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Helicobacter pylori infection is associated with high methane production during lactulose breath test

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Abstract. – OBJECTIVE: Despite a growing interest toward the interplay between *H. pylori* and gastric microbiota, few data are available about this correlation. The aim of this study was to explore the relationship between *H. pylori* infection and gas production during lactulose breath test.

MATERIALS AND METHODS: Data of patients undergoing both ^{13}C-urea breath test (UBT) and lactulose breath test (LBT) under standard conditions in our GI unit were retrospectively analyzed. GI symptoms, such as dyspepsia, bloating, abdominal pain/discomfort, and epigastric pain on an eleven-point scale were also analyzed and correlate with the results of those tests. H_2 and CH_4 were calculated using the trapezoidal rule; a considerable CH_4 production was defined by AUC_{CH4} ≥1200 ppm*4h. Statistical analyses were performed with Fisher's exact test and independent samples Mann-Whitney test.

RESULTS: Data of 136 patients during a period of time of 3 months were analyzed. 36 patients (26.5%) showed a positive UBT. We do not find any difference as regards age, sex, symptom complaints, and small intestinal bacterial overgrowth between HP negative and positive patients. A greater methane production was observed in infected rather than non-infected patients (47.2% *vs.* 26% respectively, p=0.02). Furthermore, 25% infected and 10% non-infected produced greater amounts of CH_4 compared to H_2, resulting in a AUC_{CH4}/AUC_{H2} ratio >1 (p=0.046).

CONCLUSIONS: This study shows for the first time, a significant association between *H. pylori* infection and methane production, suggesting that *H. pylori* might influence gut microbiota composition. Further studies are needed to clarify mechanisms underlying this phenomenon.

Key Words
H. pylori, Methane production, Breath test.

Introduction

H. pylori is a Gram-negative bacterium involved in the development of several gastric and extra-gastric diseases (e.g. gastritis, peptic ulcer, gastric malignancies, sideropenic anaemia, idiopathic thrombocytopenic purpura) through its ability of surviving in the gastric acid environment and activate the immune system[1]. Increasing evidences focused on the relationship between *H. pylori* and other actors of microbiota. As suggested by several studies in both mice and humans[2-4], gastric microbiota harbors in a context of atrophic gastritis and promotes gastric carcinogenesis synergistically with *H. pylori*[5]. To date, only one published study describes *H. pylori*'s capability of promoting changes of microbiota composition along the entire gastrointestinal tract in Mongolian gerbils[6]. Furthermore, several studies suggest a protective role of *H. pylori* infection toward the development of microbiota-related disease, such as inflammatory bowel diseases, even though this association is still controversial[7-10].

The most reliable diagnostic test for *H. pylori* infection is ^{13}C-urea breath test (UBT) a non-invasive test consisting in the measurement of the $^{13}C/^{12}C$ ratio in exhaled air prior and following the oral assumption of a dose of ^{13}C urea. A Delta-Over-Baseline (DOB) >3.50 per mille after thirty minutes is commonly considered diagnostic for *H. pylori* infection[11].

Lactulose breath test (LBT) is a non-invasive test used to assess oro-caecal transit time and the presence of a small intestinal bacterial overgrowth (SIBO)[12]. Although its diagnostic targets are achieved through H_2 measurement (in parts per million, p.p.m.), increasing evidence supports the importance of measuring CH_4 concentration in exhaled air after a 10 g lactulose challenge. Methane is produced along the gastrointestinal tract by specific microbes belonging to the phyla of *Archaea* and *Bacteria*, mainly *Methanobrevibacter smithii*, by converting H_2 produced by other bacteria of gut microflora[13-16]. Clinically, methane production has been associated with several

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conditions, such as colonic cancer[17], chronic constipation[18,19], constipation-predominant irritable bowel syndrome[20], and obesity[21,22].

Despite a growing interest in discovering the interplay between *H. pylori* and the gastric microbiota, few data are available about the relationship between *H. pylori* and the gut microbiota. Since *H. pylori* is able to modify the gastric pH, which in turn may alter the gut microbiota composition, we have designed a study aimed at assessing any possible relation between *H. pylori* infection and enteric flora at a level recognisable by LBT.

Materials and Methods

We have analyzed data of patients who underwent both LBT and UBT in our Gastroenterology Unit from November 2013 through June 2014. Only patients whose tests were performed in a maximum time lapse of 14 days were considered. Breath tests were all performed under conditions mentioned below.

For LBT, exhaled air was sampled once in fasting conditions and at time intervals of 15 minutes for 4 hours following the intake of 10 g lactulose in a watery solution. H_2 and CH_4 concentrations corrected for CO_2 were measured with a gas cromatograph (Quintron Breathtracker SC, QuinTron, Milwaukee, WI, USA) within 6 hours after sample collection. Tests were judged as positive for SIBO when an early peak of H_2 production (±10 parts per million, ppm) was observed. Areas Under the Curve (AUCs) of H_2 and CH_4 were assessed with the trapezoidal rule and methane producer patients were defined by an $AUC_{CH4} \geq 1200$ ppm*4h, equal to a mean CH_4 production of 5 ppm. Patients underwent lactulose breath test at least one month after the last antibiotic assumption and 14 days after the last probiotic assumption.

UBT was performed in fasting conditions by sampling exhaled air prior to and 30 minutes after the ingestion of a 75 mg dose of ^{13}C-urea dissolved in a citric acid solution. Samples were analyzed using an isotope ratio mass spectrometer (ABCA, Sercon, UK). A DOB ≥3.50 per mille was considered positive for *H. pylori* infection. Patients were required to be off proton-pump inhibitors in the previous 2 weeks.

We have also analyzed the recorded GI symptoms, such as dyspepsia, bloating, abdominal pain or discomfort, and epigastric pain using an eleven-point validated scale (0 to 10).

The study was conducted in accordance with the Declaration of Helsinki. None of the patients or authors received any honorary or economic benefits for the participation in this work.

Statistical analyses were performed using Fisher's exact test and independent samples Mann- Whitney U test with 95% confidence intervals at a significance level of 0.05.

Results

Data of 136 patients (95F/41M, mean age 42.5±16.4 years), were analyzed. UBT was positive in 36 patients (26.5%) (Table I); no significant differences were observed between *H. pylori*-positive and negative patients concerning the intensity of GI symptoms, such as dyspepsia, bloating, abdominal pain or discomfort and epigastric pain (Figure 1). No significant differences were found concerning the results of H2 LBT between *H. pylori*-positive and negative patients (13.9% *vs.* 12% respectively, *p=0.77*) (Figure 2).

Twenty-six out of 100 (26%) *H. pylori*-negative subjects and 17 out of 36 (47.2%) *H. pylori*-positive patients showed a cumulative CH_4 production after lactulose ingestion of at least 1200 ppm*4h (OR=2.55; 95% C.I. 1.15 to 5,62; *p=0.02*). Further-

Table I. General characteristics of patients.

Patients characteristics	UBT-positive group (n=36)	UBT-negative group (n=100)	*p*-value (2-sided)
Sex	69.0% F	72.2% F	0.83
Age (mean ± SD)	41.4 ± 16.9	46.7 ± 15.7	0.1
SIBO	13.9%	12.0%	0.77
Dyspepsia (mean ± SD)	4.7 ± 2.6	5.0 ± 2.5	0.37
Bloating (mean ± SD)	6.3 ± 3.0	7.3 ± 1.6	0.22
Abdominal pain/discomfort (mean ± SD)	3.9 ± 2.9	4.0 ± 2.7	0.80
Epigastric pain (mean ± SD)	4.1 ± 2.8	3.8 ± 2.4	0.52

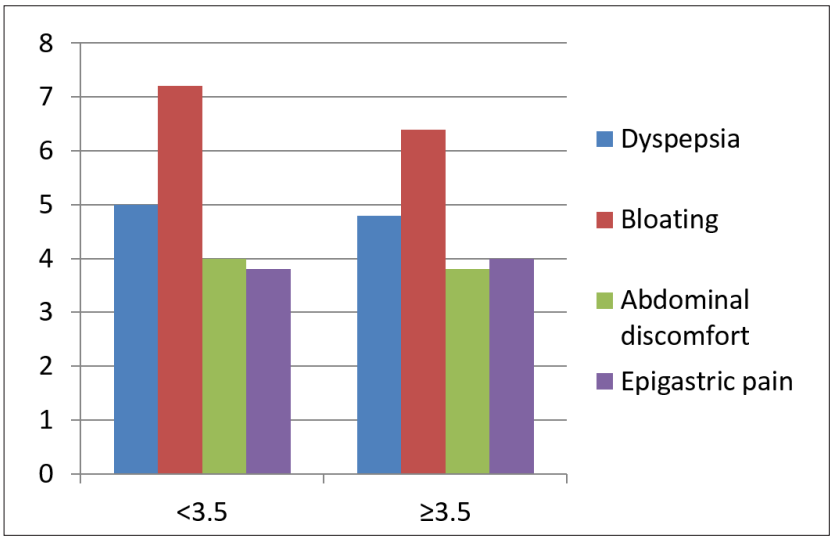

Figure 1. Intensity of GI symptoms between *H. pylori*-positive (DOB>3.5 ppm) and negative patients (DOB<3.5 ppm).

more, 9 out of 36 (25%) *H. pylori*-positive patients and 10 out 100 (10%) *H. pylori*-negative patients produced more CH_4 than H_2, resulting in a AUC_{CH4}/AUC_{H2} ratio >1 (OR=3; 95% C.I. 1.11 to 8.14; *p=0.046*) (Figure 3).

Discussion

We explored for the first time the relationship between the results of UBT and LBT in patients for whom indications were decided by their physicians. We have then analyzed the results of UBT and LBT and correlated them with the intensity of GI symptoms reported by patients. Interestingly, we found that *H. pylori*-infected patients were more frequently methane producers. Moreover, we have also found a specific pattern in *H. pylori*-positive subjects, such as the production of at least 1200 ppm*4h during LBT. Such an area under the curve is equal to a mean value of 5 ppm of methane at each sample. These data suggest that the presence of *H. pylori* in the stomach could influence the composition of enteric flora with a peculiar pattern. Since this observation was made in just 47% of *H. pylori*-infected patients, it seems quite possible that this bacterium might influence gut microbiota even in different ways, not necessarily appreciable by LBT. Interestingly, we found that *H. pylori*-positive patients were 2.5 times more likely to overproduce CH_4 instead of H_2 during LBT, thus reinforcing our previously exposed data. Notably, except for CH_4 production, there was no difference in the results of LBT between the two groups.

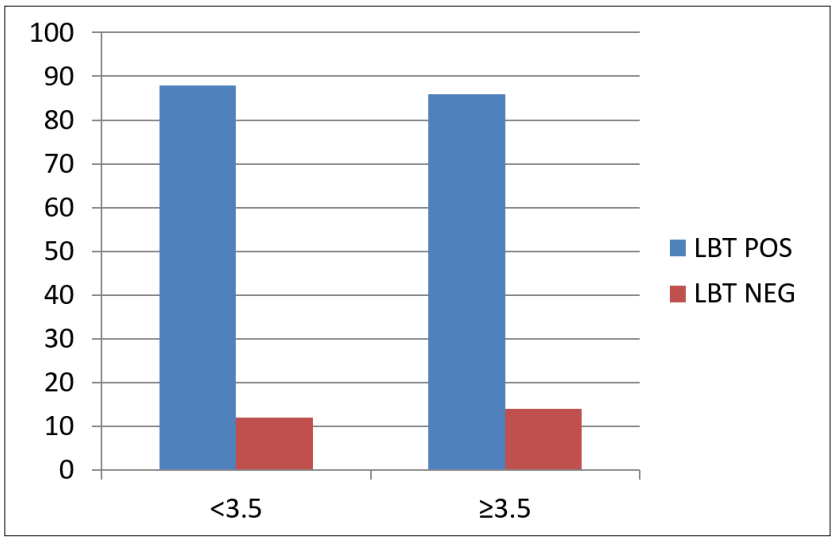

Figure 2. Differences between *H. pylori*-positive and negative patients concerning the results of LBT, except for methane production.0

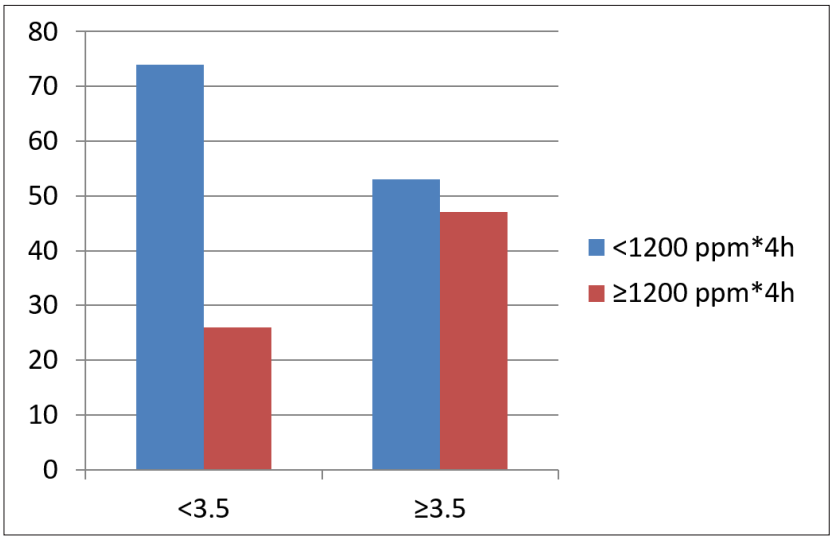

Figure 3. Differences in methane production between *H. pylori*-positive and negative patients.

Since our findings does not allow to clearly explain the occurrence of such a phenomenon, we may hypothesize that *H. pylori* may act in two different ways. First, *H. pylori* is a cause of hypochlorhydria, while pH elevation produces alterations in gut microbiota composition, similarly to what found in chronic proton pump inhibitors users[23,24]. Secondly, we may hypothesize that *H. pylori* may alter the gut microbiota composition through a direct interaction with other bacteria[13].

Conclusions

We have demonstrated a positive correlation between *H. pylori* infection and CH_4 production, thus suggesting a role of this bacterium in modulating gut microbiota composition. Further studies are now needed to determine the mechanisms involved in this process.

Competing interests:
The authors have no competing interests.

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