.

Conclusion

In a cohort of patient with advanced HF, exhaled H_2 concentration after lactulose breath test—a surrogate of SIBO—was positively associated with surrogates of inflammatory activity, clinical status, and a higher risk of the death and hospitalizations.

Disclosures

None.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cardfail.2018.10.004.

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