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ORIGINAL ARTICLE

A Prospective Evaluation of Ileocecal Valve Dysfunction and Intestinal Motility Derangements in Small Intestinal Bacterial Overgrowth

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Abstract

Background Small intestinal bacterial overgrowth (SIBO) is an increasingly recognized clinical syndrome; however, its etiophathogenesis is poorly understood. We hypothesized that loss of gastric acid, a delayed intestinal transit, and ileocecal valve dysfunction may contribute to the genesis of this syndrome.

Aims Our primary aim was to assess these parameters using wireless motility capsule (WMC) testing and to correlate them with the presence or absence of SIBO.

Methods We prospectively evaluated 30 consecutive patients at a tertiary care center with suspected SIBO, diagnosed by lactulose hydrogen breath testing (LBT), and small bowel aspirate microbiology. Patients underwent WMC testing to assess ileocecal junction pressure (ICJP), small bowel transit time (SBTT), and regional gastrointestinal pH.

Results Thirty patients completed testing; 15 had a positive LBT, and 11 had a positive aspirate culture. As compared with LBT-negative patients, ICJP was lower (27.8 vs. 72.7 mmHg, $p = 0.027$), SBTT was longer (10.0 vs. 1.1 h, $p = 0.004$), gastric pH was higher (3.63 vs. 2.42,

$p < 0.01$), and small bowel pH was higher (6.96 vs. 6.61, $p = 0.05$). A hypotensive ICJP (<46.61 mmHg) was more prevalent in LBT-positive patients as compared with LBT-negative patients (73.3 vs. 14.29%, $p = 0.003$). Logistic regression models were used to assess the magnitude of each measured WMC parameter and the presence of SIBO. p values ≤0.05 were considered statistically significant.

Conclusions Patients with SIBO have significantly lower ICJP, prolonged SBTT, and a higher gastrointestinal pH as compared to those without SIBO. These abnormalities may play different roles in the pathogenesis of SIBO, facilitating more targeted treatment to prevent recurrences of SIBO.

Abbreviations

SIBO	Small intestinal bacterial overgrowth
ICV	Ileocecal valve
WMC	Wireless motility capsule
LBT	Lactulose hydrogen breath test
ICJP	Ileocecal junction pressure
SBTT	Small bowel transit time
PPIs	Proton-pump inhibitors
H2RBs	H2 receptor blockers
NPO	*Nothing per os*
H$_2$	Hydrogen
CH$_4$	Methane
EGD	Esophogastroduodenoscopy
WGTT	Whole-gut transit time
CTT	Colonic transit time
GET	Gastric emptying time
OR	Odds ratio

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MMC   Migrating motor complex
SD   Standard deviation

Background

Small intestinal bacterial overgrowth (SIBO) is an important and increasingly recognized clinical syndrome [1–3]. A significant problem among patients with SIBO is frequent relapse or recurrence following antimicrobial therapy [4, 5]. This likely results from our current lack of understanding about the underlying pathophysiological mechanisms predisposing to SIBO [6, 7]. The presumed endogenous mechanisms that prevent bacterial overgrowth in the small bowel include a high gastric acid concentration, microbial clearance by intestinal motility and an intact ileocecal valve (ICV) [8]. Disruption of these protective mechanisms is often not clinically evident; thus, SIBO is frequently associated with hypochlorhydria (typically as a result of therapy with proton pump inhibitors), small bowel dysmotility (e.g., scleroderma, diabetes) and anatomic abnormalities (ileocecal resections and surgical blind loop). In addition, it has been postulated that physiological malfunction of the ileocecal barrier, predisposing to colo-intestinal reflux of bacterial contents may be a significant factor [9–12]. However, until recently, functioning of the ICV has been difficult to reliably measure and this mechanism has remained more of a theoretical consideration.

The wireless motility capsule (WMC, SmartPill) is multivitamin sized and utilizes technology to measure pH, pressure, and temperature throughout the gastrointestinal tract. This technology provides a simple, non-invasive and innovative approach to evaluate regional gastrointestinal transit. In addition, the capsule allows for measurements of pressure contraction patterns throughout the gastrointestinal tract. With these capabilities, WMC testing offers the potential to evaluate gastrointestinal pH, transit, and luminal pressure in a regionally specific manner.

In our prior retrospective studies, we found that small bowel transit time (SBTT) was significantly prolonged among patients with SIBO (based on LBT) as compared to those without. We also observed significantly higher gastrointestinal pH in SIBO. Interestingly, ICV pressures, based on WMC measurements (see methods below), were significantly lower in the SIBO positive as compared to negative group [13, 14]. In an effort to validate these important findings, we carried out this prospective study, with the primary aim to assess the relationship between small bowel transit time (SBTT), ICV pressures, and gastrointestinal pH with SIBO.

Methods and Study Design

Subjects

We performed an observational study of 30 consecutive, adult patients with suspected SIBO, who were referred to an academic tertiary care center over a 6-month period (March 2014 to September 2014). Symptoms included chronic unexplained abdominal discomfort, bloating, distention, nausea, weight loss, flatulence, and loose stool. All subjects included in the study underwent lactulose hydrogen breath testing, upper endoscopy to obtain small bowel aspirates for quantitative cell count and cultures, and WMC testing. Patients were assigned to a cohort based on results of their LBT and aspirate culture results (positive vs. negative). For the purposes of our study, SIBO was defined as positive if either the LBT or the aspirate culture results was positive. Clinical and demographic characteristics among the groups were collected and used for further analysis. Normative data were developed using the results of original WMC testing in healthy controls, as previously published [13–15].

Measurements and Definitions

Wireless Motility Capsule (SmartPill) Testing

Prior to WMC ingestion, all patients were instructed to hold proton pump inhibitors (PPIs) and H2 receptor blockers (H2RBs) for 7–10 days prior to the study. Patients were additionally asked to remain *nothing per os* (NPO) beginning at midnight prior to the ingestion. On the day of the study, all patients were given a standardized *Smartbar* (*255 calories, 75% carbohydrate, 21% protein, 3% fat, 3% fiber*) prior to WMC ingestion and were instructed to remain NPO for 6 h with the exception of small quantities of water (up to half a cup). Participants were also asked to keep the SmartPill data receiver within 3 feet at all times during the 5 day study period and were prompted to push the button on their external data receiver whenever they had a bowel movement. Patients were instructed to remain on the toilet bowl for 2 min after having a bowel movement, in order to ensure communication with the data receiver if the pill had passed. At the completion of 5 days, patients were instructed to return their data receivers and diary/log of events. Data from each receiver was subsequently downloaded and analyzed using the *MotiliGI* software. All WMC tests were interpreted by a single reader, who was blinded to results of LBT and aspirate cultures.

We recently reported a novel method [13, 14] to estimate the ileocecal junction pressure or ICJP, as a surrogate marker for ICV function. This was based on the simple

time stamping of the characteristic drop in pH as the pill exits the ileum into the cecum and then identifying the highest/peak pressure over a 5-min window prior to this event. This pressure was taken to represent the ICJP.

Small bowel transit time measurements were obtained as per standard WMC criteria [13]. Additionally, gastric and small bowel pH measurements were obtained and compared among patients with and without positive LBT and among patients with and without positive aspirate cultures. Patients on long-term, chronic acid suppressive therapy (proton pump inhibitors and/or H2 receptor blockers) were excluded from all pH analyses.

Normative Data

Based on the wireless capsule motility studies of 47 healthy control patients, normative values were established for ileocecal junction pressures, small bowel transit time, small bowel mean pH, and gastric mean pH. The mean value plus twice the standard deviation of the healthy control group was used as the cut off point for normal values of SBTT. Because of skewedness of small bowel mean pH, gastric mean pH and ileocecal junction pressures, interval estimation using Horn's method was used to establish a normal cutoff.

Lactulose Hydrogen Breath Testing

Lactulose breath testing was performed using a Quintron BT Digital Microlyzer calibrated with Quingas-5. A 10-g dose of lactulose was administered followed by standard protocol, obtaining samples every 20 min for a total duration of 3 h. The test was considered positive if it showed one or more of the following: (a) a baseline breath concentration of >12 parts per million (ppm) for hydrogen (H2) or >7 ppm for methane (CH4); (b) an increase within 90 min that was followed by a larger peak (with a decrease in at least 5 ppm following the first peak). The first increase had to meet one of the following to be considered positive: (1) an increase in CH4 of at least 12 ppm above the baseline by 90 min, and (2) If producing H2 only, an increase in H2 of at least 20 ppm (parts per million) over the baseline by 90 min. A positive LBT result indicated the presence of SIBO. Patients were instructed to avoid taking antibiotics for a minimum of 4 weeks prior to the test and to avoid laxatives and any other medications that effect GI motility for a least 1 week prior to breath testing. On the day prior to the test, patients were instructed to avoid high-fiber foods such as bran, coarse breads, pasta, nuts, beans and uncooked vegetables along with all caffeinated beverages. Twelve hours prior to their test, patients were instructed to remain NPO. All breath tests were evaluated by a single, experienced reader who was blinded to the results of the WMC testing.

Small Bowel Aspirates for Quantitative Cell Count and Culture

EGD with small bowel aspirates were obtained using standard sterile technique and transferred to the growth media, BBL Port-a-Cul (Beckton, Dickinson and Company, Franklin Lakes, NJ). All cultures were stored on ice and transported to the microbiology lab at Johns Hopkins Hospital within 1 h of collection for interpretation. SIBO was defined by total (aerobic and anaerobic) viable bacterial counts of $>10^5$ colony-forming units (CFU) per mL of luminal fluid.

Statistical Analysis

We compared summary statistics of demographic, clinical and WMC-measured variables (gender, age, race, presenting symptoms, WMC testing results) between the SIBO group and the control group (without SIBO by definition), using two-sample t tests and Chi-square tests for continuous and categorical variables, respectively. Each study subject's gastrointestinal pH, transit time, and ICJ pressure were presented as numerical as well as binary variables (using the normal cutoffs) for comparisons. When the continuous variables were significantly skewed (tested by normality analysis), such as SBTT, CTT, WGTT, and ICJP, or any count data in the 2×2 contingency table were below 5, nonparametric Wilcoxon rank-sum tests and Fisher's exact tests were used, respectively. Pearson correlation coefficients were calculated to examine the possible linear relationship among IJP, SBTT, small bowel pH, and gastric pH. All patients on chronic acid suppressive therapy (>60 days) were excluded from our pH analyses.

Univariate logistic regression models were used to assess the magnitude of association between each measured WMC parameter and the presence of SIBO. Multivariate logistic regression models were then performed to investigate whether a significant association in univariate analysis is confounded by other parameters. Odds ratios and 95% confidence intervals were presented. All analyses were conducted using STATA 13. p values ≤ 0.05 were considered statistically significant.

Results

All 30 patients completed LBT and had small bowel aspirates obtained. Twenty-nine of the 30 patients completed WMC testing (one subject withdrew from WMC testing). Of our subjects, 15 (50%) had a positive LBT, 11 (36.7%) had a positive aspirate culture and 11 (36.7%) had a positive LBT or a positive aspirate culture (Table 1),

Table 1 Concordance and discordance between LBT and aspirate culture results

LBT	Aspirate culture		
	Negative	Positive	Total
Negative (N)	13	2	15
Positive (N)	6	9	15
Total	19	11	30

confirming the presence of SIBO. Further sub-analysis was performed to compare and contrast gastrointestinal physiological parameters among LBT-positive and aspirate culture-positive subjects.

Demographics

The majority of our patients were female (83%), Caucasian (80%), and the average age was 41 years old. The most common primary presenting symptom in our patient population was abdominal bloating. There were no significant demographic or clinical differences between the SIBO positive and negative groups (Table 2).

Small Bowel Transit Times Are Slower in Patients with SIBO

Small bowel transit time (SBTT) in patients with SIBO was about twice as slow as compared to patients without SIBO (mean of 7.89 vs. 3.93 h, $p = 0.02$, Table 4c). Furthermore, with a prolonged SBTT defined as taking longer than 6 h, we found that prolonged SBTT was present in 47% of patients with SIBO, while in none of those without SIBO ($p = 0.01$, Table 4). There was no statistical difference in SBTT when SIBO was defined by LBT versus aspirate culture positivity ($p = 0.276$, Table 3).

Gastric Emptying Times, Colonic Transit Times, and Whole-Gut Transit Times Are Similar Among Patients With and Without SIBO

While patients with SIBO had a slightly slower colonic transit time (CTT), there were no significant difference in CTT among patients with and without SIBO (mean of 39.95 vs. 37.94 h, $p = 0.0676$, Table 4c). There was no statistical difference in CTT when SIBO was defined by LBT versus aspirate culture positivity ($p = 0.145$, Table 3).

Similarly, while patients with SIBO had a slightly slower gastric emptying time (GET), there were no significant difference in GET among patients with and without SIBO (mean of 3.74 vs. 3.394 h, $p = 0.568$, Table 4c). There was no statistical difference in GET when SIBO was defined by LBT versus aspirate culture positivity ($p = 0.419$, Table 3).

Finally, while patients with SIBO had an overall slower whole-gut transit time, there were no significant differences when compared to patients without SIBO (mean WGTT of 53.77 vs. 45.29 h, $p = 0.246$, Table 4c). Similarly, there were no statistical differences in WGTT when SIBO was defined by LBT versus aspirate culture positivity ($p = 0.259$, Table 3).

Gastrointestinal Environments Are Less Acidic Among Patients with SIBO

The pH of both the stomach and small intestine were significantly higher in patients with SIBO as compared to those without SIBO (mean gastric pH of 3.59 vs. 2.28, $p < 0.01$; mean small bowel pH of 7.01 vs. 6.49, $p = 0.002$, Table 4c). There was no statistical difference in gastric pH ($p = 0.357$) or small bowel pH ($p = 0.401$) when SIBO was defined by LBT versus aspirate culture positivity (Table 3).

Ileocecal Valves Are Less Competent in Patients with SIBO

The mean ileocecal junction pressure (ICJP) was significantly lower among patients with SIBO (LBT or aspirate positive results) as compared to those without SIBO (LBT or aspirate negative results) (47.2 vs. 98.9 mmHg, $p = 0.001$, Table 4c). Similar to the above parameters, there was no statistical difference in ICJP when SIBO was defined by LBT or aspirate culture positivity ($p = 0.244$, Table 3).

Furthermore, using ICJP cutoff from previous WMC study of healthy patients, we found that a hypotensive ICJP (<46.61 mmHg) was significantly more prevalent in SIBO positive as compared to negative patients (71 vs. 8.33%, $p = 0.002$, Table 4c).

Delayed SBTT, an Elevated Gastrointestinal pH, and a Lower ICJP Are Independent Risk Factors in the Development of SIBO

Small bowel transit, gastrointestinal pH, and ICJP appear to be independent risk factors in the development of SIBO. Univariate logistic regression analyses showed a significant association between LBT positivity and ICJP (OR 0.97,

Table 2 Demographics and clinical characteristics

Characteristic	All	LBT		$p^{\dagger}$
		Positive	Negative	
Patients (%)	30 (100)	15 (50)	15 (50)	–
Age (years), mean (SD)	41.2 (12)	42.1 (12.2)	38 (12)	0.31
Gender, *N* (%)				1.00
Male	5 (16.7)	3 (20)	2 (13.3)	
Female	25 (83.3)	12 (80)	13 (86.7)	
Race, *N* (%)				0.56
Black	5 (16.7)	3 (20)	2 (13.3)	
White	24 (80)	12 (80)	12 (80)	
Other	1 (3.3)	0 (0)	1 (6.7)	
Presenting symptoms, *N* (%)				
Bloating	15 (50)	7 (46.7)	8 (53.3)	1.00
Abdominal pain/discomfort	8 (26.7)	2 (13.3)	6 (40)	1.00
Diarrhea	6 (20)	3 (20)	3 (20)	1.00
Constipation	4 (13.3)	2 (13.3)	2 (13.3)	1.00
Nausea	4 (13.3)	2 (13.3)	2 (13.3)	1.00
Distension	3 (10)	1 (6.7)	2 (13.3)	1.00
≥2 symptoms (vs. 1 symptom)	15 (50)	8 (53.3)	7 (46.7)	1.00
	All	Aspirate		$p^{\dagger}$
		Positive	Negative	
Patients (%)	30 (100)	11 (36.7)	19 (63.3)	–
Age (years), mean (SD)	41.2 (12)	43 (13.5)	38.1 (10.9)	0.29
Gender, *N* (%)				1.00
Male	5 (16.7)	2 (18.2)	3 (15.8)	
Female	25 (83.3)	9 (81.8)	16 (84.2)	
Race, *N* (%)				0.19
Black	5 (16.7)	3 (27.2)	2 (10.5)	
White	24 (80)	7 (63.6)	17 (89.5)	
Other	1 (3.3)	1 (9.1)	0 (0)	
Presenting symptoms, *N* (%)				
Bloating	15 (50)	8 (72.7)	11 (57.9)	0.47
Abdominal pain/discomfort	8 (26.7)	1 (9.1)	7 (36.8)	0.35
Diarrhea	6 (20)	1 (9.1)	5 (26.3)	0.37
Constipation	4 (13.3)	1 (9.1)	3 (15.8)	1.00
Nausea	4 (13.3)	3 (27.2)	3 (15.8)	0.64
Distension	1 (9.1)	1 (9.1)	2 (10.5)	1.00
≥2 symptoms (vs. 1 symptom)	15 (50)	4 (36.4)	11 (57.9)	0.45
	All	LBT and aspirate		$p^{\dagger}$
		Positive	Negative	
Patients (%)	30 (100)	9 (30)	13 (43.3)	–
Age (years), mean (SD)	41.2 (12)	45.7 (13.5)	38.7 (12.1)	0.22
Gender, *N* (%)				1.00
Male	2 (6.7)	1 (11.1)	1 (7.7)	
Female	25 (83.3)	8 (88.9)	12 (92.3)	

Table 2 continued

| | All | LBT and aspirate | | $p^{\dagger}$ |
		Positive	Negative	
Race, N (%)				0.32
Black	5 (16.7)	3 (33.3)	2 (15.4)	
White	17 (56.7)	6 (66.7)	11 (84.6)	
Other	0 (0)	0 (0)	0 (0)	
Presenting symptoms, N (%)				
Bloating	14 (46.7)	7 (77.8)	7 (53.8)	0.38
Abdominal pain/discomfort	9 (30)	3 (33.3)	6 (46.2)	0.67
Diarrhea	4 (13.3)	1 (11.1)	3 (23.1)	0.62
Constipation	3 (10)	1 (11.1)	2 (15.4)	1.00
Nausea	5 (16.7)	3 (33.3)	2 (15.4)	0.61
Distension	3 (10)	1 (11.1)	2 (15.4)	1.00
≥ 2 symptoms (vs. 1 symptom)	11 (36.7)	4 (44.4)	7 (53.8)	1.00

SD standard deviation

Statistical significance set at a *p* value ≤ 0.05

† Chi-square test for categorical variables and Student *t* test for continuous variables

Table 3 Discordance among GI pathophysiological parameters in LBT-positive versus aspirate culture-positive cohorts

Parameter	LBT positive (mean)	Aspirate culture positive (mean)	*p* value
GET (h)	3.95	4.40	0.42
SBTT (h)	8.19	10.02	0.28
CTT (h)	42.21	30.50	0.15
WGTT (h)	57.09	48.51	0.26
Gastric pH	3.63	3.45	0.36
Small bowel pH	6.96	6.92	0.40
ICJP	47.73	40.82	0.24

p values obtained from Fischer's exact tests performed for categorical variables

Statistical significance set at a *p* value ≤ 0.05

$p = 0.047$), between LBT positivity and SBTT [(OR 2.31, $p = 0.049$) (Table 5)], and between LBT positivity and small bowel pH (OR 8, $p = 0.04$) and gastric pH [(OR 2.14, $p = 0.03$), (Fig. 1b)]. On the contrary, among patients with SIBO, there was no significant correlation between ICJP with SBTT ($r = -0.25$, $p = 0.36$), gastric pH with SBTT ($r = -0.29$, $p = 0.29$), or small bowel pH with SBTT ($r = 0.18$, $p = 0.53$) (Fig. 1a, b).

Predictive Value of SIBO Using WMC Parameters

Each 1-h increase in SBTT was associated with an increased odds of LBT positivity (OR 2.3, $p = 0.049$), small intestine aspirate culture positivity (OR 2.5, $p = 0.026$), or combined (OR 1.95, $p = 0.068$) (Table 5). Each 1 mmHg decrease in ICJP was significantly associated with a 3–5% increase in odds of LBT positivity (OR 0.97, $p = 0.047$), small intestine aspirate culture positivity

(OR 0.95, $p = 0.039$), or combined (OR 0.96, $p = 0.026$) (Fig. 2). Similarly, an increased gastric and small bowel pH were significantly associated with LBT positivity [(gastric pH: $p = 0.017$, small bowel pH: $p = 0.056$) (Table 4a)]. The accuracy of SBTT in predicting SIBO positivity was 70.4% and ICJP was 79.3%. The accuracy was further improved when using a combination of SBTT and ICJP of 86.2% (Table 6).

Concordance in GI Parameters Among LBT- and Aspirate Culture-Positive Patients

While there was not a direct overlap in patients who tested positive with both LBT ($n = 15$) and with aspirate culture ($n = 11$), further sub-analysis of the aforementioned GI pathophysiological parameters as measured by WMC testing, illustrate that these associations are present for SIBO positive subjects, regardless of whether SIBO is

Table 4 Statistical comparisons of gastrointestinal pathophysiologic parameters in SIBO positive versus SIBO negative (control) cohorts

	SIBO LBT positive Mean (SD) or N (%)	Control LBT negative Mean (SD) or N (%)	$p^{\dagger}$
(A)			
SBTT (h)	8.19 (5.77)	3.88 (1.12)	0.01
Prolonged SBTT (≥6.12 h)	7/13 (53.85%)	0/14 (0%)	0.00
GET (h)	3.95 (1.72)	3.19 (1.17)	0.152
Prolonged GET (>4 h)	2/14 (14.29%)	6/13 (46.15%)	0.103
CTT	41.21 (37.40)	35.97 (27.60)	0.408
Prolonged CTT (>59 h)	2/14 (14.29%)	3/14 (21.43%)	1.000
WGTT	57.09 (34.66)	43.18 (27.58)	0.066
Prolonged WGTT (>120 h)	1/14 (7.14%)	0/14 (0%)	1.000
Gastric pH	3.63 (1.17)	2.42 (1.34)	0.02
Small bowel pH	6.96 (0.39)	6.61 (0.43)	0.06
ICJP	47.73 (30.20)	91.00 (60.41)	0.01
Hypotensive ICJP (<46.61)	11/15 (73.33%)	2/14 (14.29%)	0.00

	Aspirate positive	Aspirate negative	$p^{\dagger}$
(B)			
SBTT (h)	10.02 (6.37)	1.07 (1.03)	0.00
Prolonged SBTT (≥6.12 h)	7/10 (70%)	0/17 (0%)	<0.01
GET (h)	4.40 (1.71)	3.31 (0.96)	0.07
Prolonged GET (>4 h)	6/10 (60%)	2/17 (11.76%)	0.03
CTT	30.50 (18.53)	43.86 (37.67)	0.39
Prolonged CTT (>59 h)	1/10 (10%)	4/18 (22.22%)	0.63
WGTT	48.51 (19.99)	51.04 (36.96)	0.63
Prolonged WGTT (>120 h)	0/10 (0%)	1/18 (5.56%)	1.00
Gastric pH	3.45 (1.16)	2.79 (1.47)	0.19
Small bowel pH	6.92 (0.46)	6.72 (0.42)	0.20
ICJP	40.82 (13.59)	85.61 (58.52)	0.01
Hypotensive ICJP (<46.61)	8/11 (72.73%)	5/18 (27.78%)	0.03

	LBT or aspirate positive	LBT or aspirate negative	$p^{\dagger}$
(C)			
SBTT (h)	7.89 (5.71)	3.93 (1.16)	0.02
Prolonged SBTT (≥6.12 h)	7/15 (46.76%)	0/12 (0%)	0.01
GET (h)	3.74 (1.75)	3.39 (1.10)	0.57
Prolonged GET (>4 h)	6/15 (40%)	2/12 (16.67%)	0.24
CTT	39.95 (35.59)	37.94 (29.12)	0.68
Prolonged CTT (>59 h)	2/16 (12.50%)	3/12 (25%)	0.62
WGTT	53.77 (33.78)	45.29 (29.01)	0.25
Prolonged WGTT (>120 h)	1/16 (6.25%)	0/12 (0%)	1
Gastric pH	3.59 (1.13)	2.28 (1.37)	0.01
Small bowel pH	7.01 (0.38)	6.49 (0.32)	0.00
ICJP	47.24 (28.81)	98.92 (61.56)	0.00
Hypotensive ICJP (<46.61)	12/17 (70.59%)	1/12 (8.33%)	0.00

Statistical significance set at a p value ≤0.05

$\dagger$ p values from Wilcoxon rank-sum tests and Fisher's exact tests performed for continuous and categorical variables, respectively

Table 5 Univariate logistic regression modeling SIBO

	OR	95% CI	*p* value
LBT			
SBTT	2.31	1.00–5.32	0.05
ICJP	0.97	0.94–1.00	0.05
Aspirate			
SBTT	*2.50*	1.12–5.62	0.03
ICJP	0.95	0.90–1.00	0.04
LBT or aspirate			
SBTT	1.95	0.95–3.98	0.07
ICJP	0.96	0.93–1.00	0.03

CI confidence interval

p values from Wilcoxon rank-sum tests and Fisher's exact tests performed for continuous and categorical variables, respectively

Statistical significance set at a *p* value ≤0.05

defined by lactulose hydrogen breath testing or small bowel aspirate culture. There was no significant difference in any of parameters among the LBT-positive and aspirate culture-positive cohorts (Table 3).

Discussion

We previously reported our findings from consecutive patients with suspected SIBO who underwent both lactulose hydrogen breath testing and WMC studies and found that low ileocecal junctional pressures, prolonged small bowel transit times, and elevated gastric/intestinal pH were all significantly associated with SIBO [13, 14]. However, we recognize the limitations of our preliminary findings including the retrospective nature of the study, small

sample size, challenges with the use of only lactulose hydrogen breath testing to establish a diagnosis [15–18] and an inability to eliminate potential confounders (e.g., chronic acid suppressive therapy, antibiotic therapy).

In the present study, we have prospectively confirmed our previous findings using both hydrogen breath testing and the gold standard of small bowel aspirate. We have additionally confirmed that ileocecal junction pressures, as measured by a WMC, are significantly lower among SIBO patients as compared to those without, suggesting that the ICVs of patients with SIBO are likely less competent than the ICV in patients without SIBO. We have also shown that patients with SIBO have prolonged small bowel transit times and elevated gastrointestinal pH (gastric and small bowel). Similar to our prior investigation, our present study again suggests minimal overlap among these subgroups. While our findings suggest that a delayed SBTT, an elevated small bowel pH, and a lower ICJP are all associated with LBT positivity and thus indirectly with the development of SIBO, there is no interrelation among these factors.

Prior studies, employing gastroduodenal manometry to evaluate SIBO, have all demonstrated significant abnormalities in small intestinal motility [19, 20]. In healthy individuals, an absence of phase III migrating motor complex (MMC) activity has been associated with increased small intestinal bacterial counts [1]. Octreotide, a somatostatin analog and an effective small bowel prokinetic agent, has been reported to increase MMC frequency in both healthy controls and in scleroderma patients. Additionally, this agent has been shown to effectively reduce small intestinal bacterial overgrowth [3]. The importance of preserved small bowel motility in this syndrome has also been emphasized in prior studies showing
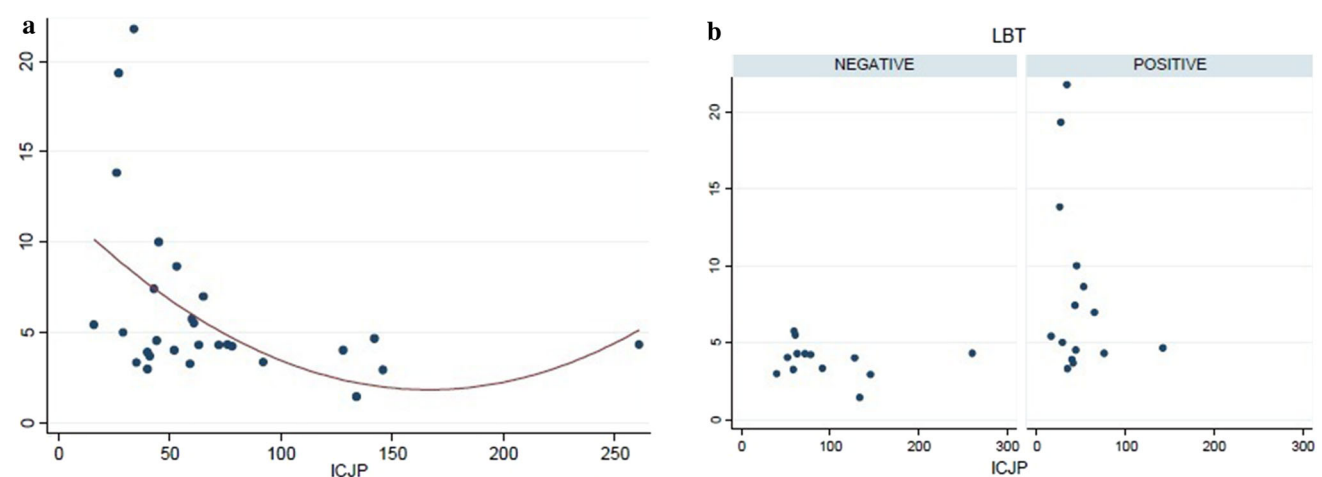

Fig. 1 **a** Scatter plot of SBTT versus ICJP among all subjects. Hypotensive ICJP is associated with longer SBTT, however the relationship is not linear. **b** Scatter plot of SBTT versus ICJP stratified by LBT test positivity. There is a marked difference in the relationship between SBTT and ICJP among LBT-positive and

LBT-negative subjects. Among the SIBO patients, there was no significant correlation between ICJP with SBTT ($r = -0.25$, $p = 0.36$), gastric ($r = -0.29$, $p = 0.29$) or small bowel pH ($r = 0.18$, $p = 0.53$)
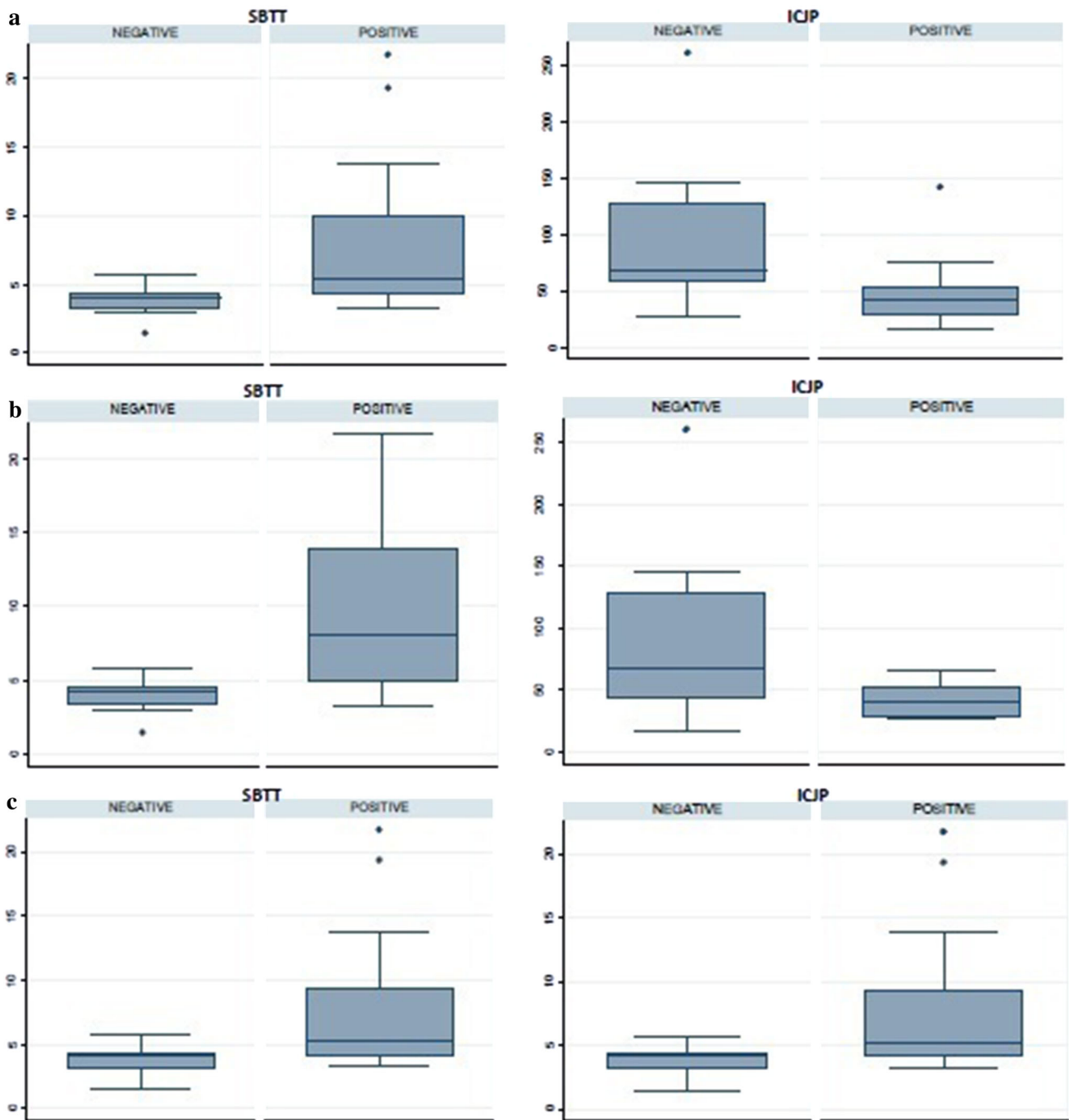
Fig. 2 Box plots of SBTT and ICJP in SIBO versus control (nonsymmetric distribution). **a** LBT, **b** aspirate culture, **c** LBT or aspirate culture. Each 1-h increase in SBTT was significantly associated with increased odds of LBT positivity (OR 2.3, $p = 0.049$), small intestine aspirate culture positivity (OR 2.5, $p = 0.026$), or combined (OR 1.95, $p = 0.068$) (see Table 6). Each

1 mmHg decrease in ICJP was significantly associated with a 3–5% increase in odds of LBT positivity (OR 0.97, $p = 0.047$), small intestine aspirate culture positivity (OR 0.95, $p = 0.039$), or combined (OR 0.96, $p = 0.026$). Negative LBT/aspirate culture serves as the control cohort. Positive LBT/aspirate cultures serves as the SIBO cohort

that the presence of SIBO is associated with a loss and reduced frequency of MMC cycling [21]. Another study using antroduodenal manometry to evaluate patients with SIBO, demonstrated an increase in clustered contractions

observed in obese patients with SIBO as compared to obese patients without SIBO [22].

The ICV remains a relatively unexplored sphincter in the gastrointestinal tract; however, prior studies looking at

Table 6 Relationship between prolonged SBTT and hypotensive ICJP among SIBO patients

	Normal SBTT	Prolonged SBTT	p value
LBT positive			
Normotensive ICJP	2	2	–
Hypotensive ICJP	4	5	1.000
Aspirate positive			
Normotensive ICJP	1	2	–
Hypotensive ICJP	2	5	1.000
LBT and/or aspirate positive			
Normotensive ICJP	3	2	–
Hypotensive ICJP	5	5	1.000

p value obtained from Fisher's exact test for correlation between prolonged SBTT and hypotensive ICJP

Statistical significance set at a p value ≤0.05

manometric assessment of ICV pressures among SIBO positive and negative patients have been in agreement with our findings. Miller et al. [11] investigated subjects who underwent colonoscopy with manometric ICV measurements following cecal distention. These authors showed that patients with SIBO (as established by lactulose hydrogen breath testing), had no reflexive increase in ICV pressures, while an appropriate increase was observed in SIBO negative subjects. Unlike colonic manometry which has associated problems related to the invasive nature of the procedure, patient discomfort, and need for sedation, our method can evaluate ICV pressures in a simple and noninvasive manner, thus facilitating further studies on this important mechanism.

One of the most well-described protective factors of SIBO in healthy subjects is preserved gastrointestinal acidity. Inhibition of normal gastric acid secretion, either by acid suppressive agents such as PPIs and H2RBs, or physiological hypochlorhydria of aging, are all presumed to be significant risk factors for SIBO as demonstrated by several observational and prospective studies [23–25]. However, there is scant literature reporting on direct measurements of gastrointestinal pH among patients with this syndrome. In the present study, we found that patients with SIBO had significantly higher gastric and small bowel pH and thus reduced acidity of their gastrointestinal environment, as compared to SIBO negative patients. This remained true even after excluding those on chronic PPI therapy. The mean difference was approximately 1 pH unit, which translates into a clinically significant tenfold reduction in acid concentration.

Some limitations of this study include a small sample size, referral bias due to the tertiary nature of the institution, inherent limitations of lactulose breath testing for the diagnosis of SIBO, and possible contamination of small bowel aspirate cultures leading to a reduced diagnostic utility. Another limitation that deserves mentioning is the lack of complete concordance among LBT and small bowel aspirate results. This discrepancy is likely multifactorial; the learning curve of the laboratory personal performing this test, the intrinsic limitations of each test, and the fact that lactulose is not absorbed in the small bowel are all likely responsible. Additionally, differences among bacterial flora determine the response of breath testing, further complicating this matter.

Nonetheless, our study adds significant value to the literature. At present, there are limited studies utilizing small bowel cultures—the most direct method of assessing bacterial colonization—in the diagnosis of SIBO. The fact that small bowel aspirates were used in conjunction with LBT results likely adds a level of accuracy to this study. Results of our study are further valuable given the paucity of existing literature evaluating pathophysiological mechanisms in SIBO, including evaluation of the ICV. Another strength of our study is the demonstration that pathophysiological mechanisms in SIBO can be studied in a simple and noninvasive manner.

In summary, this study clarifies the mechanisms that may predispose to SIBO in different patients, allowing us to propose a working classification of the phenotype based on three pathophysiological groups: Type 1: High gastric/intestinal pH ("low acid" group); Type 2: Slow transit ("intestinal dysmotility" group); and Type 3: Low ICJP ("dysfunctional ICV" group). A stratification scheme into subgroups, based on etiopathogenesis, may provide a key framework for understanding this increasingly common, but undoubtedly heterogeneous syndrome. This stratification system may also provide the foundation for the development of more mechanistic and targeted therapies to effectively treat individuals with recurrent SIBO.

Compliance with ethical standards

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