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The ^{13}C-Urea Blood Test Accurately Detects Active *Helicobacter pylori* Infection: A United States, Multicenter Trial

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OBJECTIVES: Current nonendoscopic tests for *Helicobacter pylori* include antibody tests and the urea breath test. After the administration of ^{13}C-urea, serum bicarbonate measurement can identify those infected with *H. pylori*. In this study, our aims were to determine the accuracy of the urea blood test, and to compare the accuracy of the urea blood test with that of rapid urease testing of gastric biopsies.

METHODS: This was a multicenter trial conducted at five sites within the U.S. Patients scheduled for endoscopy were recruited. During endoscopy, biopsies were obtained from the gastric body and antrum for histology and rapid urease testing. Patients underwent the urea blood test, which required the ingestion of 125 mg of ^{13}C-urea after endoscopy. Thirty minutes later, a 3-ml blood sample was obtained and later analyzed by mass spectrometry for ^{13}C-bicarbonate. Performance characteristics for the urea blood test were calculated using the endoscopic biopsy tests as a gold standard.

RESULTS: One hundred and twenty-one patients (54 infected) were enrolled. The urea blood test yielded sensitivity of 89%, specificity of 96%, positive predictive value of 94%, negative predictive value of 91%, and accuracy of 93% using histology as a gold standard. There was no difference between results obtained with the urea blood test and rapid urease testing of gastric biopsies.

CONCLUSIONS: The urea blood test accurately identified active *H. pylori* infection. The performance characteristics of this nonendoscopic test were similar to those of endoscopic rapid urease testing. (Am J Gastroenterol 1999;94: 1522–1524. © 1999 by Am. Coll. of Gastroenterology)

INTRODUCTION

Helicobacter pylori (*H. pylori*) plays an important role in the pathogenesis of peptic ulcer disease and gastric malignancy (1). Diagnostic tests for *H. pylori* infection can be divided into those that rely upon endoscopy with gastric mucosal biopsy and those that can be performed without endoscopy. Currently available nonendoscopic tests for *H. pylori* include antibody tests and the urea breath test. Enzyme-linked immunosorbant assays and serum-based qualitative tests identify the presence of specific IgG antibodies to *H. pylori* (2, 3). Unfortunately, a positive serology test does not equate with active *H. pylori* infection. These tests can remain positive even years after successful eradication of the infection (4). As such, antibody tests are not useful to establish eradication of *H. pylori* after a course of antibiotics (5). In addition, as awareness of *H. pylori* becomes more widespread and larger numbers of patients are treated, it is possible that the specificity of antibody testing will decrease in the coming years (2).

The urea breath test relies upon the administration of ^{13}C- or ^{14}C-labeled urea with the subsequent collection of timed breath samples. In the presence of urease, an enzyme produced in large quantities by *H. pylori*, urea is metabolized to ammonia and $^{13}CO_2$ or $^{14}CO_2$. This labeled carbon dioxide is rapidly absorbed across the gastric epithelium into the blood stream, where it is carried to the lungs and eventually expired in the breath. The urea breath test sensitively and specifically identifies active *H. pylori* infection both before and after therapy (2, 3, 5). Recent reports in small numbers of patients suggest that testing can be performed using serum ^{13}C-bicarbonate determination instead of breath analysis for ^{13}C or $^{14}CO_2$ (6). In this current U.S. multicenter trial, we attempted to determine the performance characteristics of the ^{13}C-urea blood test for *H. pylori* infection using endoscopic biopsy based tests as a gold standard.

MATERIALS AND METHODS

Patient Population and Study Protocol

Five centers from geographically diverse locations within the U.S. participated in this study. Patients scheduled for endoscopy were recruited. Exclusion criteria included therapy for *H. pylori* within a yr of endoscopy, antibiotics or bismuth for any reason within a month of endoscopy, and proton pump inhibitors within 7 days of endoscopy. During endoscopy, biopsies were obtained from the body and an-

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Table 1. Performance Characteristics of the Urea Blood Test and a Rapid Urease Test for *H. pylori* Infection

	Sensitivity	Specificity	PPV	NPV	Accuracy
Urea blood test	48/54	64/67	48/51	64/70	112/121
	89% (85–93)	96% (94–98)	94% (91–97)	91% (88–94)	93% (91–95)
Rapid urease test	47/53	65/68	47/50	65/71	112/121
	89% (85–93)	96% (94–98)	94% (91–97)	92% (89–95)	93% (91–95)

PPV = positive predictive value; NPV = negative predictive value.
There was no statistically significant difference between performance characteristics of the urea blood test and the rapid urease test ($p > 0.2$).

trum for histology (hematoxylin and eosin in all cases and Giemsa when chronic active gastritis but no organisms was seen) and rapid urease testing (Pyloritek, Serim Research, Elkhart, IN). A patient was considered infected if biopsies from either the body or antrum demonstrated *H. pylori* organisms. Rapid urease testing was done according to the manufacturer's instructions by trained personnel at the participating study sites. Histological evaluation of the gastric biopsies was performed by experienced pathologists at each participating site.

After endoscopy, patients underwent the urea blood test (Ez-HBT, Metabolic Solutions, Inc., Nashua, NH) For this test, patients ingested 237 ml Ensure (250 kcal; Ross Products, Columbus, OH) to delay gastric emptying, followed 5 min later by 125 mg ^{13}C-urea dissolved in 75 ml water. Thirty minutes after administration of the ^{13}C-urea, a 3-ml blood sample was obtained by standard venipuncture and analyzed by gas isotope ratio mass spectrometry at a centralized location (Metabolic Solutions, Nashua, NH). A modified protocol, described by Moulton-Barrett *et al*. (7), was used to analyze samples for ^{13}C-bicarbonate. The central laboratory was blinded to the results of the endoscopic tests until completion of the blood sample analysis for ^{13}C-bicarbonate.

Data Interpretation and Statistical Analysis

Sensitivity (TP/TP + FN), specificity (TN/TN + FP), positive predictive value (PPV) (TP/TP + FP), negative predictive value (NPV) (TN/TN + FN), and accuracy (TP + TN/TP + TN + FP + FN) were determined for the urea blood test and rapid urease test using histology as a gold standard. The 95% confidence intervals for sensitivity, specificity, PPV, NPV, and accuracy were also calculated. Performance characteristics for the urea blood test and the rapid urease test were compared for statistically significant differences using McNemar's test for paired observations. We further determined the characteristics of the urea blood test when infection was defined as positive histology and rapid urease test results. Finally, test characteristics were determined when infection was defined as positive histology or rapid urease test.

RESULTS

Patient Characteristics

One hundred and twenty-one patients participated in the protocol (59 women, 62 men, mean age 49 ± 15 yr). Fifty-four (45%) were found to be infected by histology. For the purposes of this study, a serum bicarbonate result of ($> -17\Delta$ ^{13}C per mil) was defined as a positive urea blood test (unpublished data, Dr. David Wagner, Metabolic Solutions). No adverse events were reported in association with the urea blood test.

Performance Characteristics of the Urea Blood Test

When histology was used as a gold standard, performance characteristics of the urea blood test were as follows: sensitivity, 89%; specificity, 96%; PPV, 94%; NPV, 91%; and accuracy, 93%. The endoscopically based rapid urease test yielded a sensitivity of 89%, specificity of 96%, PPV of 94%, NPV of 92%, and accuracy of 93%. The raw data, as well as the 95% confidence intervals for the performance characteristics of the urea blood test and the rapid urease test, are provided in Table 1. The performance characteristics of the urea blood test and rapid urease test were not statistically significantly different ($p > 0.2$).

Acknowledging that histology alone is an imperfect gold standard, we recalculated performance characteristics of the urea blood test when *H. pylori* infection was defined as *positive histology and rapid urease testing*. Patients with discordant histology and rapid urease test results were considered uninfected. This analysis should overestimate sensitivity and NPV, while underestimating specificity and PPV. Under these conditions, the urea blood test yielded excellent sensitivity (94%), specificity (91%), PPV (86%), NPV (96%), and accuracy (92%).

In our final analysis, active infection was defined as a *positive histology or rapid urease test* result. Uninfected patients had negative histology and rapid urease test. We believed this analysis important, as both histology and rapid urease testing rarely yield false-positive results (2, 3). This analysis should optimize the urea blood test's specificity and PPV while presenting a worst case scenario for sensitivity and NPV. In this analysis, the test had a sensitivity of 88%, specificity of 98%, PPV of 98%, NPV of 90%, and accuracy of 93%.

DISCUSSION

Endoscopic methods including histology and the rapid urease test accurately identify *H. pylori* infection (2, 3). Histology is currently considered the gold standard for *H. pylori* diagnosis, with sensitivity and specificity exceeding 90% (3). Rapid urease testing is a well-accepted means of con-

firming the presence of *H. pylori* infection at the time of endoscopy. The sensitivity of the currently available rapid urease tests exceeds 85% while their specificity is greater than 95% (8, 9). Unfortunately, the need for endoscopy makes these tests costly and inconvenient for patients.

At present, the urea breath test is the only available nonendoscopic test that can identify active *H. pylori* infection (10). Versions of both the ^{14}C- and ^{13}C-urea breath test have recently been approved for use in the United States by the Food and Drug Administration (FDA). Despite FDA approval and numerous studies confirming its accuracy, the urea breath test has not been widely adopted by community physicians in the U.S. Much of this lack of enthusiasm for the urea breath test in the U.S. stems from problems with availability, cost to the patient, and unreliable physician reimbursement. Another issue that has likely slowed the widespread acceptance of the urea breath test involves the lack of familiarity of clinicians and patients with breath tests in general. Most hospitals and offices have to make special arrangements to perform the urea breath test. On the other hand, both clinicians and patients are quite familiar with tests requiring venipuncture. All hospitals and most physician offices have the capability to obtain blood samples. As such, urea blood testing, unlike breath testing, requires minimal additional training or equipment. Finally, the ^{14}C- and ^{13}C-urea breath tests require the acquisition of a basal breath sample, with the subsequent collection of one or more samples after urea ingestion. The urea blood test, on the other hand, requires no basal blood sample and the collection of only one sample after urea ingestion.

When histology was used as a gold standard, the urea blood test yielded excellent performance characteristics. Our results validate a recently published study by Kim *et al*. (6), who reported a sensitivity of 90.5% and specificity of 85.7% in patients undergoing upper endoscopy. This interesting preliminary study was hampered by its small sample size (20 infected and seven uninfected patients). Unlike the previous study, we collected blood 30 rather than 60 min after ingestion of ^{13}C-urea. From a practical standpoint, this time savings could have important implications for both patients and physicians. In addition to validating the urea blood test in a large number of patients from different geographical regions of the U.S., we also found that this nonendoscopic test was as accurate as the endoscopic rapid urease test. Finally, performance characteristics were outstanding, regardless of how we combined endoscopic tests (histology and rapid urease test) to define active *H. pylori* infection.

The urea blood test accurately identified patients with *H. pylori* infection with a single, 30-min venous blood sample collection in this multicenter U.S. trial. In this age of cost-consciousness, nonendoscopic means of evaluating patients with suspected peptic ulcer disease have taken center stage (11). In clinical practice, the usefulness of serology is limited not only by the inherent performance characteristics of the tests themselves but also by the widespread treatment of *H. pylori* and the use of antibiotics in general, which can lead to false-positive results. Unlike serological testing, the urea blood test identifies active *H. pylori* infection. In conclusion, the urea blood test should provide an easy-to-perform, accurate, nonendoscopic means of identifying active *H. pylori* infection in patients with suspected peptic ulcer disease.

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