**Introduction**

*Helicobacter 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. Increasing evidences focused on the relationship between *H. pylori* and other actors of microbiota. As suggested by several studies in both mice and humans, gastric microbiota harbors in a context of atrophic gastritis and promotes gastric carcinogenesis synergistically with *H. pylori*. 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. 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.

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

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). Although its diagnostic targets are achieved through H2 measurement (in parts per million, p.p.m.), increasing evidence supports the importance of measuring CH4 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 H2 produced by other bacteria of gut microbiota.* Methane production has been associated with several extra-gastric diseases (e.g. gastritis, peptic ulcer,
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...conditions, such as colonic cancer, chronic constipation, constipation-predominant irritable bowel syndrome, and obesity.

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. H₂ and CH₄ concentrations corrected for CO₂ were measured with a gas chromatograph (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 H₂ production (±10 parts per million, ppm) was observed. Areas Under the Curve (AUCs) of H₂ and CH₄ were assessed with the trapezoidal rule and methane producer patients were defined by an AUC_{CH₄} ≥1200 ppm*4h, equal to a mean CH₄ 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 CH₄ 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...
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 1.** Intensity of GI symptoms between H. pylori-positive (DOB>3.5 ppm) and negative patients (DOB<3.5 ppm).

**Figure 2.** Differences between H. pylori-positive and negative patients concerning the results of LBT, except for methane production.
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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\textsuperscript{23,24}. Secondly, we may hypothesize that H. pylori may alter the gut microbiota composition through a direct interaction with other bacteria\textsuperscript{13}.

**Conclusions**

We have demonstrated a positive correlation between H. pylori infection and CH\textsubscript{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.

**References**


