

# Effect of Proton Pump Inhibitors on the Continuous Real Time $^{13}\text{C}$ -Urea Breath Test

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**OBJECTIVE:** The aim of this study was to evaluate the accuracy of a new, continuous real time  $^{13}\text{C}$ -urea breath test, BreathID, for the diagnosis of *Helicobacter pylori* in patients taking proton pump inhibitors (PPIs).

**METHODS:** Fifty-two consecutive patients, positive for *H. pylori* by BreathID, were prospectively evaluated. Patients were randomized to receive either omeprazole 20 mg/day or pantoprazole 40 mg/day for 14 days. A repeat breath test was performed on day 14 while patients received their last PPI pill. Patients were given a test drink containing 75 mg  $^{13}\text{C}$ -urea and 4.0 g citric acid. Real time, continuously sampled expired  $^{13}\text{CO}_2$ , obtained within 6–20 min, was compared with measurement of expired  $^{13}\text{CO}_2$  by isotope ratio mass spectrometry (IRMS).

**RESULTS:** A full set of test data was available for 43 patients. After 14 days of treatment with PPIs, false negative detection of *H. pylori* occurred in only 1/43 (2.3%) patients examined by continuous real time  $^{13}\text{C}$ -urea breath test compared with 2/43 (4.6%) patients examined by IRMS. With the exception of one case, complete agreement was observed between BreathID and the IRMS breath tests at both baseline and after PPI treatment. PPI treatment was associated with three different types of responses on UBT: 1) one third of the patients developed a significant decrease in the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  excretion, 2) roughly one third developed a significant increase in the post-PPI breath test results, and 3) results did not change significantly in the remaining patients. Linear regression analysis of 43 *H. pylori*-positive subjects indicated a significant positive association between baseline and post-PPI  $\Delta^{13}\text{CO}_2/^{12}\text{CO}_2$  excretion.

**CONCLUSIONS:** The use of a single test drink containing 4.0 g citric acid in BreathID, resulted in a low number of false negative results associated with sustained PPI treatment. Although there were some differences between BreathID versus IRMS, the type of PPI and the sampling method used do not appear to play a critical role in the detection of *H. pylori* by BreathID. According to these results, BreathID is a reliable tool for testing *H. pylori* in

patients taking PPIs. (Am J Gastroenterol 2003;98:46–50. © 2003 by Am. Coll. of Gastroenterology)

## INTRODUCTION

Proton pump inhibitors (PPIs) are among the most commonly prescribed drugs worldwide. They act by blocking the  $\text{H}^+$ ,  $\text{K}^+$  adenosine triphosphatase (ATPase) ion pump, thus inhibiting acid secretion from the gastric parietal cells (1). They are highly effective in the treatment of peptic ulcer and gastroesophageal reflux diseases. Together with antibiotics, PPIs are the current gold standard of the triple therapy regimens used for the eradication of *Helicobacter pylori* (2). PPIs inhibit the growth of *H. pylori*, probably by a pH-dependent mechanism (3–5). The use of PPIs and of histamine  $\text{H}_2$ -blockers may result in false negative results during diagnostic urea breath tests (UBT); therefore, cessation of PPIs and  $\text{H}_2$ -blockers 7–14 days before UBT is currently recommended (6, 7). However, for “PPI dependent” patients this may be a critical issue, because PPI withdrawal is strongly associated with symptom recurrence. Recently, Chey *et al.* demonstrated that intragastric acidification by citrate administration before and during the UBT decreases the false negative results in patients on PPI treatment (8).

UBTs detect active *H. pylori* infection with a high sensitivity and specificity. They are considered the preferred method for epidemiological studies, for screening dyspeptic patients, and for the assessment of eradication or recurrence of the infection (9). The  $^{13}\text{C}$ -UBT BreathID is a novel, office-based, rapid, continuous real time urea breath test (CRT-UBT) for the detection of *H. pylori* that has been approved for use by the U.S. Food and Drug Administration. Using the molecular correlation spectrometry method, BreathID displays graphically continuous real time rapid results within 20 min. The time to final result may be further reduced when a conclusive positive or negative case of *H. pylori* has been identified (10). Because we were aware that alkalinization of the gastric secretions by PPIs may cause false negative UBT results, this new breath test assay incorporated from the outset a test drink that includes a 75-mg,

<sup>13</sup>C-labeled urea tablet, dissolved in 200 ml water with 4.5 g of citric acid-based powder.

In the present study, we prospectively evaluated the accuracy of this CRT-UBT to detect the presence of *H. pylori* in patients treated with PPIs. We also compared the CRT-UBT results with those from conventional isotope ratio mass spectrometry (IRMS), to determine whether other parameters, such as the sampling method (continuous sampling in CRT-UBT *versus* two point sample collection in IRMS), rather than increase in the gastric pH, might lead to the false negative results in these patients.

## MATERIALS AND METHODS

### Study Patients and Protocol

CRT-UBT was performed in healthy volunteers more than 18 yr of age after a 3-hr fast. Those with a positive UBT were included in the study. Exclusion criteria included 1) administration of antibiotics and/or bismuth preparations within 4 wks before the date of entry to the study, 2) administration of PPIs within 4 wk before the date of entry to the study, 3) being pregnant or currently breast-feeding for women, and 4) previous gastric surgery. Patients were randomized to treatment with either omeprazole 20 mg/day or pantoprazole 40 mg/day, taken at 8:00 AM, 30–60 min before breakfast, for 14 days. A repeat breath test was performed on day 14, 1–3 h after patients received their last PPI pill. An additional subgroup of *H. pylori*-negative subjects performed a urea breath test before and 14 days after PPI therapy in a similar fashion.

### Urea Breath Tests

The effect of breath test sampling method on detection was examined using the newer CRT methodology (BreathID, Oridion, Jerusalem, Israel), and conventional intermittent sampling, using IRMS (Analytical Precision, AP 2003, Northwich, UK). The BreathID system comprises the following components: 1) a kit containing 75 mg <sup>13</sup>C urea (tablet form of 99% <sup>13</sup>C-enriched urea), 2) a packet of granulated Citrica. Each 4.5-g packet contains 4 g citric acid, 0.149 mg aspartame, orange aroma, and FD&C yellow #6, 3) IDcircuit-sampling device, and 4) the BreathID device. All patients received 75 mg <sup>13</sup>C-urea with 4.5 g citric acid-based powder (Citrica). The IDcircuit, a continuous nasal breath sampling device, transports the breath sample from the patient to the BreathID and does not require active patient cooperation. Based on molecular correlation spectrometry, the BreathID continuously measures <sup>13</sup>CO<sub>2</sub> and <sup>12</sup>CO<sub>2</sub> concentrations from the patient's breath and establishes the <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> ratio, which is displayed *versus* time on the screen. Results are obtained within 10–15 min and printed on a thermal printer.

Breath samples at baseline and at 10–15 min after ingestion of the <sup>13</sup>C-labeled urea tablet with the granulated Citrica dissolved in 200 ml water were collected for the isotope

ratio analysis by IRMS in parallel with the continuous assessment of <sup>13</sup>C excretion by CRT-UBT (10).

The cut-off point or threshold for the BreathID test has been determined to be 5 delta over baseline (DOB). Therefore, a test result is defined as positive if the final reading of DOB is greater than 5. A test result is defined as negative if the final reading of DOB is less than 5. The cut-off point in the IRMS test was defined as DOB of above 3.5.

Informed consent was obtained from each patient before enrollment in the study. The study protocol was approved by the Institutional Review Boards of the E. Wolfson Medical Center. This study was not supported by a commercial company.

### Data Analysis

Data were stored on Excel 97 with Hebrew Language support (Microsoft, Redmond, WA). Analysis of data was carried out using SPSS statistical analysis software (SPSS, Chicago, IL). The Kolmogorov-Smirnov test was used to determine whether the distributions of continuous variables (age, breath test results) significantly deviated from normal. Breath test results were highly skewed and were therefore ln-transformed prior to analysis. Descriptive statistics for continuous variables were calculated and are reported as median (minimum, maximum). Categorical variables such as gender and medication were described using frequency distributions. The *t* test for independent samples was used to detect differences in the ln-transformed means of breath test results using gender and, separately, treatment medication, as the categorical variable. Ln-transformed pre- and post-treatment breath test results were compared using the paired *t* test. Univariate linear regression analysis (least squares method) was used to model ln-transformed posttreatment breath test results. Multivariate linear regression analysis was also undertaken using a backward, stepwise approach. All tests were considered significant at *p* < 0.05.

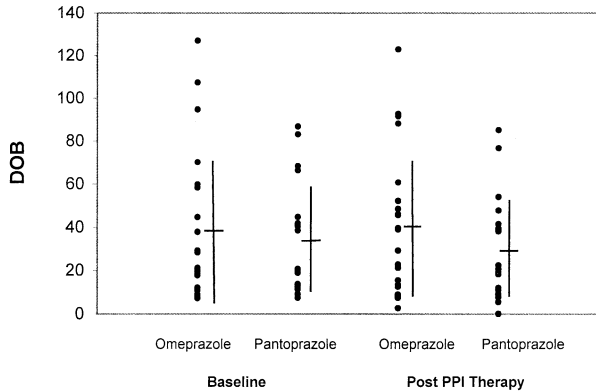
## RESULTS

### Patient Data

Eighty-nine consecutive volunteers were tested by CRT-UBT; of these, 52 (58.4%) were found to be *H. pylori* positive and enrolled in the study. None of the patients had significant ongoing medical problems. Forty-three positive patients (20 men and 23 women, mean age 43, range 18–72 yr), as well as 13 additional patients (7 men and 6 women) found to be *H. pylori* negative, completed the study with a full set of test data. All patients had complied fully with the medication schedule. Nine patients were excluded because they returned for follow-up testing after day 14 of PPI therapy.

### Urea Breath Tests

All patients tolerated the test drink. Although one third of the patients defined it as a having a poor taste, only one patient did not perform the second breath test because of

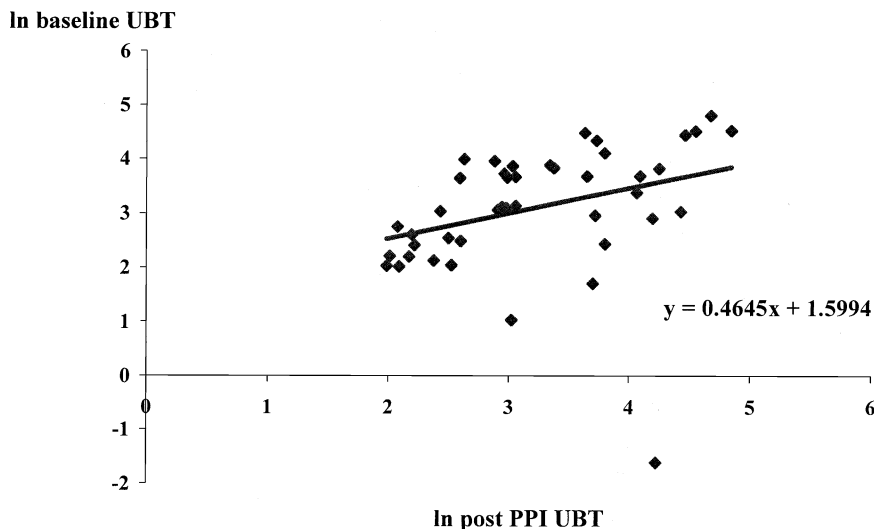


**Figure 1.** Distribution of the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  DOB results at baseline and after 14 d PPI treatment on the CRT-UBT. Comparison between omeprazole 20 mg/day and pantoprazole 40 mg/day. Data are shown as means  $\pm$  SD.

heartburn related to the test drink. After 14 d of treatment, only two patients examined by CRT-UBT, one receiving omeprazole and the other pantoprazole, demonstrated a negative breath test, whereas all the others remained positive. In a third breath test, performed 2 wk after cessation of PPIs in these two patients, one of the patients (treated by omeprazole) became positive, confirming that this was a real false negative result, and the second patient, who became negative after pantoprazole treatment, remained negative, suggesting a false positive result in the first urea breath test or, alternatively, true eradication of the bacteria by pantoprazole. Another patient treated by pantoprazole became false negative with IRMS but remained positive with the CRT-UBT. With the exception of this single patient, complete agreement was observed between CRT-UBT and IRMS in both baseline and post-PPI breath tests. All *H. pylori*-negative patients remained negative on repeated UBT.

Although there were no differences in the distribution of breath test results at baseline and after PPI therapy between the omeprazole and pantoprazole groups (Fig. 1), further evaluation of the results revealed three different types of response to PPI therapy: 1) 12 of 43 patients (28%) developed a significant decrease in the  $^{13}\text{CO}_2/^{12}\text{CO}_2$  excretion ratio, 2) conversely, in 35% (15/43) of the subjects, an opposite phenomenon of significant increase in the post-therapy  $^{13}\text{CO}_2/^{12}\text{CO}_2$  DOB was detected, and 3) equivocal results without significant changes were noted in the remaining 37% (16/43 patients).

The Kolmogorov-Smirnov test indicated that distributions of the breath test results significantly differed from normal, so these variables were normalized by transforming them to their natural logs. A series of univariate linear regression analyses were used to model ln-transformed postintervention breath test results, including the following variables, each in a separate model: ln-transformed baseline breath test results, age, gender, and treatment medication. Gender was significantly associated with ln-transformed posttreatment breath test results ( $r = 0.4, p = 0.007$ ). Indeed, a *t* test for independent samples revealed that both pre- and posttreatment ln-transformed breath test results were significantly higher in females ( $44.63 \pm 35.43$  vs.  $24.95 \pm 19.69$  in the pretreatment group and  $46.04 \pm 32.91$  vs.  $19.56 \pm 15.98$  in the posttreatment group). A significant positive association was also detected between pre- and posttreatment ln-transformed breath test results ( $r = 0.3, p = 0.04$ ) (Fig. 2). A paired *t* test indicated that ln-transformed pre- and posttreatment breath test results did not significantly differ from one another ( $p = 0.24$ ). Because of the association between gender and ln-transformed pre- and posttreatment breath test, the paired *t* test was also carried out in each gender separately. No significant difference



**Figure 2.** Linear regression analysis of 43 *H. pylori*-positive subjects, defining the post-PPI UBT as the outcome variable and the baseline UBT as the predictor variable, indicated a significant positive association but not negative association between baseline and post-PPI UBT results ( $r = 0.3, p = 0.04$ ).

between either of these variables was detected in either gender separately. Treatment medication and age were not significantly associated with posttreatment ln-transformed breath test results. In a backward, stepwise approach in which all aforementioned predictors were included in the initial model, only gender remained in the final model. This significance was lost when gender was added back to the model, probably because of the association between gender and breath test results.

## DISCUSSION

The results of the present study show that 14 d of PPI treatment with omeprazole or pantoprazole had minimal influence on the detection of the bacteria by CRT-UBT. The accuracy of the BreathID was comparable to mass spectrometry, suggesting that the false negative results induced by PPIs are unrelated to the sampling method. One of the possible explanations for the false negative urea breath test induced by PPIs is growth inhibition of the bacteria. Both omeprazole and lansoprazole have direct selective bactericidal activity against *H. pylori*, which is four-fold stronger for lansoprazole and comparable to that of bismuth salts (11–13). An alternative hypothesis is that the rise in gastric pH caused by PPIs prevents the neutralization of ammonia produced by the bacterial urease, and this by itself may be toxic and cause bacterial damage (14). A decrease in bacterial urease activity could occur secondary to alkalinization of the gastric content (15–18). More than 90% of the bacterial urease that generates ammonia to buffer the bacteria from the acidic milieu, is located in the cytoplasm. Urease activity is low at neutral pH, but as the external pH decreases to between 6.5 and 5.5, there is a 10- to 20-fold increase in activity, which remains high through approximately pH 2.5 (15, 16). The transport of urea into the bacteria is regulated by UreI-dependent specific H<sup>+</sup>-gated urea channels that are also pH dependent (19). In this way alkalinization of gastric juice by PPIs may reduce both the entrance of urea into *H. pylori* and the activity of its cytoplasmic urease, the cornerstone of the UBT. This may result in a false negative test. To minimize these pH dependent effects we used citric acid, which delays gastric emptying (20) and decreases gastric pH, although recently Shiotani et al. hypothesized that these two factors *per se* appear unlikely to be the critical determinants in the increased access of urea to the urease enzyme *in vivo* (21).

Evaluation of different <sup>13</sup>C-UBT protocols demonstrates that there is no consensus regarding the dosage of the <sup>13</sup>C-urea, the time and interval of breath sample collection, or the test meal chosen to delay gastric emptying used in UBTs. Each clinical center uses its own test protocol, and this makes the comparison of results almost impossible. Although Dominguez-Munoz *et al.* reported identical sensitivity and 100% specificity of <sup>13</sup>C-UBT for three different test meals (0.1 N citric acid solution, semiliquid fatty meal,

and semiliquid meal) the  $\Delta$  peak values of <sup>13</sup>CO<sub>2</sub> were much higher when citric acid solution was used as the test drink (22). Moreover, Graham *et al.*, using 1, 2, and 4 g citric acid, reported that the increase in urease activity is dose dependent (23). This may play a role in the accuracy of the test and decrease the false negative results in specific circumstances. Indeed, many UBTs now use citric acid as the test drink. However, because of the unappealing taste of citric acid, which can reduce compliance, orange juice has been proposed as an alternative. The sensitivity of the <sup>13</sup>C-UBT is lower with orange juice compared with 0.1 mol/L citric acid, probably because decrease in pH is less significant with orange juice (smaller content of citric acid), and gastric emptying was significantly faster (20). Comparison between UBT studies using orange juice is almost impossible because in many cases there is no data about the ingredients and citric acid concentrations of the juice.

Because of the limitations of pH change on urease activity, the BreathID device protocol was originally devised for a test drink that includes a 75-mg <sup>13</sup>C-labeled urea tablet, dissolved in 200 ml water with a high concentration (4.5 g) of citric acid-based powder. With this protocol we have recently shown that BreathID has comparable sensitivity and specificity to the other commercially available UBTs (10). In the current study, we demonstrate that in patients taking PPIs, the frequency of the false negative results with the protocol we used is much lower than in previously published reports that described false negative results in 40% of patients (3, 4, 24). Our study confirms that this is most likely related to the citric acid dose rather than the method of <sup>13</sup>C analysis.

The evidence that PPI treatment may induce three variant effects on the  $\Delta$  <sup>13</sup>CO<sub>2</sub> values (increase, decrease, or no change) has been reported previously for both H<sub>2</sub> receptor antagonists and PPI therapies (4, 7, 24–26). Although UBT is characterized by its very high diagnostic reproducibility, variations in the  $\Delta$  <sup>14</sup>CO<sub>2</sub> (after 1-wk re-examination), which may vary by a factor of above two, has already been shown (27). However, interestingly, our results demonstrate that with high dose citric acid as a test drink there is a significant positive association between baseline and post-PPI results (Fig. 2). In conclusion, the use of a single test drink containing 4.5 g citric acid powder given simultaneously with <sup>13</sup>C-urea resulted in a low number of false negative results associated with sustained PPI treatment, using both CRT-UBT and IRMS. The type of PPI and the sampling method used do not appear to alter detection of *H. pylori* by CRT-UBT.

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