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 $= 45 \pm 13$, 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

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, hepatobiliary, 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 14C 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 (14C) 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 14C 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 14C 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-