Comparison of Noninvasive Diagnostic Tests for *Helicobacter pylori* Infection

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Key Words

Capsule ¹³C-urea breath test • *Helicobacter pylori* • Serology

Abstract

Objectives: Since the ¹³C-urea breath test (UBT) has become a highly reliable method for the noninvasive diagnosis of *Helicobacter pylori* infection, this study was performed in order to compare the sensitivity, specificity and accuracy among noninvasive tests including capsule UBT, conventional UBT and serology in the diagnosis of *H. pylori* infection. **Patients and Methods:** One hundred patients received capsule UBT, conventional UBT and gave blood samples for the diagnosis of *H. pylori* infection. Upper gastrointestinal endoscopy was performed in all patients. *H. pylori* infection was defined as the presence of a positive culture or positive results of both histology and rapid urease test (CLO test). McNemar's test was used to determine the significance of differences among capsule UBT, conventional UBT and serology. Differences were considered significant at $p < 0.05$. **Results:** According to the predefined criteria, the sensitivity, specificity, positive predictive value and negative predictive value of capsule UBT, conventional UBT and serology was 100, 95.7, 96.4 and 100%; 100, 85.1, 88.3 and 100%, and 90.6, 85.1, 82.7 and 88.9%, respectively. The accuracy of capsule UBT was higher than that of conventional UBT and serology (98 vs. 93 and 88%, respectively). Capsule UBT had a similar ability for the detection of *H. pylori* infection compared with conventional UBT and serology (McNemar's test, $p > 0.05$). **Conclusions:**

According to our study, capsule UBT was highly accurate compared with other noninvasive tests including conventional UBT and serology. It could become a good alternative to endoscopy for the diagnosis of *H. pylori* infection.

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Introduction

This study was performed in order to compare the sensitivity, specificity and accuracy among noninvasive tests including capsule ¹³C-urea breath test (UBT), conventional UBT and serology in the diagnosis of *Helicobacter pylori* infection. *H. pylori* infection can be diagnosed by invasive and noninvasive techniques. Invasive methods require endoscopy- and biopsy-based tests, including microbiological culture, histology, rapid urease test (CLO test) and polymerase chain reaction. Noninvasive tests include serology, stool antigen test and breath test. The choice of a diagnostic test should depend on the clinical circumstances, sensitivity and specificity of the tests, and the cost-effectiveness of the testing strategy.

According to our prior articles [1, 2], the sensitivity of culture, histology and CLO test was 77.8–94.4, 88.9–90.9 and 82.1–94.4%, respectively, and the specificity was 100, 90.6–100 and 95.5–96.9%, respectively. The overall accuracy of serological assays average 78% [3]. The sensitivity and specificity of UBT have been shown to range from 90 to 100%, compared with biopsy-based tests for *H. pylori*

infection [4]. Because of the excellent diagnostic accuracy, this test has been proposed for replacing endoscopy to diagnose *H. pylori* infection [5–8]. It is also an ideal method in monitoring treatment success following anti-*H. pylori* therapy, because it can avoid the false-negative result from biopsy-based tests due to focal colonization of bacteria and the false-positive result from serology due to the presence of anti-*H. pylori* IgG antibody [9].

UBT is based on the high urease activity of *H. pylori* in vivo. The orally administered ^{13}C -labeled urea is readily hydrolyzed by the urease of the organism to ammonia and $^{13}\text{CO}_2$. The latter can be detected through collection of expired samples. We recently reported a trial of a capsule UBT for the detection of *H. pylori* infection [2]. This diagnostic method may avoid contamination of urea from oral urease. Additionally, it can rapidly discriminate between *H. pylori*-positive and *H. pylori*-negative patients with 100% sensitivity and specificity. Its diagnostic accuracy was significantly higher than that of culture, histology, and CLO test.

Patients and Methods

Patients

One hundred patients who presented for routine upper gastrointestinal endoscopy and were willing to cooperate with this study were included in the present study. Fifty-six were male and 44 female, with a mean age of 55 years (range, 18–83 years). Criteria for exclusion included (1) use of proton pump inhibitors within 1 month before endoscopy; (2) antibiotic ingestion within 1 month before endoscopy; (3) serious medical illness, and (4) previous history of anti-*H. pylori* therapy. The study was approved by the institutional review board and the hospital's ethics committee. All participants gave written informed consent.

Upper Gastrointestinal Endoscopy, Culture, Histology and CLO Test

During endoscopy, four gastric biopsy specimens were taken from the lesser curvature 2 cm from the pylorus. Two specimens were fixed immediately in 10% neutral buffered formalin for histological examination, one for culture and the other for urease test (CLO test, Delta-West, Bently, Australia). Samples were sent to different laboratories that were blind to the results of other tests. The CLO test was monitored for color change up to 24 h after the addition of the gastric tissue. The gel was not warmed above ambient temperature at any time during the incubation period. The specimens for microbiological examination were transferred with brain-heart infusion in ice and inoculated onto the CDC anaerobic blood agar (Becton Dickinson Microbiology System, Cockeysville, Md., USA). The agar was incubated at 35°C for 7 days in a microaerophilic gas mixture composed of 5% O_2 , 10% CO_2 , and 85% N_2 . Culture-positive patients were those with bacterial colonies grown in culture within 7 days. The organisms were identified as *H. pylori* by Gram staining, colony morphology and posi-

tive oxidase, catalase and urease reaction. Histology-positive patients were those with curved organisms seen in hematoxylin and eosin-stained sections under the microscope. Although there is no real gold standard for the diagnosis of *H. pylori*, cultures should be 100% specific if the procedures are performed properly. Use of culture alone as the gold standard may yield false-negative results due to the inherent difficulties of culture. Hence, in the present study we defined patients with *H. pylori* infection as those with positive culture or positive results from histology and CLO test.

Conventional UBT and Capsule UBT

Conventional UBT and capsule UBT were performed 2 days apart within 7 days after endoscopy before any antibiotic treatment was given. No test meal was used in these tests. The patients were asked to fast at least 6 h. The ^{13}C -urea, 100 mg of 99 atom % of ^{13}C -labeled urea, was supplied with a commercial package from Japan (UBiT). On conventional UBT, the ^{13}C -urea solution was prepared by dissolving 100 mg ^{13}C -urea in 50 ml sterile water in a drinking vessel. For capsule UBT, a 100-mg capsule of ^{13}C -urea was packed in a gelatin capsule. Patients drank the 100-mg ^{13}C -urea solution. Immediately after ^{13}C -urea consumption, patients underwent a mouth washing by gargling to avoid oral ^{13}C -urea activity. Breath samples were collected before and 15 min after consumption of ^{13}C -urea. All breath samples were collected breathing out to a 200-ml gas storage bag for infrared spectrometer. The excess $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio samples were analyzed by an infrared spectrometer at our department (UBiT-IR300, Photol Otsuka Electronics Co., Japan). According to our previous studies [2, 8], the cutoff values were set at 4.8‰ for conventional UBT and 2‰ for capsule UBT to judge the agreement of *H. pylori*-positive and *H. pylori*-negative. The conventional UBT used in this study is identical to our previous study [8] except for the abolishment of the test meal because the test meal did not affect UBT results at 15 min, but increased values at 30 min and thereafter [10].

Values were expressed as an excess $\delta^{13}\text{CO}_2$ ‰ excretion. The $\delta^{13}\text{CO}_2$ is the ratio of ^{13}C to ^{12}C in the sample compared to the Pee Dee Belemnite standard. The equation is given as: $\delta^{13}\text{CO}_2 = (\text{R}_{\text{samp}} - \text{R}_{\text{std}})/\text{R}_{\text{std}} \times 1,000$. R_{samp} and R_{std} represent the ratios of ^{13}C to ^{12}C in sample and standard, respectively. Excess $\delta^{13}\text{CO}_2$ is the value of $\delta^{13}\text{CO}_2$ detected at 15 min minus that at baseline.

Serology Test

Blood samples for serologic evaluation were taken in the morning of the first UBT test. A serological assay for IgG antibodies against *H. pylori* was performed by a commercial test kit (ASSURE™ *H. pylori* rapid test kit, Genelabs Diagnostics, Cavenish Singapore Science Park, Singapore). The test is an indirect solid-phase immunochromatographic assay where antibodies in the test sample form antibody-antigen complexes with immobilized *H. pylori* antigens on the membrane as the test samples migrate upwards from the sample well. The bound antibody-antigen complexes are subsequently detected by anti-human IgG conjugated to colloidal gold. In brief, 25 μl of a whole blood sample was added to a sample well followed by 1 drop of chase buffer to the well. When the sample front reached the pink control line, three drops of chase buffer were added to the buffer well. If colored bands appeared at (1) all the control line, CIM line and test line, or (2) both the control line and test line, the test was regarded as positive. If only the control line was visible, the test was regarded

Table 1. Comparison of the sensitivity, specificity, PPV, NPV and accuracy of capsule UBT, conventional UBT and serology in the diagnosis of *H. pylori* infection expressed as percentages

	Serology	Conventional UBT	Capsule UBT
Sensitivity (CI)	90.6 (82.7–98.5)	100 (100–100)	100 (100–100)
Specificity (CI)	85.1 (74.9–95.3)	85.1 (74.8–95.2)	95.7 (89.9–100)
PPV (CI)	87.2 (78.5–96.1)	88.3 (80.2–96.4)	96.4 (91.5–100)
NPV (CI)	88.9 (79.7–98.1)	100 (100–100)	100 (100–100)
Accuracy (CI)	88 (81.6–94.4)	93 (88–98)	98 (95.3–100)

Table 2. Matched reading of capsule UBT versus conventional UBT and serology

Capsule UBT	Conventional UBT		Serology	
	positive	negative	positive	negative
Positive	55	5	50	5
Negative	0	40	5	40
McNemar's test	p = 0.063		p = 1	

as negative. If the control line was absent or both control line and CIM line were present but the test line absent, the test was regarded as invalid.

Statistical Analysis

Sensitivity, specificity and accuracy of capsule UBT, conventional UBT and serology were calculated according to the pre-defined gold standard. Proportions of positive and negative results of capsule UBT, conventional UBT and serology for the diagnosis of *H. pylori* infection were compared using the two-tailed McNemar's test for matched pairs [11]. A p value less than 0.05 was considered as statistically significant. Ninety-five percent confidence intervals (CIs) were also calculated. Narrow and high CIs are desirable. Wide and low CIs indicate poor agreement [12]. All calculations were performed using SPSS version 12.0 (SPSS Inc., Chicago, Ill., USA).

Results

In the total of 100 patients, 53 were *H. pylori*-positive and 47 *H. pylori*-negative according to the predefined criteria. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the capsule UBT, conventional UBT and serology are shown in table 1. The sensitivity of the capsule UBT was higher than that of serology (100 vs. 90.6%), and equal to that of conventional UBT (100 vs. 100%). The specificity of the capsule UBT was higher than that of conventional UBT and serology (95.7 vs. 85.1 and 85.1%, respectively). The PPV of the capsule UBT was higher than that of con-

ventional UBT (96.4 vs. 88.3 and 87.2%, respectively). The NPV of the capsule UBT was higher than that of serology (100 vs. 88.9%), and equal to that of conventional UBT (100 vs. 100%). The capsule UBT had a higher accuracy compared with conventional UBT and serology (98 vs. 93 and 88%).

Table 2 shows the matched reading of capsule UBT versus conventional UBT and serology. Based on the two-tailed McNemar's test, capsule UBT detected the same number of positive and negative *H. pylori* as serology ($p = 1$), and a slightly different number compared with conventional UBT ($p = 0.063$). Therefore, capsule UBT had similar ability for the detection of *H. pylori* infection compared with conventional UBT and serology (McNemar's test, $p > 0.05$).

Discussion

Conventional UBT with administration of liquid solution has been regarded as an accurate method for the noninvasive diagnosis of *H. pylori* infection. Its sensitivity and specificity have been shown to range from 90 to 100%, compared with biopsy-based tests [4]. Most investigators collected the breath samples at least 10 min after consumption because urease from oral flora may cause false-positive results if breath samples are taken too soon [4]. According to our previous studies and this study, the false-positive rates of conventional UBT still ranged from 4.3 to 14.9% [8, 9]. The decrease in specificity of conventional UBT may be related to a number of factors. Urease from the oral bacterial flora is an important false-positive factor in the breath test for *H. pylori*. Cleansing the patients' mouths and delayed sample collection have commonly been used to avoid this factor. Tests performed at 15 min were less specific than those performed at 30 min, probably because of the interference of oral urease-producing organisms. Another factor is the cutoff value used. Based on our previous studies, we chose 4.8‰ as the cutoff value and achieved 100% sensitivity and 85.1% speci-

ficity on conventional UBT. Increased cutoff values might achieve higher specificity, but lower sensitivity.

We have previously demonstrated that the timing of sample collection can be shortened to 2 min with 100% sensitivity and specificity through endoscopic administration [1, 13]. However, endoscopy is invasive, expensive, causes patient discomfort, and introduces the risk of endoscopy cross-infection. Patient-to-patient transmission of *H. pylori* infection by gastroduodenoscopy may result from the biopsy procedure or the insertion of an endoscope that is not properly cleaned [14, 15]. Because the biopsy forceps are usually reused after sterilization in Taiwan and in many developing countries, gastric biopsy may increase the risk of cross-infection. Application of encapsulated urea has the same effect as endoscopic UBT to prevent the release of urea before reaching the stomach and avoids the contamination of oral urease. We have recently reported a trial of a capsule UBT for the detection of *H. pylori* infection [2]. This diagnostic method avoids contamination of urea from oral urease. Additionally, it can rapidly discriminate between *H. pylori*-positive and *H. pylori*-negative patients. Its diagnostic accuracy was significantly higher than that of culture, histology, and CLO test [2]. In the present study we compared the capsule UBT with conventional UBT in the same patients and showed that the capsule UBT has a similar ability for the detection of *H. pylori* infection compared with conventional UBT (McNemar's test, $p = 0.063$) and a higher accuracy than conventional UBT (98 vs. 93%).

According to the predefined criteria, there were 2 false-positive capsule UBTs. Conventional UBT and serology were also found to be positive in these 2 subjects. We doubted the diagnostic accuracy of predefined criteria because biopsy-based tests may lead to false-negative results from suboptimal sampling due to focal colonization of bacteria. Based on our prior article, the sensitivity of culture, histology and CLO test were generally around 77.8–94.4, 88.9–90.9 and 82.1–94.4%, respectively [1, 2], but 66, 43 and 37% in patients with bleeding peptic ulcer, respectively [16]. However, the sensitivity of serology and UBT was both 94% in patients with bleeding peptic ulcers [16]. Since patients with peptic ulcer bleeding were not excluded in the study, the false-positive UBTs might be due to the low sensitivity of the biopsy-based tests in patients with bleeding peptic ulcers with which the UBT has been compared. Therefore, the low sensitivity of biopsy-based tests in patients with bleeding peptic ulcers may increase the proportion of false-positive results in UBTs. UBT effectively integrates the enzyme activity of bacteria over the entire surface of the stomach, and avoids the pos-

sibility of false-negative results from biopsy-based tests. That is also the reason why conventional UBT has been proposed for replacing endoscopy to diagnose *H. pylori* infection [5–8]. In the present study, capsule UBT demonstrated higher accuracy than conventional UBT. In addition, the ^{13}C -urea solution was prepared by dissolving 100 mg ^{13}C -urea in 50 ml sterile water in a drinking vessel for conventional UBT. For capsule UBT, a 100-mg capsule of ^{13}C -urea was packed in a gelatin capsule. Application of the capsule UBT shortened the preparation time and avoided contamination during preparation. We therefore suggest the capsule UBT as a good alternative to endoscopy and conventional UBT as the gold standard for the diagnosis of *H. pylori* infection.

The accuracy of our serological test was 88%. In an independent study by Rahman et al. [17], the accuracy of the test was also 88%. In our study, the accuracy of capsule UBT was higher than that of serology (98 vs. 88%). The values of capsule UBT are able to reflect the bacterial load in the stomach, whereas those of serology are not. Several authors have proposed a correlation between UBT values and *H. pylori* bacterial load [18–20]. Suto et al. [20] have found positive correlations between endoscopic UBT values and *H. pylori* colonization and activity score in the antrum and corpus, and negative correlations between the endoscopic UBT values and the atrophy and intestinal metaplasia scores in the corpus. Endoscopic and capsule UBT theoretically are the most accurate methods to quantitatively assess bacterial load in the stomach since they avoid the influence of urease activity in the oral cavity. Furthermore, capsule UBT is also an ideal method for monitoring treatment success following anti-*H. pylori* therapy, where serology is invalid due to the presence of anti-*H. pylori* IgG antibody [9]. The presence of anti-*H. pylori* IgG antibody implies prior exposure to these organisms, but does not imply the presence of a current infection. Therefore, capsule UBT is superior to serology for noninvasive diagnosis of *H. pylori* infection.

In this study, we demonstrated that capsule UBT was an extremely accurate method for the diagnosis of *H. pylori* infection. Because urease is not present in a healthy stomach, capsule UBT rarely causes a false-positive result. UBT effectively integrates the enzyme activity of bacteria over the entire surface of the stomach, and avoids the possibility of false-negative results. Capsule UBT provides an excellent method both for the initial diagnosis of *H. pylori* infection and for the confirmation of its eradication after treatment. Nowadays, the strategy of 'test and treat' (diagnose the *H. pylori* infection and treat it)

was recommended for patients with dyspeptic symptoms. However, failure of 'test and treat', >45 years of age or the presence of alarm features still deserve prompt endoscopy. Although the diagnostic accuracy of capsule UBT is very high, it still cannot replace endoscopy. It can be used along with invasive tests to improve diagnostic accuracy and in all conditions not requiring endoscopy. Our study showed that capsule UBT is a simple, noninvasive, and extremely accurate method for the diagnosis of *H. pylori* infection. The new test had a high accuracy

compared with conventional UBT and serology in the present study. It could be a good alternative to endoscopy for the diagnosis of *H. pylori* infection.

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References

- Peng NJ, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, Tseng HH, Huang WK, Lo GH, Hsu PI: Endoscopic ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection. *Dig Liver Dis* 2003;35:73-77.
- Peng NJ, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, Tseng HH, Huang WK, Lo GH, Hsu PI: Capsule ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection. *World J Gastroenterol* 2005;11:1361-1364.
- Stevens M, Livsey S, Swann R: Evaluation of Sixteen EIAs for the Detection of Antibodies to *Helicobacter pylori*. London, Department of Health, 1997, pp 1-46.
- Gisbert JP, Pajares JM: Review article: ^{13}C -urea breath test in the diagnosis of *Helicobacter pylori* infection - a critical review. *Aliment Pharmacol Ther* 2004;20:1001-1017.
- Slomiansk A, Shubert T, Cutler AF: [^{13}C]urea breath test to confirm eradication of *Helicobacter pylori*. *Am J Gastroenterol* 1995;90:224-226.
- Eppl HJ, Kirstein FW, Bojarski C, Frege J, Fromm M, Riecken EO, Schulzke JD: ^{13}C -urea breath test in *Helicobacter pylori* diagnosis and eradication: correlation to histology, origin of 'false' results, and influence of food intake. *Scand J Gastroenterol* 1997;32:308-314.
- Katellaris PH, Jones DB: Testing for *Helicobacter pylori* infection after antibiotic treatment. *Am J Gastroenterol* 1997;92:1245-1247.
- Peng NJ, Hsu PI, Lee SC, Tseng HH, Huang WK, Tsay DG, Ger LP, Lo GH, Lin CK, Tsai CC, Lai KH: A 15-minute ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection in patients with non-ulcer dyspepsia. *J Gastroenterol Hepatol* 2000;15:284-289.
- Peng NJ, Hsu PI, Lee SC, Tseng HH, Huang WK, Tsay DG, Lo GH, Lin CK, Cheng JS, Lai KH: Follow-up of *Helicobacter pylori* status by using ^{13}C -urea breath test in nonulcer dyspeptic patients after eradication therapy. *Chin Med J (Taipei)* 2001;64:337-342.
- Atherton JC, Washington N, Blackshaw PE, Greaves JL, Perkins AC, Hawkey CJ, Spiller RC: Effect of a test meal on the intragastric distribution of urea in the ^{13}C -urea breath test for *Helicobacter pylori*. *Gut* 1995;36:337-340.
- Fleiss J: Statistical Methods for Rates and Proportions, ed 2. New York, Wiley, 1981, pp 113-115.
- Grant JM: The fetal heart rate trace is normal, isn't it? Observer agreement of categorical assessments. *Lancet* 1991;337:215-218.
- Peng NJ, Lai KH, Liu RS, Lee SC, Tsay DG, Lo CC, Tseng HH, Huang WK, Lo CC, Hsu PI: Clinical significance of oral urease in diagnosis of *Helicobacter pylori* infection by ^{13}C -urea breath test. *Dig Dis Sci* 2001;46:1772-1778.
- Langenberg W, Rauws EAJ, Oudbier JH, Tytgat GNJ: Patient-to-patient transmission of *Campylobacter pylori* infection by fiberoptic gastroduodenoscopy and biopsy. *J Infect Dis* 1990;161:507-511.
- Miyaji H, Kohli Y, Azuma T, Ito S, Hirai M, Ito Y, Kato T, Kuriyama M: Endoscopic cross-infection with *Helicobacter pylori*. *Lancet* 1995;345:464.
- Lo CC, Lai KH, Peng NJ, Lo GH, Tseng HH, Lin CK, Shie CB, Wu CM, Chen YS, Huang WK, Chen A, Hsu PI: Polymerase chain reaction: a sensitive method for detecting *Helicobacter pylori* infection in bleeding peptic ulcers. *World J Gastroenterol* 2005;11:3909-3914.
- Rahman SHZ, Azam MG, Radman MA, Arfin MS, Alam MM, Bhuiyan TM, Ahmed N, Rahman M, Nahar S, Hassan MS: Non-invasive diagnosis of *H. pylori* infection: evaluation of serological tests with and without current infection marker CIM. *World J Gastroenterol* 2008;28:1231-1236.
- Perri F, Clemente R, Pastore M, Quitadamo M, Festa V, Bisceglia M, Li Bergoli M, Lauriola G, Leandro G, Ghos Y, Rutgeerts P, Andriulli A: The ^{13}C -urea breath test as a predictor of intragastric bacterial load and severity of *Helicobacter pylori* gastritis. *Scand J Clin Lab Invest* 1998;58:19-28.
- Debonnie JC, Pauwels S, Raat A, de Meeus Y, Haot J, Mainguer P: Quantification of *Helicobacter pylori* infection in gastritis and ulcer disease using a simple and rapid carbon-14-urea breath test. *J Nucl Med* 1991;32:1192-1198.
- Suto H, Azuma T, Ito S, Ito Y, Miyaji H, Yamazaki Y, Kohli Y, Kuriyama M: Endoscopic ^{13}C -urea breath test for quantification of *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 2000;15:161-167.