Functional $^{13}$C-urea and glucose hydrogen/methane breath tests reveal significant association of small intestinal bacterial overgrowth in individuals with active *Helicobacter pylori* infection

Dietmar Enko *, Gernot Kriegshäuser

1. Introduction

*Helicobacter pylori* is still reported to be a common pathogen worldwide [1,2]. Since this Gram-negative bacterium was recognized as a risk factor of peptic ulcer disease, chronic atrophic gastritis, gastric cancer, and mucosa-associated lymphoid tissue lymphoma, numerous invasive and non-invasive diagnostic methods for accurate detection of *H. pylori* have been established. The functional $^{13}$C-urea breath test ($^{13}$C-UBT) is considered as a valid laboratory method for the diagnosis of *H. pylori* infection [3].

$H. pylori$ is well known as a bacterium that hydrolyses urea into ammonia and carbonic acid [4,5]. This chemical reaction is the basis for the detection of active infected individuals with the $^{13}$C-UBT [6] and protects the bacterium against gastric acid [4,7], which plays an important protective role in the prevention of bacterial colonization in the stomach and small intestine [8]. The low intra-gastric pH value may increase due to *H. pylori* infection because of production of ammonia [9]. Therefore, this bacterium may be a causative agent of small intestinal bacterial overgrowth (SIBO).

SIBO is a complex condition of the upper gastrointestinal tract, which is defined as the presence of abnormal numbers of bacteria in the small intestine [10,11]. The etiology of SIBO is multifactorial comprising achlorhydria, previous surgery of the upper gastrointestinal tract, mechanically defective ileocecal valve, and/or impaired motility of the intestine [10]. The laboratory diagnostic glucose hydrogen (H$_2$)/methane (CH$_4$) breath test (HMBT) represents a non-invasive highly reproducible and inexpensive tool for SIBO diagnosis [12].

To date, SIBO rates in patients with active *H. pylori* infection have not been systematically evaluated by laboratory functional breath tests. Therefore, it is of great interest to investigate whether a close association exists between these two gastrointestinal conditions.

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**ABSTRACT**

**Objectives:** *Helicobacter pylori* infection is considered to alter the bacterial flora in the upper gastrointestinal tract. This study aimed at investigating the presence of small intestinal bacterial overgrowth (SIBO) in patients with active *H. pylori* infection assessed by functional breath testing.

**Design and methods:** A total of 109 outpatients, who were referred for the *H. pylori* $^{13}$C-urea breath test ($^{13}$C-UBT) by general practitioners and specialists, were also tested for the presence of SIBO by the glucose hydrogen (H$_2$)/methane (CH$_4$) breath test (HMBT). A detailed anamnesis was carried out about the history of *H. pylori* infection, eradication therapies, proton pump inhibitor intake, and comorbidities.

**Results:** In total, 36/109 (33.0%) patients had a positive *H. pylori* $^{13}$C-UBT, and 35/109 (32.1%) patients had a positive glucose HMBT, the latter being indicative of SIBO. Interestingly, individuals with a positive *H. pylori* $^{13}$C-UBT were significantly more often associated with a positive glucose HMBT ($p = 0.002$). Cohen's $\kappa$ measuring agreement between the $^{13}$C-UBT and the glucose HMBT was 0.31 (confidence intervals: 0.12–0.50) ($p = 0.001$). Altogether, 19 of 54 (35.2%) patients, who had completed up to four eradication therapies, were diagnosed with SIBO by HMBT.

**Conclusions:** *H. pylori* infection was found to be significantly associated with the presence of SIBO as determined by functional breath testing. In addition, SIBO rates appeared to have increased after completed eradication therapies. However, further longitudinal studies are warranted to fully elucidate the relationship and treatment modalities of coincident *H. pylori* infection and SIBO.

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SIBO is a complex condition of the upper gastrointestinal tract, which is defined as the presence of abnormal numbers of bacteria in the small intestine [10,11]. The etiology of SIBO is multifactorial comprising achlorhydria, previous surgery of the upper gastrointestinal tract, mechanically defective ileocecal valve, and/or impaired motility of the intestine [10]. The laboratory diagnostic glucose hydrogen (H$_2$)/methane (CH$_4$) breath test (HMBT) represents a non-invasive highly reproducible and inexpensive tool for SIBO diagnosis [12].

To date, SIBO rates in patients with active *H. pylori* infection have not been systematically evaluated by laboratory functional breath tests. Therefore, it is of great interest to investigate whether a close association exists between these two gastrointestinal conditions.

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As proton pump inhibitors (PPIs) are included in the treatment regime of *H. pylori* infections, the interference of this class of drugs with the gastric acidic barrier must also be considered [13]. PPIs can block the proton pump (H+/K+-ATPase) of parietal cells in the stomach. Nevertheless, the side effects of PPI use, such as altering the bacterial flora of the upper gastrointestinal tract through changes in gastric pH, may not be underestimated [14].

The aim of this study was to assess the presence of SIBO in patients with active *H. pylori* infection by the functional 13C-UBT and glucose HMBT in a cohort of patients who were referred by general practitioners and specialists to our outpatient clinic. Additionally, a detailed anamnesis was carried out on gastrointestinal symptoms, the history of *H. pylori* infection, eradication therapies, PPI use, and comorbidities.

## 2. Materials and methods

### 2.1. Ethics

The ethical approval for this study was obtained from the Ethical Committee of Upper Austria, Linz, Austria. The study was carried out in accordance with the latest version of the Declaration of Helsinki. Written informed consent was obtained from all patients.

### 2.2. Patients

Overall, 109 outpatients, who were referred for laboratory *H. pylori* 13C-UBT by general practitioners and specialists, were also tested for SIBO by functional glucose HMBT during a second consultation. Thirty-six (33.0%) individuals were males and 73 (67.0%) were female. The mean age was 44 ± 16 years. The basic characteristics of the study participants are illustrated in Table 1. Taken together, 97/109 (89.0%) patients presented one or more gastrointestinal symptoms (i.e., upper abdominal pain, bloating, nausea, diarrhea, or obstipation), 21/109 (19.2%) individuals had a preexisting gastrointestinal or hepatic disorder (i.e., reflux esophagitis and hepatitis) in their case histories, and 12/109 (11.0%) individuals were diagnosed with a psychiatric disorder. All patients had a negative case history of previous surgery of the upper gastrointestinal tract (i.e., esophagus, stomach, and small intestine).

The inclusion criteria for this study were a minimum age of 15 years, an overnight fasting state, and a non-smoking period >12 h before breath testing. Patients who underwent antibiotic-based therapy at least 4 weeks before and/or PPI therapy at least 2 weeks before the breath test performance were excluded from the study. A detailed anamnesis was carried out on the history of *H. pylori* infection, the intake of PPI, completed eradication therapies, and comorbidities.

### 2.3. *H. pylori* 13C-UBT

The 13C-UBT was performed by isotope ratio mass spectrometry using an IRIS®-13C-Infrared Isotope Analyzer System (Wagner Analysen Technik GmbH, Bremen, Germany). The test protocol was implemented according to the manufacturer's instructions. In brief, after a 12-h fasting period, breath samples were obtained before (baseline) and 30 min after the test drink intake (75 mg 13C-urea from the capsule dissolved in 200-ml fruit juice) early in the morning (8:00–10:00 a.m.). After giving the patient the 13C-urea dose in liquid form, an immediate mouth rinsing was carried out to prevent false-positive results by oral bacteria with urease activity [15,16]. The 13C/12C-isotope ratio on CO2 in the breath samples was determined as the delta (δ) value (%) versus PDB (Pee Dee Belemnite, international limestone standard). The increase in the δ value 30 min after ingestion of the tracer was expressed in delta over baseline (DOB) (‰) (formula: DOB, ‰ = δ value of baseline breath sample − δ value 30 min after ingestion of the tracer) [15]. A sample was considered positive if the 30 min value was above a 4‰ cut-off level [6,17]. Eating, drinking, and/or smoking were not allowed until the 13C-UBT had been completed.

### 2.4. Glucose HMBT

A glucose HMBT protocol was established with the QuinTron Model DP Plus MicroLyzer™ (QuinTron, Milwaukee, Wisconsin, United States of America) to detect patients with SIBO. After an overnight fasting state of 12 h, 50 g glucose dissolved in 200 ml of water was orally administered [12]. The H2 and CH4 breath concentrations were measured at 0 (baseline before sugar ingestion), 15, 30, 45, 60, 90, and 120 min by gas chromatography. According to the literature [12,18], patients were classified to have SIBO if a H2 and/or CH4 increase of ≥ 10 ppm above the baseline was observed. During the test procedure, patients were instructed to avoid physical effort, smoking, and/or eating and to report clinical symptoms.

### 2.5. Statistical analysis

Descriptive statistics were performed to analyse and compare the functional 13C-UBT and glucose HMBT results. Fisher's exact test (2 × 2 tables) was calculated for subgroup comparisons of categorical parameters. The agreement between the 13C-UBT and the HMBT results was calculated using Cohen's kappa (κ) with 95% confidence intervals (CIs). All statistical tests were used in an explorative way; therefore, no correction of the type I error (two-sided, 5%) was made. This means that the results were only descriptive. For all calculations, the Analyse-it® software version 2.30 (Analyse-it Software, Ltd., Leeds, United Kingdom) was used.

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**Table 1**

<table>
<thead>
<tr>
<th>Basic characteristics of the study population.</th>
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<tr>
<td>Study population (n = 109)</td>
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<td>Gender</td>
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<td>Male</td>
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<td>Age (years, mean ± SD)</td>
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<td>Presence of gastrointestinal symptoms (anamnesis)</td>
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<td>Upper abdominal pain</td>
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<td>Depression</td>
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<td>Eating disorder</td>
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<td>Chronic comorbidities (case histories)</td>
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<tr>
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<td>Bechterew's disease</td>
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<td>Hereditary angioedema</td>
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3. Results

3.1. Association between H. pylori infection and SIBO

The results of the $^{13}$C-UBT, which was indicative of active H. pylori infection, and of the glucose HMBT, which detected individuals with SIBO, were available in all 109 ambulatory patients and are presented in Table 2. Fisher’s exact test revealed that individuals with active H. pylori infection were significantly associated with SIBO ($p = 0.002$). Taken together, 19/36 (52.8%) individuals with a positive H. pylori $^{13}$C-UBT also had a positive glucose HMBT compared with 16/73 (21.9%) patients with a negative H. pylori $^{13}$C-UBT. The positivity rates of the $^{13}$C-UBT and the glucose HMBT were 36/109 (33.0%) and 35/109 (32.1%), respectively. Calculating Cohen’s $\kappa$ with 95% CIs, a fair agreement was observed between the $^{13}$C-UBT and the glucose HMBT ($\kappa = 0.31$, CI: 0.12–0.50; $p = 0.001$).

Thirty-one (24.8%) of all the patients developed one or more symptoms (i.e., abdominal pain, bloating, nausea, heartburn, burping, or headaches) during the glucose HMBT. Of these, 21/31 (67.7%) individuals had a positive result, whereas 10/31 (32.3%) individuals showed a negative glucose HMBT result.

3.2. $^{13}$C-UBT results in H. pylori anamnesis, completed eradication therapies, and PPI intake

As shown in Table 3, 58/109 (53.2%) patients had a positive H. pylori anamnesis. Of these, 54/58 (93.1%) individuals completed up to four eradication therapies; however, 4/58 (6.9%) patients did not undergo any kind of antibiotic treatment. Furthermore, 71/109 (65.1%) individuals reported previous PPI use, of which 14/71 (19.7%) had a constant PPI intake for >6 months.

The $^{13}$C-UBT results in patients with positive and negative H. pylori history are presented in Table 4. A total of 27/58 (46.6%) patients with a positive H. pylori history had a positive $^{13}$C-UBT result, whereas 9/51 (17.6%) patients with a negative H. pylori history showed a positive $^{13}$C-UBT result. H. pylori history and $^{13}$C-UBT and glucose HMBT results in individuals with PPI intake and eradication therapies are listed in Table 5. In total, 24/54 (44.4%) individuals who had completed eradication therapies and 30/71 (42.3%) patients with reported previous PPI intake showed a positive $^{13}$C-UBT. Six of 38 (15.8%) individuals, who had no PPI use in the anamnesis, achieved a positive $^{13}$C-UBT result.

3.3. Glucose HMBT results in patients with PPI use and completed eradication therapies

In total, 11/38 (28.9%) patients, who had a negative anamnesis of PPI intake, had a positive glucose HMBT result (Table 5). Interestingly, 6/14 (42.9%) patients with a previous constant PPI intake for >6 months and 18/57 (31.6%) individuals with intermittent PPI use (i.e., in context with eradication therapies and/or gastrointestinal complaints) had a positive glucose HMBT result.

Additionally, 13/38 (34.2%), 4/12 (33.3%), 1/2 (50.0%), and 1/2 (50.0%) patients with one, two, three, and four completed eradication therapies (i.e., combined antibiotics/PPI intake), respectively, were diagnosed with SIBO by HMBT (Table 5). All these individuals had a positive history of H. pylori infection.

4. Discussion

In this study, 109 patients, who were referred by general practitioners and specialists to our outpatient clinic, were tested for active H. pylori infection and SIBO by the laboratory $^{13}$C-UBT and glucose HMBT, respectively. We identified 36/109 (33.0%) individuals with active H. pylori infection (IRIS®:$^{13}$C-Infrared Isotope Analyzer System). This prevalence rate is in agreement with a previous German study, which reported 57/180 (31.7%) adults (age: 18–62 years) to be H. pylori positive [2,19].

To the best of our knowledge, this is the first report on the assessment of SIBO in patients with active H. pylori infection by functional breath testing. Taken together, 19/36 (52.8%) individuals with a positive H. pylori $^{13}$C-UBT also had a positive glucose HMBT, whereas 17/36 patients (47.2%) with a positive H. pylori $^{13}$C-UBT were negative for the glucose HMBT test. The present study results demonstrate that individuals with a positive H. pylori infection showed significantly more often a positive glucose HMBT (19/36 (52.8%), which is indicative of SIBO, compared to individuals without active H. pylori infection (16/73 (21.9%)) ($p = 0.002$), respectively. These findings support the hypothesis that a positive $^{13}$C-UBT in patients with H. pylori infection increases the risk of SIBO. All individuals who had a negative H. pylori $^{13}$C-UBT and a positive glucose HMBT result had a negative case history of previous surgery of the upper gastrointestinal tract. In addition, no other agent that causes SIBO (e.g., anatomical abnormalities, motility disorders) [10] could be found in the anamnesis.

One possible explanation for the observed association between H. pylori infection and SIBO could be the influence of the gastric pH on the gastric and small intestinal bacterial flora [20]. In healthy subjects, gastric pH is highly acidic (range: 1.0–2.5) [21]. The acidity of the gastric juice is an important factor in regulating both the quality and quantity of the upper gastrointestinal bacterial flora [20]. The H. pylori bacterium produces a 550-kDa multimeric urease, which metabolizes urea into ammonia and carbonic acid [5]. The increase in the ammonia concentration raises the pH in the gastric mucus [9], which may promote bacterial growth in the stomach and small intestine.

Moreover, the production of ammonia by H. pylori urease is involved in direct cytotoxic gastric epithelial cell damage [22–24]. Additionally,
H. pylori-activated neutrophils enhance gastric mucosal cell injury [25], and the H. pylori bacterium has the potential to diminish the acid-induced tightening of gastric epithelial cell junctions. The subsequent loss of epithelial cell barrier function further aggravates the progression of mucosal damage [26]. Gastric epithelial cell injury may lead to H. pylori-induced atrophy of gastric mucosa, which is considered a crucial risk factor in the development of SIBO [13].

The drug-induced inhibition of acid secretion may be another risk factor of SIBO [10,13,27,28]. In the present study, individuals with previous constant or intermittent PPI intake presented SIBO more often than PPI non-users. These data are in line with a previous study, which found longer durations of PPI therapy to be associated with an increased risk of SIBO [10]. Moreover, about one-third (19/54 (35.2%)) of the individuals with one or more completed eradication therapies were also diagnosed with SIBO by HBMT in the present study. These data indicate that H. pylori eradication therapies may alter the small intestinal bacterial flora and are in line with a previous study, which reported an increased presence of non-H. pylori flora in the culture of gastric and duodenal mucosa of 18 patients after eradication therapy [30]. Furthermore, gastric acid suppression might have a downstream effect on the small intestinal bacterial overgrowth. The major advance of this work is that the study population is well characterized. Nevertheless, several limitations of this study may be described: Invasive biopsy-based methods were not performed. It was difficult to collect precise anamnestic data about the antibiotics, which were used for eradication therapies. Treatment modalities of coincident H. pylori infection and SIBO were not considered.

In conclusion, our data suggest that active H. pylori infection is significantly associated with the presence of SIBO as determined by functional laboratory breath tests. In addition, SIBO rates were found to have increased after completed eradication therapies. However, further longitudinal studies are warranted to fully elucidate the relationship and treatment modalities of coincident H. pylori infection and SIBO.

Conflict of interest

The authors declare that there was no conflict of interest regarding the publication of this article.

References

