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ORIGINAL ARTICLE

Could the simplified ^{14}C urea breath test be a new standard in noninvasive diagnosis of *Helicobacter pylori* infection?

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Abstract
Objective The carbon-14 (^{14}C) urea breath test (UBT) is a reliable and noninvasive technique for the diagnosis of *Helicobacter pylori* (HP) infection. The diagnostic performance of a new practical and low dose ^{14}C UBT system (Heliprobe, Stockholm, Sweden) was compared with those of other diagnostic tests, namely, rapid urease test (RUT), histopathology, and DNA detection using polymerase chain reaction (PCR).
Methods Eighty-nine patients (mean age = 45 ± 13, 30 men) with dyspeptic complaints who underwent an endoscopic procedure were studied. Biopsy specimens acquired during the procedure were subjected to RUT, histopathological examination using hematoxylin and eosin (HP-HE) and PCR. All patients underwent UBT using the Heliprobe system on a different day. The gold standard for HP positivity was defined as any two of the three tests being positive, excluding UBT, and the sensitivity and specificity of any single test alone were determined using this gold standard. Whenever only one test was positive, it was considered to be a false-positive one.
Results With the gold standard used in this study, 59 (66%) patients were diagnosed HP positive. The Heliprobe method detected HP infection with 96.6% sensitivity and 100% specificity and had the best diagnostic performance when compared with all the other methods. The sensitivity and specificity of the other methods for the detection of HP positivity were 89.8% and 100% for RUT, 93.2% and 63.3% for PCR, and 93.2% and 76.6% for HP-HE, respectively. Areas under the receiver-operating characteristic were 0.977 for UBT, 0.947 for RUT, 0.84 for HP-HE, and 0.775 for PCR.
Conclusions Using a combination of invasive diagnostic tests as the gold standard, Heliprobe UBT was found to be highly sensitive and specific for the diagnosis of HP infection in patients with dyspeptic complaints.

Keywords Urea breath test · *Helicobacter pylori* · Rapid urease test

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Introduction

Helicobacter pylori (HP) is not only a cause of gastritis, duodenitis, and peptic ulcer disease in humans but also one of the most important bacterial pathogens shown recently to be associated with the occurrence of distal gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphomas [1–5]. Moreover, it

has a potential role in certain cardiovascular and cerebrovascular, hematological, pulmonary, hepato-biliary, intestinal, and neurological diseases [6]. About 70–90% of the population is estimated to be carriers of this pathogen in developing countries. This rate drops to 25–50% in developed ones [7]. Owing to this large number of affected systems and the huge population under risk, it poses a major public health problem, necessitating cheap, noninvasive, and simple diagnostic methods.

The Heliprobe urea breath test (UBT) is a recently introduced noninvasive, simple, and cheap low-dose ^{14}C UBT system. In the Heliprobe method, breath sample is collected into a dry cartridge system, and the activity of the cartridge is counted using the Heliprobe analyzer, which is based on two Geiger Müller counters. In this study, the diagnostic performance of Heliprobe carbon-14 (^{14}C) UBT was tested in comparison with biopsy-driven methods, namely, rapid urease test (RUT), polymerase chain reaction (PCR) detection of HP DNA fragments, and histopathological examination of gastric biopsy specimens.

Materials and methods

Patients

Eighty-nine patients (30 men, 59 women, mean age 45 ± 13 years) referred for upper gastrointestinal (GI) endoscopic examination because dyspeptic complaints were studied. Patients who had previously received HP eradication treatment were excluded. All patients underwent upper GI endoscopy and three biopsy specimens were taken from the antral mucosa for histological analysis, RUT, and PCR.

Urea breath test

Prior to UBT, the subjects fasted for at least 6 h, usually overnight. Antiacids and H2 receptor antagonists were stopped for at least 24 h prior to the test. Proton pump inhibitors and sucralfate were discontinued 1 week prior to the test, and antibiotics were stopped for 1 month.

Patients swallowed 37 kBq (1 μCi) of encapsulated ^{14}C urea/citric acid composition (Helicap, Noster system, Stockholm, Sweden) with 25 ml water. Breath samples of patients were collected into Heliprobe Breath Cards (Noster system) in 10 min after administration of ^{14}C urea. Patients exhaled into the breath card until its color indicator changed from orange to yellow. The breath samples were measured using the Heliprobe analyzer (Noster system), and the activity was counted for 250 s. Results were expressed as counts per minute (cpm) and counts <25 cpm were defined as Heliprobe 0 = not infected, counts between 25 cpm and 50 cpm as Heliprobe 1 = equivocal and counts >25 cpm as Heliprobe 2 = infected [8].

Rapid urease test

The biopsy specimens for the RUT were removed from the biopsy forceps with a sterile needle and placed immediately into the RUT (CLOtest Kimberly-Clark, Roswell, GA, USA). The tests were read first after half an hour and at 24 h and the test results were recorded as positive if the indicator turned into red or orange; otherwise it was considered as negative. To prevent false-negative RUT results, the endoscopic procedure was postponed for 4 weeks in patients on regular anti-acid treatment or antibiotics.

Histopathological examination

Specimens for histopathological examination were transported in 4% formalin solution. Sections of paraffin-embedded specimens were stained with hematoxylin and eosin (HE) and examined under light microscopy for the detection of HP by a single pathologist who was totally blinded to the results of other tests used in the study.

Detection of point mutations in the 23S rRNA gene of HP by real-time PCR

A real-time PCR-based PCR-hybridization assay was used directly on DNA obtained from gastric biopsies for detecting point mutations conferring resistance to clarithromycin. The method included amplification of a fragment of the 23S rRNA gene of HP coupled with simultaneous detection of the product by probe hybridization and analysis of the melting curve by using LightCycler thermocycler (Roche Diagnostics, Neuilly sur Seine, France) [9]. A 267-bp fragment of the 23S rRNA gene of HP was amplified using primers HPYS and HPYA as described earlier [10].

Data evaluation/statistics

The gold standard for HP positivity was defined as any two of the three tests (RUT, PCR, and histopathology) being positive. Whenever only one test was positive, it was considered to be false-positive. Sensitivity, specificity, diagnostic accuracy, predictive values of negative and positive results, and the validity of the tests were evaluated in accordance with the standard methods. Φ coefficient was used to evaluate the agreement level between various tests used in the detection of HP. Diag-

nostic performance of the tests was evaluated with receiver-operating characteristic (ROC) curve. A P value of <0.01 was considered to be statistically significant.

Results

A total of 89 patients were evaluated for HP using four different tests. All four tests were positive in 46 patients (51%) and negative in 14 (15%). Fifty-seven (64%) subjects had a positive UBT test result, whereas the remaining 32 (36%) were HP negative with UBT.

The gold standard was defined as any two of the three tests (RUT, PCR, and histopathology) being positive, excluding UBT. Accordingly, 59 patients (66%) were HP positive and 30 (34%) negative. Table 1 shows the sensitivity, specificity, diagnostic accuracy, positive and negative predictive values of the four tests against the gold standard as defined earlier. When the sensitivity and specificity and diagnostic performance characteristics of all tests were compared via the ROC curve analysis, the area under the ROC curve (95% CI) was 0.977 (0.956–0.997) for UBT, 0.947 (0.902–0.996) for RUT, 0.849 (0.752–0.947) for histopathology, and 0.775 (0.669–0.896) for PCR (Fig. 1).

All the tests were positively correlated as shown in Table 2. The highest correlation was found between UBT and RUT ($\Phi = 0.861$, $P < 0.001$). The results of the UBT were highly correlated with histopathological findings ($\Phi = 0.728$, $P < 0.001$). There was a positive correlation, albeit weaker, between UBT results and PCR findings ($\Phi = 0.574$, $P < 0.001$). A weaker correlation was noted between PCR findings and the results of the other tests utilized in this study, the weakest one being between PCR and histopathological findings ($\Phi = 0.392$, $P < 0.001$). On the other hand, the strongest correlation was observed between UBT and the gold standard used in this study ($\Phi = 0.878$, $P < 0.001$; Table 2).

Discussion

In this study, the diagnostic performance of the Heliprobe ^{14}C UBT has been tested in comparison with

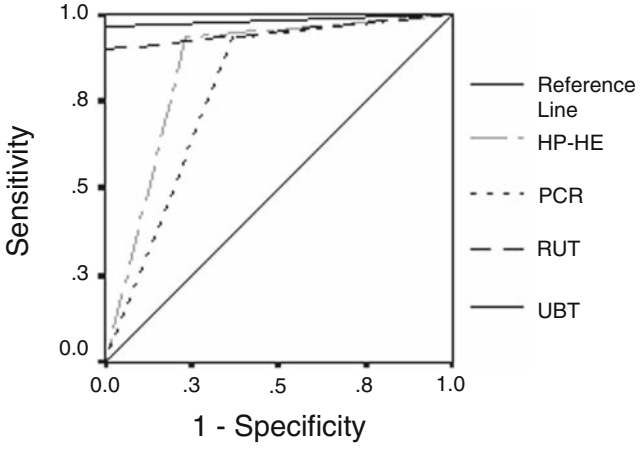

Fig. 1 Receiver-operating characteristic curve: diagnostic performances of the tests. Area under curve (95% confidence interval): urea breath test 0.977 (0.956–0.997), rapid urease test 0.947 (0.902–0.996), polymerase chain reaction 0.775 (0.669–0.896), and histopathology 0.849 (0.752–0.947)

Table 1 Sensitivity, specificity, diagnostic accuracy, negative and positive predictive values of the four tests for the detection of *Helicobacter pylori*

Test	Sensitivity (%)	Specificity (%)	Accuracy (%)	NPV (%)	PPV (%)
UBT	96.6	100	97.7	100	93.7
RUT	89.8	100	93.2	100	83.3
PCR	93.2	63.3	83.1	83.3	82.6
Histopathology	93.2	76.6	87.6	88.7	85.1

UBT urea breath test, *RUT* rapid urease test, *PCR* polymerase chain reaction, *PPV* positive predictive value, *NPV* negative predictive value

Table 2 Correlation analysis, with Φ coefficients displayed

	UBT	RUT	Histopathology	PCR	Gold standard	P*
UBT	1.000	0.861	0.728	0.574	0.878	<0.001
RUT	0.861	1.000	0.651	0.507	0.844	<0.001
Histopathology	0.728	0.651	1.000	0.392	0.793	<0.001
PCR	0.574	0.507	0.392	1.000	0.630	<0.001
Gold standard	0.878	0.844	0.793	0.630	1.000	<0.001

Φ correlation coefficient; correlation is significant at the 0.01 level (two-tailed)
* All P values were found less than 0.001

biopsy-driven methods, namely, RUT, PCR, and histopathological examination with the definition of the gold standard as positivity of any two of the three invasive tests (RUT, PCR, and histopathology). We do know that the methods used in this study are based on different principles for the detection of HP. These are the enzyme "urease" content of HP (UBT and RUT), the existence of the HP bacillus on the gastric epithelium (histopathological examination), and the presence of DNA fragments of the HP bacillus on biopsy specimens (PCR). On the basis of the assumption that no single test should be considered the gold-standard for the diagnosis of HP infection, we tested the sensitivity and specificity of the four tests against a gold standard, defined as positivity of any two of the three invasive tests. It is well known that in daily practice, a combination of tests is recommended and the simultaneous positivity of at least two different tests may therefore be considered diagnostic for HP presence and therefore a gold standard for the detection of HP infection [11, 12].

Various invasive and noninvasive methods had previously been compared for the diagnosis of HP infection, but, to our knowledge, ours is the first study to compare Heliprobe UBT with RUT, PCR, and histopathological examination [12–16].

Urea breath testing with Heliprobe detected HP infection with 96.6% sensitivity and 100% specificity, and the diagnostic yield of Heliprobe UBT ranked the highest among the four tests compared in this study. Previous studies reported sensitivity and specificity of 87–100% and 95–100%, respectively, with classical ^{13}C–^{14}C UBT [12–15]. Hegedus et al. [8] who first defined Heliprobe testing reported a complete concordance between the Heliprobe method and the classical ^{14}C UBT that utilizes liquid scintillator. With histopathological positivity as the gold standard, Öztürk et al. [17] reported a sensitivity of 100% and a specificity of 76% with the Heliprobe method. They only had six false positives in their study. In contrast, in our study we found no false positives but two false negatives with Heliprobe testing. False-negative results with the Heliprobe method may be owing to an inadequate time interval between the cessation of acid-lowering therapy and Heliprobe testing, as the activity of the urease enzyme is closely linked to the gastric pH [18, 19].

Classical ^{13}C–^{14}C UBT utilizes mass spectrophotometry and liquid scintillator that are expensive equipments and are only available in a few centers. The Heliprobe equipment, on the other hand, is composed of two Geiger-Müller counters, is cheap and user friendly, and the results are available within only 20 min, which make it an attractive alternative for centers that cannot afford the classical ^{13}C–^{14}C UBT technology. When compared with other tests used in the diagnosis of HP infection, the advantages of UBT are its noninvasive nature, practicality, and the availability of test results within a short time. Cumulative lifetime radiation exposure from ^{14}C UBT has been calculated to be not more than 0.3 mrem/μCi [20], and it is less than the natural background radiation in an ordinary day. The major disadvantage of UBT and other urease-based methods is that most patients with dyspeptic complaints are on either proton pump inhibitors, or H2 receptor antagonists and these drugs have to be interrupted for an adequate time interval before Heliprobe UBT to maximize the yield of testing.

Hegedus et al. [8] defined an optimal cut-off value between 25 cpm and 41 cpm with Heliprobe testing and recommended that 25–50 cpm, which yields similar results, might be used as lower and upper cut-off count values. In our study, with a cut-off range of 25–50 cpm, we found UBT to be 96.6% sensitive and 100% specific for the detection of HP infection, verifying the validity of this previously defined cut-off range.

Although invasive, its low cost and the availability of test results within a day make RUT a practical test for the detection of HP. It has been reported to be 82–90% sensitive and 99–100% specific to diagnose HP infection [12, 14–16]. Our finding of an 89.8% sensitivity and a 100% specificity is in accordance with these figures. No false positives were detected in our study. The six false negatives most probably were owing either to the patchy distribution of the HP bacillus on the gastric mucosa or to the utilization of proton pump inhibitors or H2 receptor antagonists by patients, although recommended not to do so [21, 22].

In our study, detection of HP bacillus by HE was 93.2% sensitive and 76.6% specific to diagnose HP infection. Low-grade infections have been reported to be missed with HE. Diagnostic methods based on gastric biopsy specimens all carry the risk of being false negative owing to the patchy distribution of HP on the gastric mucosa [23]. False positives, on the other hand, may stem out from HP-like microorganisms and immunohistochemical examination utilizing polyclonal antibodies against HP may help in these instances [24].

The sensitivity and specificity of PCR for detecting HP infection have been reported to range between 80–91% and 82–96%, respectively, in previous reports [25–27]. In our study, we detected a concordantly sensitivity figure of 93.2% but a discordantly low specificity figure of 63.3%. The PCR was positive in 36% of HP negative patients in our study. False positives with PCR testing have been attributed to inappropriate cleaning of the endoscopic equipment [28]. In busy hospital settings, this highly costly method seems to be of limited clinical use except for the detection of drug resistance.

Although not utilized in this study, serological testing is another noninvasive method to detect HP which has been reported to be 88–99% sensitive and 88%–95% specific for the diagnosis of HP infection [12, 14, 15]. The major advantage of serological testing is that it does not require cessation of proton pump inhibitor (PPI) therapy. The Maastricht III Consensus Report recommends serological testing as a diagnostic test in occasions where other tests could be false negative owing to low bacterial density like in patients with bleeding ulcers, gastric atrophy, and MALT lymphoma and in patients who are on recent or current use of PPI and antibiotics [29].

There are multiple important factors to determine the most appropriate method for the diagnosis of HP infection. Rapid urease testing seems to be appropriate for patients off acid-lowering therapy undergoing endoscopy because it is cheap, highly sensitive, and specific, and the results are available in a short time. Histopathological examination adds the advantage of determination of the gastric mucosal status and detecting probable precancerous changes. PCR should best be reserved for detecting drug resistance.

Besides being a major etiological factor in peptic ulcer disease, HP infection has a potential role in the development of distal gastric carcinoma, MALT lymphoma, and some cardiovascular, respiratory tract, neurological, dermatological, and autoimmune disorders [1–6]. Owing to this large list of diseases potentially related to HP infection, practical and cheap diagnostic tests with high sensitivity and specificity are obviously needed. The issue of who is to be given HP eradication therapy is important and in patients below 45 years of age, with dyspeptic complaints and no alarming symptoms (weight loss, anorexia, anemia, vomiting, dysphagia, malabsorption, and palpable mass), a "test and treat" strategy is recommended by consensus reports and guidelines [29–33]. Therefore, a positive noninvasive test result may render HP eradication therapy indicated without an endoscopic examination. One has the choice of using either ^{13}C or ^{14}C UBT or fecal HP-specific antigen analysis for this purpose and Heliprobe ^{14}C UBT, which has been shown in this study to be extremely sensitive and specific for the diagnosis of HP infection, may be recommended as the test of the "test and treat" strategy approved by consensus reports.

In conclusion, using a combination of invasive diagnostic tests as the gold standard, Heliprobe ^{14}C UBT was found to be highly sensitive and specific for the diagnosis of HP infection. With the advantages of simplicity, very low level of radiation exposure, short test time and low cost, we strongly recommend this noninvasive and reliable test for the diagnosis of HP infection.

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