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Appropriate Timing of the ^{14}C-Urea Breath Test to Establish Eradication of *Helicobacter pylori* Infection

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OBJECTIVE: The aim of this study was to determine the performance characteristics of the ^{14}C-urea breath test (UBT) performed 2 wk after the completion of therapy for *Helicobacter pylori* using a 4 to 6 wk study as the gold standard.

METHODS: Patients with active *Helicobacter pylori* infection at four medical centers received proton pump inhibitor-based triple or quadruple therapy for 10–14 days. Patients underwent the ^{14}C-UBT 2 and 4–6 wk after the completion of therapy. A positive test was defined as $^{14}CO_2$ excretion of $>$200 dpm, a negative test as $<$50 dpm, and an equivocal test as $>$50 but $<$200 dpm. Performance characteristics of the 2-wk UBT were calculated using the 4 to 6-wk result as a gold standard.

RESULTS: Eighty-five patients were enrolled and 82 patients (mean $\pm$ SD age, 62 $\pm$ 15 yr; 15 women) completed the protocol. Four patients had equivocal UBT results and were excluded from the analysis. Of the 78 patients, 68 (87%) had a negative 4 to 6-wk UBT. The 2-week UBT yielded a sensitivity of 90% (95% confidence interval 72–100%), specificity of 99% (97–100%), and accuracy of 97% (93–100%). In patients with a persistently positive UBT, $^{14}CO_2$ excretion at 2 wk was significantly lower than at 4–6 wk after therapy ($p = 0.03$).

CONCLUSIONS: A UBT performed 2 wk after therapy yielded results comparable to 4 to 6 wk testing. Further studies to evaluate the optimal time of confirmatory testing in the age of more effective proton pump inhibitor-based triple therapies are warranted. (Am J Gastroenterol 2000;95: 1171–1174. © 2000 by Am. Coll. of Gastroenterology)

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection has been linked to the pathogenesis of peptic ulcer disease (PUD) (1) and gastric malignancy (2). The eradication of *H. pylori* in patients with PUD significantly reduces the likelihood of ulcer recurrence (1). More recently a number of medical organizations have advocated the eradication of *H. pylori* in young patients with uncomplicated dyspeptic symptoms (3). *H. pylori* is usually treated with an acid suppressive medication in combination with antimicrobials. Eradication rates with the currently available therapeutic regimens vary considerably, ranging from 50 to 95% (4).

Currently, testing to prove *H. pylori* eradication is only recommended in patients with a complicated gastric or duodenal ulcer, mucosa-associated lymphoid tissue lymphoma, after resection of an early gastric cancer, and in patients with persistent dyspeptic symptoms despite appropriate therapy (5). *H. pylori* eradication can be established reliably by histology, rapid urease testing, and the urea breath test (UBT) (5). Preliminary studies suggest that the stool antigen test may also be useful as a means of testing for eradication, although this requires confirmation in the United States (6).

The UBT uses labeled urea (^{13}C or ^{14}C) that, in the presence of *H. pylori*, is metabolized by urease to yield CO_2. The labeled gas is absorbed across the gastric mucosa and subsequently measured in the patient's expired breath. Both the ^{13}C- and ^{14}C-UBT have been approved for commercial use by the Food and Drug Administration. Because of favorable cost, accuracy, convenience, and safety, the UBT has become the standard means of determining *H. pylori* eradication (7, 8). Largely related to the fear of false-negative test results, it is currently recommended that eradication testing be performed no less than 4 wk after the completion of antimicrobial therapy (9). Although this recommendation has been accepted by clinicians and clinical investigators, few studies have attempted to determine the optimal timing of eradication testing.

We attempted to determine the performance characteristics of the ^{14}C-UBT performed 2 wk after the completion of therapy using the standard 4- to 6-wk assessment for *H. pylori* eradication as gold standard.

Part of this work was presented at Digestive Diseases Week, Orlando, Florida, May 1999.

PATIENTS AND METHODS

Patient Population

Patients with active *H. pylori* infection documented by histology (at least one biopsy each from the body and antrum evaluated by an experienced gastrointestinal pathologist after hematoxylin and eosin staining), rapid urease testing (Pyloritek, Serim Research, Elkhart, IN; CLOtest, Ballard Medical Products, Draper, UT) or urea breath testing (Pytest, Ballard Medical Products, Draper, UT) within 30 days of recruitment were asked to participate in this study at four geographically diverse medical centers within the United States. The choice of therapy for *H. pylori* was left to the discretion of the physician who identified the presence of the infection. Children under the age of 18 yr, pregnant women, and those unable to understand or provide written informed consent were not eligible. This protocol was approved by the Institutional Review Board at each of the participating study sites.

Experimental Protocol

After providing written informed consent, eligible patients were asked to undergo the ^{14}C-urea breath test 2 wk and 4–6 wk after the completion of therapy for *H. pylori*. Patients were instructed not to take antibiotics, bismuth-containing compounds, or proton pump inhibitors (PPI) between the completion of therapy for *H. pylori* and their return for breath testing. The UBT was performed according to the manufacturer's instructions. A single 10-min breath sample was collected and analyzed using a calibrated scintillation counter at each of the participating study sites. Appropriate quality control measures were carried out to assure the accuracy of the scintillation counter at each study site. A positive test was defined as $^{14}CO_2$ excretion of >200 disintegrations per minute (dpm); a negative test was defined as <50 dpm; and an equivocal test was defined as >50 but <200 dpm.

Statistical Analysis

The sensitivity, specificity, positive predictive value, negative predictive value (NPV), accuracy, and associated 95% confidence intervals (CI) for the 2-wk UBT were determined using the 4 to 6-wk UBT as a gold standard. Patients with equivocal UBT results were not included in the determination of performance characteristics of the 2-wk UBT.

The mean ($\pm$SD) dpms for positive and negative studies at 2 wk and 4–6 wk were determined. Results were compared for statistically significant differences using a paired Student's *t* test. A *p* value of <0.05 defined a statistically significant difference between values.

Table 1. Individuals With Equivocal UBT Results (dpm)

	UBT Results (dpm)	
Therapy	2 wk	4 wk
Quadruple therapy	149	0
Quadruple therapy	38	73
Quadruple therapy	7	63
Quadruple therapy	144	0

Equivocal results >50 dpm and <200 dpm.

RESULTS

Between 11/98 and 10/99, 85 patients with active *H. pylori* infection were enrolled in this protocol. Three patients did not comply with the instructions provided at the start of the protocol (1) or did not attend one of the two UBT appointments (2). Eighty-two patients (mean age $\pm$ SD of 62 $\pm$ 15 yr), 15 women and 67 men) completed the protocol. All patients were treated with PPI-based triple or quadruple therapy for 10–14 days. Four patients had equivocal 2-wk (2) or 4- to 6-wk (2) UBT results and were excluded from further analysis (Table 1). One patient with an equivocal 2-wk UBT result and a negative 4- to 6-wk test result was found to have a negative UBT 12 wk after therapy. The other three patients with equivocal UBT results have not undergone a third UBT. Sixty-eight of 78 evaluable patients had no evidence of *H. pylori* infection on the basis of the 4 to 6-wk UBT, yielding a cure rate for all therapies of 87%.

Of 10 patients with a positive UBT result at 4–6 wk, nine had a positive test 2 wk after the completion of therapy, yielding a sensitivity of 90% (95% CI 72–100%). Sixty-seven of 68 patients with a negative 4- to 6-wk UBT had a negative 2-wk test result, yielding a specificity of 99% (95% CI 97–100%). The patient with a false-positive 2-wk UBT result underwent a third UBT 12 wk after the completion of therapy, which yielded a negative result. The 2-wk UBT test result was consistent with the 4- to 6-wk test result in 76 of 78 patients (97%; 95% CI 93–100%). Performance characteristics of the 2-wk UBT using the 4- to 6-wk UBT as a gold standard are provided in Table 2.

When the excretion of $^{14}CO_2$ (absolute dpm values $\pm$SD) for the 10 patients with a positive 4-wk UBT result were evaluated, we found that $^{14}CO_2$ excretion at 2 wk (1007 $\pm$ 612 dpm) was significantly lower than $^{14}CO_2$ excretion at 4–6 weeks (1455 $\pm$ 590 dpm, $p = 0.03$). There was no significant difference in $^{14}CO_2$ excretion at 2 wk (10 $\pm$ 25 dpm) *versus* 4–6 wk (8 $\pm$ 7 dpm; $p =$ not significant) for the 68 patients with negative results.

Table 2. Performance Characteristics (%) for the 2-wk UBT Using the 4- to 6-wk UBT as a Gold Standard

	Sensitivity	Specificity	PPV	NPV	Accuracy
	9/10 (90%)	67/68 (99%)	9/10 (90%)	67/68 (99%)	76/78 (97%)
95% CI	72–100	97–100	72–100	97–100	93–100

PPV = positive predictive value; NPV = negative predictive value; CI = confidence interval.

DISCUSSION

The UBT has proven a reliable means of establishing the presence of *H. pylori* infection before or after a course of antimicrobial therapy (7, 8). Several factors including the recent use of antibiotics, bismuth-containing compounds, and PPIs can affect the accuracy of the UBT (10–12). A number of reports have suggested that the accuracy of all tests that identify active *H. pylori* infection is decreased for an unclear period after the completion of therapy (13, 14). For this reason, it is currently recommended that testing to establish *H. pylori* eradication be performed no less than 4 wk after the completion of therapy (9). Despite the widespread acceptance of this recommendation, few studies have carefully evaluated the most appropriate time to document cure of *H. pylori* infection. Neil and colleagues (9) reported agreement between endoscopic tests (histology and in some cases rapid urease testing or culture) for *H. pylori* performed 1 and 6 months after treatment in 359 of 384 patients (94%). From this data, they concluded that confirmatory testing should be performed 1 month after therapy. It is clear that testing immediately after the completion of therapy can lead to false-negative results. However, it remains unclear whether it is necessary to delay confirmatory testing for a full month after the completion of therapy.

In this study, we found that UBT results obtained 2 wk after therapy agreed with those obtained 4–6 wk after therapy in 76 of 78 patients (97%; 95% CI 93–100%). For those with persistent infection on the basis of the 4- to 6-wk UBT, 9 of 10 (90%) had a positive test result 2 wk after therapy. The specificity (99%; 95% CI 97–100%) and negative predictive value (99%; 95% CI 97–100%) of the 2-wk UBT were excellent. Breath $^{14}CO_2$ excretion was significantly lower at 2 wk than at 4–6 wk in patients with persistent infection. This finding could be the consequence of the prolonged effects of therapy on *H. pylori* growth or urease activity. A recent study found that UBT results can remain falsely negative for up to 2 wk after the cessation of a PPI (11). In the current study, we observed a false-negative rate of 10% at 2 wk. Similar studies evaluating the effects of antibiotics, either individually or in combination, on the UBT have not been performed. Although we were not surprised to see a false-negative 2-wk UBT result, we were surprised to find a false-positive 2-wk UBT result. We confirmed the 2-wk test to be truly falsely positive by obtaining an additional UBT 12 wk after therapy, which, like the 4- to 6-wk study, was negative. Although false-positive UBT results after therapy have been reported by other investigators (15), the explanation for their occurrence remains elusive.

We acknowledge several issues that should be considered when interpreting our results. First, it could be argued that clinicians are using many different regimens to treat *H. pylori* infection and that these different antibiotic combinations may have heterogeneous effects on testing to establish cure. The physicians who participated in this trial used PPI-based triple or quadruple therapy. We would suggest that these therapies are the ones that should be and are most commonly used in clinical practice. As such, our protocol simulated the behaviors of most clinicians treating *H. pylori* infection. Next, the eradication rate for all therapies used in this protocol was 87%. As such, the high pretest probability for a negative result likely contributed to the excellent NPV for the 2-wk UBT. Regardless, the narrow 95% CI for the specificity and NPV support the reliability of a negative test result. Although the sensitivity of the 2-wk UBT was 90%, our small sample size led to wide 95% CI (72–100%). A larger study with a greater number of treatment failures will be necessary to confirm or refute our result.

We have conducted the first study to evaluate the usefulness of the ^{14}C-urea breath test to confirm *H. pylori* eradication before the current standard of 4 wk after therapy. We report encouraging results, particularly with regard to the specificity and NPV of testing 2 wk after therapy. We hope that our study will stimulate a reevaluation of the current practice standard for testing to establish *H. pylori* eradication. Certainly, the ability to perform testing earlier than 1 month after therapy would afford patients and health care professionals greater flexibility and allow more timely access to clinically relevant information.

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