

# Comparison of $^{13}\text{C}$ - urea blood test to $^{13}\text{C}$ -breath test and rapid urease test for the diagnosis of *Helicobacter pylori* infection

Lucía C Fry, Walter H Curioso, Steffen Ricketts, Glen Horton, Basil I Hirschowitz, Klaus Mönkemüller

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## Summary

At present, the available methods to diagnose active *H. pylori* infection are endoscopy with biopsy for histology, culture, rapid urease tests,  $^{13}\text{C}$  or  $^{14}\text{C}$  urea breath test, urine antibody and the stool antigen test. The aims of this study were to simplify the  $^{13}\text{C}$  urea test by measuring  $^{13}\text{C}$  in blood rather than breath, and to evaluate the usefulness of the  $^{13}\text{C}$  urea blood test for the diagnosis of *H. pylori* infection. Patients who underwent upper endoscopy for standard clinical indications (e.g. dyspepsia, abdominal pain) were enrolled. A total of 161 patients (93F, 68M, mean age  $47 \pm 14.2$ ) were evaluated; 50 (31%) of them were *H. pylori* positive, and 111 (69%) were *H. pylori* negative. *H. pylori* infection was diagnosed with a rapid urease test (CLO-test) and  $^{13}\text{C}$  urea breath test (UBT). Performance characteristics for the  $^{13}\text{C}$  urea blood test for diagnosis and evaluation of *H. pylori* eradication were calculated using UBT and CLO as gold standards. The fifty *H. pylori*-positive patients were treated with triple antibiotic therapy for two weeks. Four weeks after finishing antibiotic therapy patients were retested with a commercial UBT and urea blood test. The  $^{13}\text{C}$  blood test had sensitivities of 92 and 98% and specificities of 96 and 100% as compared with urea breath test and CLO, respectively. We conclude that the  $^{13}\text{C}$  urea

blood test is highly sensitive and specific for the initial diagnosis and control of eradication of *H. pylori* infection.

## Resumen

**Comparación entre el test de urea marcada con  $^{13}\text{C}$ - en sangre, test de urea marcada con  $^{13}\text{C}$ - en aire espirado y el test rápido de ureasa para el diagnóstico de la infección por *Helicobacter pylori***

Los métodos diagnósticos disponibles para la identificación de la infección activa por *H. pylori* son endoscopia con biopsia para histología, test rápido de ureasa y/o cultivo, test del aire espirado con urea marcada con  $^{13}\text{C}$  o  $^{14}\text{C}$ , anticuerpos anti- *H. pylori* en orina y test de antígeno en materia fecal. La finalidad del presente estudio fue simplificar el test de aire espirado con urea marcada con  $^{13}\text{C}$ , midiendo  $^{13}\text{C}$  en sangre en vez de en el aire espirado y evaluar su eficacia para el diagnóstico de la infección por *H. pylori*. Pacientes a los que se les realizó esofagogastroduodenoscopia con indicaciones de dispepsia y/o dolor abdominal fueron incluidos en el estudio. 161 pacientes (93 del sexo femenino y 68 masculino, edad media  $47 \pm 14.2$ ) fueron evaluados; 50 (31%) de ellos fueron *H. pylori* positivos, y 111 (69%) fueron negativos. La infección por *H. pylori* fue diagnosticada con test rápido de ureasa y test del aire espirado con urea marcada con  $^{13}\text{C}$ . Usando estos tests como referencia, se evaluó la eficacia del test de sangre con urea marcada con  $^{13}\text{C}$  para el diagnóstico y evaluación de la erradicación del *H. pylori*. Los 50 pacientes positivos para la infección con *H. pylori* fueron tratados con triple plan de antibióticos por 2 semanas. Cuatro semanas luego de finalizado dicho tratamiento, los pa-

Division of Gastroenterology and Hepatology  
University of Alabama,  
Birmingham, Alabama, USA

**Correspondencia:** Dr. med. Klaus E. Mönkemüller  
Klinikum für Innere Medizin, Gastroenterologie, Hepatologie und Infektiologie  
Otto-von-Guericke University, Leipziger Str. 44  
39120 Magdeburg, Germany  
Phone: +49 391 6713100, Fax: +49 391 67 13105  
e-mail: Klaus.Moenkemueller@medizin.uni-magdeburg.de

cientes fueron nuevamente testeados con test de aire espirado con urea marcada con  $^{13}\text{C}$  y urea marcada con  $^{13}\text{C}$  en sangre. El test en sangre con urea marcada con  $^{13}\text{C}$  tuvo sensibilidad de 92% y 98% y especificidad de 96% y 100% comparado con UBT y test de ureasa respectivamente. Concluimos que el test en sangre con urea marcada con  $^{13}\text{C}$  es altamente sensible y específico para diagnóstico inicial y control de la erradicación de la infección por *H. pylori*.

*Helicobacter pylori* is the major etiologic agent of chronic active gastritis and is strongly associated with gastric and duodenal ulceration.<sup>1,2</sup> Gastric carcinoma and non-Hodgkin lymphoma (mucosa associated lymph node tissue or MALT lymphoma) have also been associated with this organism.<sup>3-5</sup> Elimination of *H. pylori* infection markedly reduces the relapse of duodenal and gastric ulcer and is associated with regression of early MALT lymphomas.<sup>1,4,6</sup> At present, the major methods available to diagnose active *H. pylori* infection are endoscopy with biopsy for histology, culture or rapid urease tests (RUT), urea breath test (UBT), and stool antigen test.<sup>1,7-11,14</sup> Antibody tests are not useful for the diagnosis of active *H. pylori* infection.<sup>1,9</sup> Tests which have been proven to be useful to diagnosis *H. pylori* infection before and after eradication are RUT, UBT and stool antigen determination.<sup>8,10,11,14</sup>

The UBT relies on the breakdown by *H. pylori* urease of  $^{13}\text{C}$  or  $^{14}\text{C}$ -labeled urea into ammonia and  $^{13}\text{CO}_2$  (or  $^{14}\text{CO}_2$ ), which is absorbed into the bloodstream and expelled via the lungs.<sup>8,10,12,13</sup> Thus, in theory  $^{13}\text{CO}_2$  can be measured in either blood or breath. Since gastric *H. pylori* produces a potent urease, a meal rich in  $^{13}\text{C}$ -labeled urea should lead to a measurable quantity of isotopic  $\text{CO}_2$  in the blood. The isotope-labeled carbon  $^{13}\text{C}$  or  $^{14}\text{C}$ -urea breath test (UBT) is a reliable non-invasive test to diagnose active *H. pylori* infection, but it is moderately expensive and collection of samples requires specialized equipment.<sup>8,10</sup> Recently, a  $^{13}\text{C}$ -urea blood test has been proposed as a useful test to diagnose active Hp-infection, but (few) studies have compared the  $^{13}\text{C}$ -urea blood test to other methods for the diagnosis of active Hp-infection.<sup>14</sup> The objectives of our study were to simplify the  $^{13}\text{C}$  urea test by measuring  $^{13}\text{C}$  in blood rather than in breath and to evaluate the usefulness of a  $^{13}\text{C}$  urea blood test for the diagnosis of *H. pylori* infection.

## Patients and Methods

Female and male patients (age 18 to 70 years old) who were referred for esophagogastroduodenoscopy (EGD) for upper abdominal symptoms were offered study inclusion. Indications for endoscopy included: dyspepsia, abdominal pain, heartburn, nausea. Exclusion criteria were: refusal to participate, inability to give informed consent, pregnancy, concomitant cancer or use of chemotherapy, severe lung disease or heart failure, history of a gastric surgery or resection, use of antibiotics or bismuth in the preceding 4 weeks, use of PPI or H2B in the preceding 2 weeks. The study was approved by the institutional review board of the University of Alabama at Birmingham and was conducted according to the provisions of the declaration of Helsinki (1995).

EGD was performed in standard fashion using intravenous conscious sedation with midazolam and an Olympus videogastroscope (Olympus, Lake Success, NY). All patients gave written informed consent for the procedures and tests. During EGD two biopsies for histopathology were obtained from antrum and corpus. Biopsy specimens were immediately fixed in buffered formalin, processed using standard techniques and submitted for histopathologic study. Two additional biopsies were obtained from the antrum (lesser and greater curvature) and placed in the agar media for rapid urease test (CLO test, Tri-Med Specialities, Inc., Overland, KS). CLO test was read at 2 and 24 hours. A positive test was defined according to manufacturer's instructions. UBT and  $^{13}\text{C}$ -urea blood test were performed during a different session. The patient arrived to the GI Laboratory after having fasted overnight for 12 hours. The UBT was then performed as follows: after rinsing the mouth with water the patient ate 5 oz (142 gram) of Ensure® pudding (Ross, Columbus, OH) (with the objective to delay gastric emptying), and then ingested 125 mg of  $^{13}\text{C}$ -labeled urea mixed with 75 ml of sterile water. Venous blood and end expiratory breath samples were drawn at baseline, 15 minutes and at 30 minutes for measurement of  $^{13}\text{C}$ . Whole fasting venous blood samples (5 ml) were collected in standard red top tubes and frozen until analysis. These were stored at  $-30\text{ }^\circ\text{C}$ . End expiration breath samples were collected by blowing through a straw into a 12 ml glass test tube. UBT was analyzed by Meretek® (Meretek Diagnostics, Houston, TX).  $^{13}\text{C}$  in blood was mea-

sured with the gas chromatograph (GC) selective mass-spectrometer from the University of Alabama at Birmingham (Micromass®, Beverly, MA). For testing the samples were thawed and equilibrated to room temperature. Gases in the container were sampled and then analyzed for  $^{13}\text{C}/^{12}\text{C}$  isotope ratios. The ratio (%) of stable isotope  $^{13}\text{C}$  to  $^{12}\text{C}$  was measured as an isotopic  $\Delta$  versus baseline. Normal abundance of  $^{13}\text{C}$  in nature is about 1% (1.000%). A positive result represents a change from baseline at 1.000% to a value  $>1.0015\%$ . A positive blood sample was defined as a  $\Delta > 1.5$  per mil over baseline cut-off level (ROC analysis) and a positive urea breath sample was defined as a  $\Delta > 2.4$ . We used the maximum absolute change in  $\Delta^{13}\text{C} - \text{HCO}_3$  at 15 min from the value obtained before the ingestion of the  $^{13}\text{C}$ -labeled urea as an assessment of urease activity. The performance characteristic of the  $^{13}\text{C}$  urea blood test for the diagnosis of *H. pylori* was compared to CLO test and the  $^{13}\text{C}$  -UBT (Meretek®). Performance characteristics of the  $^{13}\text{C}$  urea blood test for evaluation of *H. pylori* eradication were calculated using UBT (Meretek®) as comparator.

**Statistical analysis:** Descriptive statistics were used to summarize baseline demographical and laboratory data. All data are expressed as means and standard deviation. Sensitivity, specificity, and positive and negative predictive values were determined in the usual fashion.

## Results

A total of 213 patients underwent endoscopy during the study period. 52 patients fulfilled exclusion criteria. One hundred sixty-one patients (68 men, 93 women, average age of 47.0 +/- 19.2 yr, range 21 to 68) were included. A total of 50 of 161 patients (31%) were *H. pylori* positive. Indications for EGD were: abdominal pain (43%), heartburn (29%), dyspepsia (34%), nausea (4%). Agreement between UBT-breath (Meretek) and  $^{13}\text{C}$  urea blood test (Micromass) test results occurred in 153/161 (95%) cases. Using the Meretek UBT as the diagnostic standard, the urea blood test resulted in 44 true positive, 109 true negative, four false positive, and four false negative results, giving a sensitivity of 92%, specificity of 96%, positive predictive value of 92%, and negative predictive value of 96% (table 1).

The 30 minutes samples of both blood and breath test were more specific and sensitive than the 15

minutes. The testing for active *H. pylori* infection by a  $^{13}\text{C}$  urea blood test was 100% sensitive and specific at 30 minutes (table 2). The *H. pylori* positive patients were treated with triple antibiotic therapy for two weeks (amoxicillin 1gr PO BID, clarithromycin 500mg PO BID and omeprazole 20mg PO BID). Four weeks after finishing antibiotic therapy the patients were retested with UBT and  $^{13}\text{C}$  urea blood test. Eradication was achieved in 86% of patients according to the UBT (Meretek), and in 82% according to the  $^{13}\text{C}$  urea blood test. In this scenario the UBT (Meretek) was used as the diagnostic gold standard. Agreement between UBT (Meretek) and  $^{13}\text{C}$  urea blood test occurred in 95.3% (41 of 43 eradicated patients). There were no false positives by the  $^{13}\text{C}$  urea blood test.

**Table 1.** Comparison of  $^{13}\text{C}$  urea blood test with UBT (Meretek) and CLO test for the diagnosis and eradication of *H. pylori* infection

Reference Test	Sens	Spec	PPV	NPV
Diagnosis (vs CLO)	98%	100%	100%	98%
Diagnosis (vs UBT)	92%	96%	92%	96%
Eradication (vs UBT)	95%	100%	100%	96%

CLO-"Campylobacter-like organism" (rapid urease test)  
 PPV-positive predictive value  
 NPV-negative predictive value  
 UBT- urea breath test

**Table 2.** Comparison of  $^{13}\text{C}$  urea blood test and the  $^{13}\text{C}$  urea breath test (Meretek) for the diagnosis of *H. pylori* infection

	15 minutes		30 minutes	
	Blood Breath	Blood Breath	Blood Breath	Blood Breath
Sensitivity	0.875	0.875	1.000	0.981
Specificity	0.951	0.974	1.000	1.000

Reference standard for this comparison: CLO test

## Discussion

In the present study we investigated the feasibility, sensitivity and specificity of blood  $^{13}\text{C}$  measurement in determining *H. pylori* infection before and after eradication with antibiotics. This study shows that the  $^{13}\text{C}$  urea blood test is comparable to rapid urease test (CLO) and a validated and approved  $^{13}\text{C}$  urea breath test (Meretek) for the detection of

*H. pylori* infection. The testing for active *H. pylori* infection by the  $^{13}\text{C}$  urea blood test was both highly sensitive and specific. At present time, the diagnosis of *H. pylori* infection relies on direct or indirect demonstration of the organism on biopsies (CLO, culture and histology) obtained at the time of endoscopy, antibody measurement in blood, serum or urine (IgA or IGG), UBT or stool antigen.<sup>1,7-14</sup> Antibody measurement is sensitive and specific, but this method does not confirm active infection. One of the major disadvantages of serology is that it does not reliably correlate with eradication, because antibody titers against *H. pylori* tend to fall slowly over several months after successful antibiotic eradication, and do not always become negative.<sup>9</sup> Therefore, the three major diagnostics tests to confirm active *H. pylori* are endoscopy, stool antigen test and UBT.<sup>8-10,14</sup> Endoscopy is expensive and invasive. Stool antigen detection of *H. pylori* is a very sensitive method to diagnose active infection as well as to evaluate eradication of the organism, but collection and handling of samples can be cumbersome.<sup>11</sup> The  $^{13}\text{C}$  urea breath test is a safe and effective way of detecting *H. pylori* infection.<sup>8,10</sup> A major limitation of UBT is that it requires specialized collection and transportation equipment.<sup>8,10</sup> In addition, some individuals may have difficulty performing the exhalation component of the test, occasionally due to advanced or very young age, pulmonary chronic or upper airway diseases, or mental or physical compromise.<sup>13</sup> To date very few validation studies using  $^{13}\text{C}$ -blood test for the diagnosis of *H. pylori* have been performed.<sup>13</sup> Most previous studies evaluating urea blood test were limited by the small number of patients.<sup>12,13</sup> One of the advantages of our study was it study had large number of patients with and without *H. pylori* infection, giving the data more statistical power. Another important aspect of our study is that we have demonstrated that the  $^{13}\text{C}$  urea blood test is feasible and that it is a useful method to diagnose *H. pylori*, being equivalent to the UBT or CLO test to detect *H. pylori* infection.<sup>13,14</sup> In addition, the  $^{13}\text{C}$  urea blood test was shown to be a useful test to document *H. pylori* eradication.

Our study has several potential limitations. First, the gold standards for defining *H. pylori* infection were a positive urease test and positive UBT. The use of three tests to truly determine *H. pylori* positivity might have been more exact, but we believe that the CLO urease test and UBT have a high positive

predictive value for detecting active *H. pylori* infection.<sup>11,14</sup> Second, we used a spectrometer that is not widely available in all gastroenterology units. Nonetheless, other authors using similar spectrometers have demonstrated its accuracy for the detection of active *H. pylori* infection.<sup>12,13</sup>

In summary, this study shows that the  $^{13}\text{C}$  urea blood test is an appropriate method to diagnose *H. pylori* infection. Performance characteristics of this  $^{13}\text{C}$  urea blood test were as good as invasive (urease, CLO) and non-invasive ( $^{13}\text{C}$  urea breath) tests. Blood  $^{13}\text{C}$  analysis is a simple and noninvasive method for sensitive diagnosis and assessment of eradication of *H. pylori* infection at the fraction of the cost of endoscopy and biopsy with histology and culture. This method can be added to the armamentarium for the diagnosis and evaluation of eradication of *H. pylori* infection.

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