

Simple and accurate ^{13}C -urea breath test for detection of *Helicobacter pylori* in the remnant stomach after surgery

AKIYOSHI TOGASHI¹, NORIO MATSUKURA¹, SHUNJI KATO¹, GOTARO MASUDA¹, KEIICHI OHKAWA¹, AKIRA TOKUNAGA¹, NOBUTAKA YAMADA², and TAKASHI TAJIRI¹

¹Surgery for Organ Function and Biological Regulation, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan

²Integrated Pathology, Nippon Medical School, Tokyo, Japan

Background. The efficacy of the ^{13}C -urea breath test (UBT) for diagnosis of *Helicobacter pylori* (*H. pylori*) infection in the remnant stomach after surgery is a matter of controversy. We report a simple and accurate method of ^{13}C -UBT for diagnosis of *H. pylori* infection in the remnant stomach after gastrectomy. **Methods.** Eighty patients who had undergone gastrectomy with or without subsequent *H. pylori* eradication therapy were examined a total of 134 times for *H. pylori* infection by the ^{13}C -UBT. ^{13}C -Urea, 100 mg per test, was used in powdered form or in the form of film-coated tablets. Breath samples were collected before and 10, 20, and 30 min after ingestion. Mucosal biopsy specimens for bacterial culture and histological examination of the remnant stomach were collected endoscopically after each ^{13}C -UBT test. **Results.** Factors that confounded the ^{13}C -UBT results in the remnant stomach were oral bacteria, posture, and residual food. Lying horizontally on the left side was the best position, and film-coated tablets indicated no necessity for use of mouthwash. The method of anastomosis had no significant effect on the results of the ^{13}C -UBT. Thirty minutes and a cutoff of 4.5‰ were optimal conditions for detection of *H. pylori* in the remnant stomach. Under these conditions, sensitivity, specificity, and accuracy were 79.4% (27/34 cases), 95.7% (44/46 cases), and 88.8% (71/80 cases), respectively, in ordinary *H. pylori* diagnosis, and 100% (2/2 cases), 93.3% (14/15 cases), and 94.1% (16/17 cases), respectively, in evaluating eradication at 4 weeks after treatment of *H. pylori*. **Conclusions.** Having the patient lie horizontally on the left side, using a film-coated ^{13}C -urea tablet without using mouthwash, and measurement at 30 min provided a simple and accurate

method of ^{13}C -urea breath test for detection of *H. pylori* in the remnant stomach after gastrectomy.

Key words: ^{13}C -urea breath test, *Helicobacter pylori*, gastrectomy, eradication therapy

Introduction

Bile reflux and *Helicobacter pylori* infection may be the main causative factors of remnant-stump cancer after gastrectomy.^{1–3} The cancers in the remnant stomach after surgery for early gastric cancer are metachronous multiple cancers, and *H. pylori* infection is a candidate carcinogen, because *H. pylori* infection is associated with chronic active gastritis in the remnant stomach.^{4,5} The Maastricht Consensus Report strongly recommends *H. pylori* eradication therapy following early resection for gastric cancer,⁶ and our study showed that *H. pylori* eradication therapy markedly reduced inflammatory cell infiltration of the mucosa of the remnant stomach.⁷ We reported bacterial culture had the highest diagnostic value in the remnant stomach as well as in the unoperated stomach as an invasive method.⁸

The ^{13}C -urea breath test (UBT) is a reliable noninvasive method for detection of *H. pylori* in the intact stomach, especially for the detection of *H. pylori* after eradication therapy.^{9,10} However, there have been conflicting reports about the efficacy of ^{13}C -UBT for detection of *H. pylori* in the remnant stomach after gastrectomy.^{11–15} Many factors may influence the results of the ^{13}C -UBT test after gastrectomy. In this study we assessed factors that might influence the results of the ^{13}C -UBT: oral bacteria, posture, method of anastomosis, and residual food, in an attempt to establish a simple and accurate method for *H. pylori* diagnosis in the remnant stomach after surgery.

Materials and methods

Subjects

A total of 80 consecutive Japanese patients with remnant stomach after gastrectomy who attended the clinic of the First Department of Surgery of Nippon Medical School in Tokyo were enrolled in the study. The protocol was approved by the local ethics committees, and written informed consent was obtained from every subject. There were 55 men and 25 women, ranging in age from 47 to 86 years (mean, 68.1 years). Surgery had been performed for gastric cancer in 67 cases, peptic ulcer in 11 cases, and for leiomyoma in the other 2 cases. The interval after the operation ranged from 0.2 to 38.1 years (mean, 6.52 years). The surgical procedure was distal gastrectomy with Billroth I anastomosis in 55 cases, Billroth II in 13 cases, and Roux-en-Y in 2 cases. Pylorus-preserving gastrectomy (PPG) was performed in 2 cases and partial gastrectomy in 4 cases. Proximal gastrectomy was performed with jejunal pouch interposition in 5 cases and without interposition in 1 case. A total of 134 ^{13}C -UBTs were performed (1 to 4 times per patient; mean, 1.7 times) in the 80 patients, and they were performed 23 times to assess the results of eradication therapy.

^{13}C -Urea breath test

After an overnight fast, the ^{13}C -UBT was performed before an upper abdominal endoscopic examination. Three dosage forms containing 100 mg ^{13}C -urea were used: powder (UBIT; Otsuka Pharmaceutical, Osaka, Japan) and film-coated tablets (UBIT tablet; Otsuka Pharmaceutical). The standard protocol for powdered ^{13}C -UBT (UBIT) was (1) collection of a breath sample, (2) ingestion of ^{13}C -urea dissolved in 100 ml tap water, and two uses of mouthwash, (3) the subject then assuming a horizontal position lying on the left side until the end of the examination, and (4) breath samples obtained 10, 20, and 30 min after ingestion of the powder. The vertical and sitting postures were used in selected subjects for comparison. The standard protocol for ^{13}C -UBT tablets (UBIT) was (1) collection of a breath sample, (2) assumption of a horizontal posture lying on the left side by the subject until the end of the examination, (3) ingestion of the UBIT tablet with 100 ml tap water, and (4) breath samples obtained 10, 20, and 30 min after ingestion.

Gas samples collected separately were analyzed to determine the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio ($\Delta^{13}\text{CO}_2\%$) with an isotope mass spectrometer (MAT model Delta E; Finnegan, Germany), and the analysis was performed by Mitubishi-kagaku BCL (Tokyo, Japan). No test meals or citrate were used in this study. The results are

expressed as per thousand (‰), i.e., the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in the sample minus the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in the standard.

Histopathological examination and culture tests

Routine endoscopic examination of the esophagus, remnant stomach, and anastomosis region was performed with a gastrointestinal fiberscope (GIF Type Q-240; Olympus, Tokyo, Japan). Mucosal biopsy specimens of the remnant stomach for histological examination and culture were collected endoscopically with sterilized biopsy forceps (Olympus) from the greater curvature of the stoma and greater curvature of the upper corpus. Biopsy specimens of the remnant stomach after cardioresction for cardia cancer were collected from the greater curvature of the lower antrum and greater curvature of the lower corpus. Hematoxylin and eosin (H&E) staining and improved toluidine blue staining were used for histological diagnosis of *H. pylori* infection according to the updated Sydney System.¹⁶ *H. pylori*-specific antibody (Ab) immunostaining (Dako, Glostrup, Denmark) was used for histological diagnosis of *H. pylori* infection in selected cases. All biopsy specimens were examined by a pathologist (N.Y.) who was unaware of the results of the bacterial culture or the treatment regimen.¹⁷

Biopsy specimens for culture of *H. pylori* were immediately inoculated onto HP agar under a microaerophilic atmosphere (seed tube HP; Eiken Chemical, Tokyo, Japan). On the same day they were transferred to culture medium in Skirrow plates and incubated at 35°C under an atmosphere of 5% O_2 , 10% CO_2 , and 85% N_2 for 3–7 days. Cultures were judged to be positive for *H. pylori* based on colony morphology, the results of Gram staining, and positive oxidase, catalase, and urease activity. The cultures were performed at Mitsubishi Kagaku BCL (Tokyo, Japan).

H. pylori eradication therapy

The study design for PPI-based double and triple therapy has been reported previously.⁷ Briefly, dual therapy consisted of lansoprazole (Takeda Chemical Industries, Osaka, Japan) 30 mg bid and amoxicillin (AMPC) 500 mg tid plus a mucosal protective agent, ecabet sodium (Tanabe Seiyaku, Osaka, Japan) 1.0 g bid, for 2 weeks, and triple therapy consisted of lansoprazole 30 mg bid, AMPC 750 mg bid, and clarithromycin (CAM) 400 mg bid for 1 week.

Definition of *H. pylori* status

Infection was considered to be present when the culture was positive and negative when the culture and histo-

Table 1. Effect of posture, drug form, reconstruction method, and eradication on the diagnostic efficacy of the ^{13}C -UBT for *H. pylori* in the remnant stomach for 30 min^a

	<i>n</i>	Sensitivity	Specificity	Accuracy
Posture ^b				
Horizontal & left side	97	80.6% (29/36)	95.1% (58/61)	89.7% (87/97)
Supine or sitting	14	75.0% (3/4)	60.0% (6/10)	64.3% (9/14)
<i>P</i> value		NS	<0.01	<0.05
Drug form ^c				
Powder with mouthwashing	69	84.4% (27/32)	94.6% (35/37)	89.9% (62/69)
Film-coated tablet without mouthwashing	43	84.6% (11/13)	96.7% (29/30)	93.0% (40/43)
<i>P</i> value		NS	NS	NS
Reconstruction method ^d				
Billroth I	76	84.6% (22/26)	96.0% (48/50)	92.1% (70/76)
Billroth II	13	80.0% (4/5)	100% (8/8)	92.3% (12/13)
Roux-en-Y	6	66.7% (2/3)	100% (3/3)	83.3% (5/6)
<i>P</i> value		NS	NS	NS
Aim				
Ordinary <i>H. pylori</i> diagnosis ^e	80	79.4% (27/34)	95.7% (44/46)	88.8% (71/80)
Evaluating eradication ^f	17	100% (2/2)	93.3% (14/15)	94.1% (16/17)
<i>P</i> value		NS	NS	NS

^aCutoff value of 5.5‰ for the powder form and 4.5‰ for the film-coated tablet

^bResidual food cases excluded

^cSupine, sitting position, and residual food excluded

^dSupine and sitting position excluded

^eSupine and sitting position and residual food excluded. Without eradication or more than 6 months after eradication

^fSupine and sitting position and residual food excluded. Used for assessment of the results 4 weeks after eradication therapy

logical findings were both negative. If the culture was negative but the histological findings were positive, the case was classified “unevaluable” and was excluded.¹⁸

Statistical analysis

Statistical analyses were performed by the χ^2 analysis program for PCs (SPSS, Tokyo, Japan). A *P* value less than 0.05 was considered significant.

Results

*Effect of posture, oral bacteria, residual food, and surgical reconstruction method on the diagnostic efficacy of the ^{13}C -UBT *H. pylori* to assess the results of eradication therapy in the remnant stomach*

Factors that may confound the results of the ^{13}C -UBT are (1) oral bacteria, (2) posture, (3) residual food, and (4) method of anastomosis. Mouthwashing is essential for the ^{13}C -UBT examinations with the powder (UBIT), but mouthwashes are not required with the film-coated tablet form (UBIT tablet). The ^{13}C -urea passes through the residual stomach into the duodenum or small intestine faster without reserve function of the intact stomach. When the film-coated ^{13}C -urea tablet, was used, on the other hand, the patient lay horizontally on the left side throughout the examination, because no mouthwash was needed. Residual food in the remnant stomach also interfered with ^{13}C -UBT accuracy, and the

results of the ^{13}C -UBT were greater than 10‰ at 20 and 30 min in the *H. pylori*-negative cases (data not shown). The results of the ^{13}C -UBT at 30 min were significantly different according to posture, and horizontal posture on the left side showed significantly better specificity ($P < 0.01$) and accuracy ($P < 0.05$) than the supine or sitting position. However, ^{13}C -UBT results were not significantly different between powder form and film-coated tablet and among reconstruction methods. Thirty minutes and a cutoff of 4.5‰ were optimal conditions for detection of *H. pylori* in the remnant stomach ^{13}C -UBT with a film-coated ^{13}C -urea tablet and the patient lying horizontally on the left side. Under these conditions, sensitivity, specificity, and accuracy were 79.4% (27/34 cases), 95.7% (44/46 cases), and 88.8% (71/80 cases), respectively, in ordinary *H. pylori* diagnosis and 100% (2/2 cases), 93.3% (14/15 cases), and 94.1% (16/17 cases), respectively, in evaluating eradication at 4 weeks after treatment of *H. pylori*. There was no significant difference in ^{13}C -UBT results according evaluating eradication 4 weeks after *H. pylori* treatment or ordinary *H. pylori* diagnosis (Table 1).

Effect of drug forms on ^{13}C -UBT diagnostic accuracy in the remnant stomach: powder and film-coated tablet

With the powder form of ^{13}C -urea (UBIT), maximum accuracy was obtained under the following conditions: (1) UBIT ingested with 100ml water in the vertical position, (2) two mouthwashings, and (3) a quick

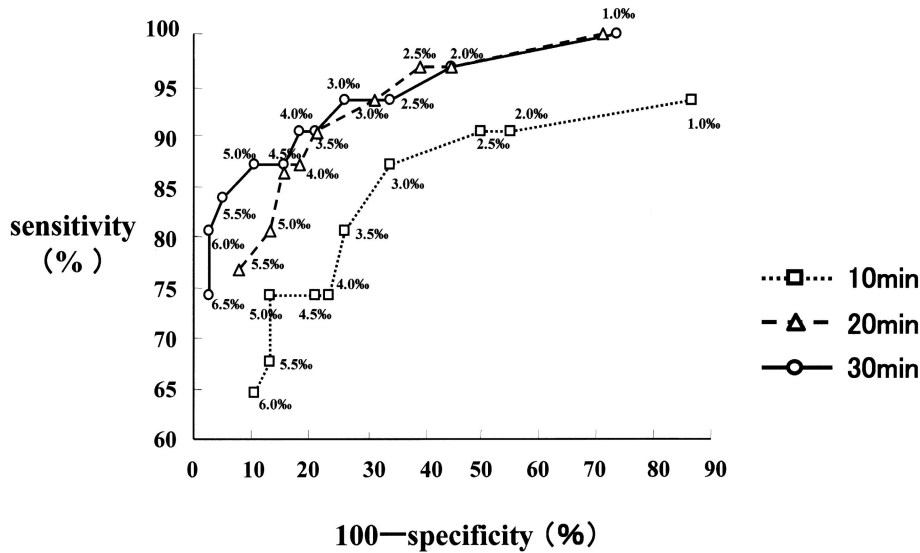


Fig. 1. Receiver operating characteristic (ROC) curves with powder form ¹³C-urea (UBIT) at 30 min (*n* = 69). The most appropriate cutoff value for diagnosis of *Helicobacter pylori* infection was identified at 5.5‰, showing sensitivity 83.9% and specificity 94.7%

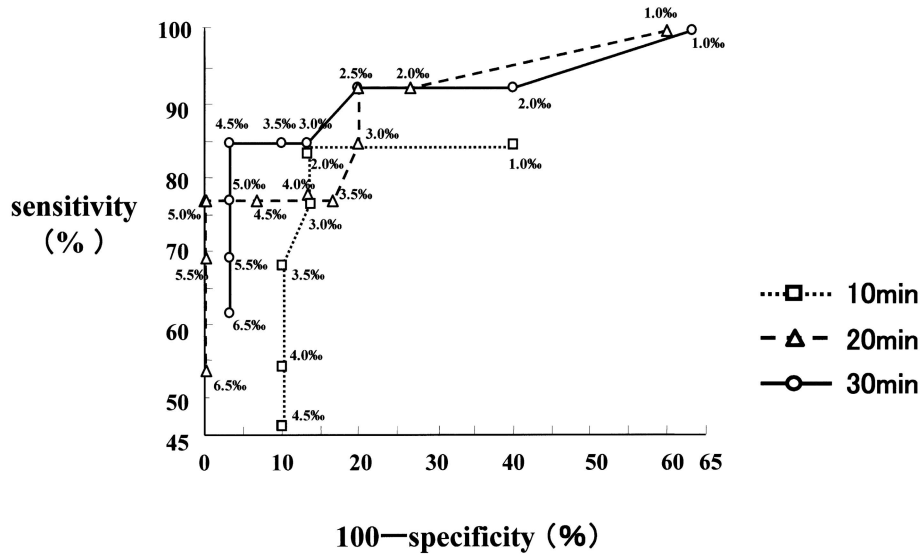


Fig. 2. ROC curves with film-coated tablet form ¹³C-urea (UBIT tablet) at 30 min (*n* = 43). The most appropriate cutoff value for diagnosis of *H. pylori* infection was identified at 4.5‰, showing sensitivity 84.6% and specificity 96.7%

change to the horizontal position on the left side until collection of the breath sample at 30min. The receiver operating characteristic (ROC) curve, which included ordinary *H. pylori* diagnosis and evaluating *H. pylori* eradication,¹⁹ showed that the highest diagnostic value with the powder form was obtained at 30min and a cutoff point of 5.5‰, at which point sensitivity was 83.9%, specificity was 94.7%, and accuracy was 89.9% (Fig. 1). With the film-coated tablet (UBIT tablet), (1) the tablet was ingested in the horizontal position on the left side with 100ml water, and (2) the horizontal position on the left side without mouthwashing was maintained until collection of the breath sample at 30min. According to the ROC curve, the highest diagnostic value for the remnant stomach with the film-coated tablet was obtained at a cutoff point of 4.5‰, at

which sensitivity was 84.6%, specificity was 96.7%, and accuracy was 93.0% (Fig. 2).

Discussion

The ¹³C-UBT is an excellent and noninvasive method for diagnosis of *H. pylori* infection in the intact stomach,^{9,10} and it is especially useful for judging the results of eradication therapy.¹⁸ However, whether ¹³C-UBT is useful for the diagnosis of *H. pylori* infection in the remnant stomach after surgery has been a matter of controversy. As shown in Table 2, several reports have shown that the ¹³C-UBT is not as accurate as histological examination and culture in detecting *H. pylori* after gastrectomy,^{12,13} but a few papers have reported excel-

Table 2. The maximum diagnostic efficacy of ¹³C-UBT in gastrectomy patients in the literature

Author	No.	Operation	Dose of ¹³ C-urea		Test Meal		Mouthwash	Position	Cutoff point (%)	Time (min)	Analysis	Standard	Sensitivity	Specificity	Useful	Reference
			¹³ C-urea	Meal												
Lotterer et al.	41	BI, R-Y	75	Yes	Yes	Left side	>18	30	Mass	Culture, smear, RUT	100	82	Yes	11		
Sheu et al.	86		100	Yes	Yes	Supine	2.5	15	Mass	Hist, culture	82.2	87.8	No	12		
Schilling et al.	68	BI, BI	75	Yes	No	Sitting	4	30	Mass	Histo, RUT	52	93	No	13		
Kubota et al.	42	PPG, BI, etc.	100	No	Yes	Left side	2	40	Infrared	Culture, histo, RUT	96.3	100	Yes	14		
Kubota et al.	65	PPG, BI, etc.	100	No	Yes	Left side	2	30	Infrared	Culture, RUT	95.7	93.3	Yes	15		
In this study																
With powder	72	BI, BI, etc.	100	No	Yes	Left side	5.5	30	Mass	Culture, hist	84.4	94.6	Yes			
With tablet	45	BI, BI, etc.	100	No	No	Left side	4.5	30	Mass	Culture, hist	84.6	96.7	Yes			

lent results.^{11,14,15} Several factors in the remnant stomach after surgery may affect the results of the ¹³C-UBT. Because ¹³C-urea flows from the small residual stomach into the duodenum (Billroth I anastomosis) or small intestine (Billroth II or Roux-en-Y anastomosis) more easily after surgery, the sitting or supine position was inadequate. Our results showed that the horizontal position on the left side was the best position until the end of the examination, as stated in Kubota’s report.^{14,15}

Mouthwashing was essential to prevent contamination by oral bacteria. Without use of mouthwash, the false-positive effect of oral bacteria possessing urease activity in this study persisted until 30min. However, there was concern that some fraction of the ¹³C-urea might pass through the residual stomach while the patient was standing to wash the mouth and during the change in position from standing to lying horizontal on the left side. We therefore compared use of the powder form of ¹³C-urea (UBIT) with mouthwash and the film-coated tablet form of ¹³C-urea (UBIT tablet) without using mouthwash. The film-coated form was adopted to prevent the effect of oral bacteria in the absence of mouthwashing. In the intact stomach, the powder form and film-coated tablets have been reported to yield similar sensitivity (98.2% vs. 98.4%), specificity (97.9% vs. 98.4%), and accuracy (98.1% vs. 98.0%), with a cut-off of 2.5‰ at 20min (from the drug package insert). The results in the remnant stomach after surgery showed that the tablet form yielded slightly higher sensitivity (84.6%), specificity (96.7%), and accuracy (93.0%) than the powder form (sensitivity 84.4%, specificity 94.6%, and accuracy 89.9%) at 30min, but the differences were not significant. Therefore, the advantage of the film-coated tablet for detection in the residual stomach is the lack of need for a quick change in position after ingestion of the ¹³C-urea.

Whether ¹³C-UBT is useful for evaluating eradication of *H. pylori* in the remnant stomach is an important question, and based on our results the answer is “yes.” Several studies on the results of ¹³C-UBT for detection of *H. pylori* in the remnant stomach have shown good specificity but low sensitivity,¹³ although sensitivity can be improved by using a different cutoff point. Our results also showed lower sensitivity and equal specificity in comparison with the intact stomach. No test meals were used in this study, because test meals are not necessary when UBIT or UBIT tablets are used for the ¹³C-UBT in subjects with an intact stomach. Because residual food cannot be detected without an endoscopic examination, subjects found to have residual food in a remnant stomach in a previous endoscopic examination should be excluded from the ¹³C-UBT.

In conclusion, our study revealed simple and optimal conditions for performing the ¹³C-UBT for detection of *H. pylori* in the remnant stomach after surgery: (1) use

of the 100-mg film-coated tablet form of ^{13}C -urea (UBIT tablet), (2) use of the horizontal position lying on the left side throughout the examination without mouthwashing, and (3) breath sampling before and 30 min after ingestion of the tablet. The most appropriate cutoff value was found to be 4.5%.

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