CLINICAL TRIAL SUMMARY REPORT

COMPARISON OF TWO METHODS FOR IN VIVO DIAGNOSIS OF *HELICOBACTER PYLORI* INFECTION, BY MEANS OF A TABLET OF ^{13}C- UREA

Administrative information	**Protocol number:** PSC-DS-BRETEX **EudraCT number:** 2016-001598-33 **Date of trial report:** March 31st, 2017 Is the trial part of a Paediatric Investigation Plan? YES☐ NO ☒
Trial design	Interventional, cross-over, open-label, single-center, sponsored clinical trial, comparing two diagnostic methods used after the assumption of EXPIROBACTER® 100 mg.
Background for conducting the trial	Helicobacter pylori is a gram-negative bacterium, which can colonize the gastric epithelium, thus leading to chronic inflammation of the mucosa. The infection, usually contracted in early childhood, if not treated becomes chronic. Untreated infections are associated with a number of several gastrointestinal diseases, e.g. dyspepsia, gastric ulcers and gastric cancer. The Urea Breath-test, performed with carbon-13 (^{13}C)-urea, is a widely used method to detect the presence of H. pylori infection with a 99% sensitivity and specificity. The classic method consisted in the collection of the basal exhaled breath sampling, followed by administration of urea enriched with ^{13}C and citric acid, and the final sampling 30 minutes after labeled urea administration. Two samples for each time point were collected and sent to an external laboratory for the mass spectrometry analysis. Recently, methods based on the continuous exhaled breath analysis have been developed. They can short the exam duration, and provide immediately available results. BreathID™ is a validated diagnostic system. The patient is connected through nasal cannula to the medical device, to obtain a continuous analysis of the exhaled breath, by molecular correlation spectrometry. The device, after the basal reading, clearly define the timing for ^{13}C-urea and citric acid administration. The patient is connected to the device until sufficient data to determinate the positivity or negativity of the test are collected. The duration of the test can vary from 10 to 20 minutes. In Italy, BreathID™ test is performed with EXPIROBACTER®, containing 100 mg ^{13}C-urea tablets and 1.4 g of citric acid. Available data support the use of BreathID™ with EXPIROBACTER® 100 mg. Aim of this study was to demonstrate that EXPIROBACTER® 100 mg tablets provide overlapping results, both with the classic method and with a faster technique based on a continuous sampling of breathing, requiring less than 30 minutes sampling.

Participants of the trial	**Eligibility criteria for participants** The study population consisted of outpatients, with clinical indication to Urea Breath-test for H. pylori. *Inclusion Criteria* 1. Male and female patients. 2. Age ≥ 18 years. 3. Clinical indication for a diagnostic test for the determination of infection by *H. pylori*. 4. Written informed consent. *Exclusion Criteria* 1. Treatment with antibiotics or bismuth preparations within 4 weeks before the date of entry into the study; 2. Treatment with proton pump inhibitors within 2 weeks before the date of entry into the study; 3. Presence of alarm symptoms (e.g. unintentional weight loss, iron deficiency anemia, gastrointestinal bleeding, dysphagia); 4. Atrophic gastritis; 5. Malignancies of the gastrointestinal system; 6. Known or suspected allergy to one or more components of EXPIROBACTER®; 7. Pregnancy or breast-feeding; 8. Recent history or suspect of alcohol or drug abuse; 9. Presence of any type of dementia, or other possible causes of progressive deterioration of the capacity of understanding and willing, or physical and mental disability that reduces the ability to take your medicine as expected; 10. Not sufficient compliance or other conditions that may result in non-compliance / adherence of the patient to Protocol; 11. Concomitant participation in another clinical trial; 12. Age < 18 years; 13. Patients unable to carry out study procedures or unwilling to give written informed consent. **Settings and locations where the data were collected** The study was conducted at one clinical site in Italy: Humanitas Research Hospital Humanitas Mirasole S.p.A. Via Manzoni 56, Rozzano (Milan), Italy
Interventions	A total number of 46 subjects were enrolled and analysed: All patients assumed the following treatment: EXPIROBACTER® 100 mg soluble tablet containing 100 mg of ^{13}C-urea and Citric Acid 1.4 gr granules for oral suspension. The patients underwent the breath test using both the classic method, requiring basal exhalation sampling before EXPIROBACTER® ingestion and a second exhalation sampling 30 minutes after, according to the Summary of the Product Characteristics, and a second method based on a continuous analysis of exhaled breath performed by Exalenz BreathID™ medical device.

Objective(s) of the Trial	The objective of this study was the comparison of the accuracy of the Urea Breath-test, carried out with either BreathID™ or the classic method (EXIPIROBACTER® Kit), using a 100 mg ^{13}C-urea tablet,administered with 1.4 g of citric acid. Aim of this study was to demonstrate that EXPIROBACTER® 100 mg tablets provide overlapping results, both with the classic method and with a faster technique based on a continuous sampling of breathing, requiring less than 30 minutes sampling.
Outcome measures	▪ Comparison of the results of H. pylori test obtained with molecular correlation spectrometry (BreathID™) and with mass spectrometry (classic method). ▪ Patient's satisfaction with the two methods assessed using two VAS scales. ▪ Adverse events (AE) recorded during the duration of the clinical trial.
Randomisation implementation	Not applicable
Blinding	Not applicable
Statistical methods	The concordance (agreement) between the two methods will be evaluated by Cohen's kappa. The degree of the agreement will be interpreted according to the following scale: ▪ if k takes on values lower than 0, there is no correlation; ▪ if k has values ranging from 0 to 0.4, the correlation is poor; ▪ if k takes values between 0.4 and 0.6, the correlation is moderate; ▪ if k takes values between 0.6 and 0.8, the correlation is good; ▪ if k has values ranging from 0.8 to 1, the correlation is excellent. The degree of patient satisfaction related to the two used diagnostic methods was evaluated using two VAS (Visual Analogue Scale), one for each method. The comparison between the two techniques was performed using Student's t test for repeated measures.
Participant flow	Forty-six (46) evaluable patients were screened and enrolled. Study procedures were correctly completed by all patients (Tab. 10.1.1); no data were missing
Recruitment	First subject enrolled: November 22nd 2016 Last subject completed: December 29th 2016
Baseline data	Forty-six Caucasian patients were included in this analysis (36 women and 10 men), with a mean age of 32 years (range 18-76 years). Most patients (58.7%) had a positive medical history; physical examination revealed no abnormality. Previous therapies were reported only by four patients (8.7%), while ten patients (21.7%) took at least one concomitant treatment Eight patients (17.4 %) were previously evaluated for H. pylori infection, between 2001 and 2016: three with BreathID™, two with the classic technique and three with gastroscopy (biopsy was taken only in one case). Four patients were positive for H. pylori: three patients were diagnosed using BreathID™ and one with gastroscopy. One subject was previously treated with eradication therapy of H. pylori (tetracycline in 2015). Concerning the symptoms leading to the current examination, seven patients (15.2%) had a sensation of postprandial fullness, four patients (8.7%) premature satiety, ten patients (21.7%) epigastric pain or burning, while three patients (6.5%) had other symptoms (gastroesophageal reflux, typical symptoms of irritable bowel syndrome, persistent cough for gastroesophageal reflux). In total, 14 patients (30.4%) had at least one of these symptoms.
Trial interruption	The trial was not interrupted.

Outcomes and estimation	**Efficacy results:** *Primary endpoint* The primary endpoint of this study was the evaluation of the overlap between the results obtained by molecular correlation spectrometry (BreathID™) and those obtained by mass spectrometry (classic method). The concordance between the two methods was excellent, with 41 subjects negative and 5 positive to both H. pylori tests (K=1.00). *Secondary endpoints* The mean patient satisfaction, measured by a Visual Analogue Scale (0-100 mm), was 82.35 (SD = 16.52) for the classic method and 90.17 (SD 8.54) for the BreathID™, with a statistically significant difference of 7.83 (95% CI = 3.35 to 12.30, p=0.001)
Ancillary analysis	Not applicable
Adverse events	During this study no adverse event was reported.
Trial termination	Study terminated prematurely YES☐ NO ☒
Discussion and interpretation of study results	Each enrolled patient performed both tests simultaneously. This approach allowed the comparison of results on the same patient at the same time, thus minimizing any intra- and inter-individual variability dependent differences, with a consequent reduction in the required sample. The results of the two methods were compared. When both methods provided the same result (positive/negative), they were considered in agreement (concordance). The positivity or negativity was determined on the basis of the "Delta Over Baseline" (DOB) of the two methods. The study demonstrated a perfect correlation between the two methods of *H. pylori* detection (kappa = 1.00). According to the VAS scales, the patients were significantly more satisfied by BreathID™. The simultaneous performance of the two tests did not cause any additional risk for the patient: the labeled urea/citric acid solution was administered only once. Both methods were safe, since no adverse event was reported.