Posttreatment ^{13}C-Urea Breath Test Is Predictive of Antimicrobial Resistance to *H. pylori* After Failed Therapy

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OBJECTIVE: We tested whether a ^{13}C-urea breath test can predict antimicrobial resistance of *Helicobacter pylori* (*H. pylori*).

METHODS: Seventy patients who had failed triple eradication therapy and 108 untreated *H. pylori*-infected patients were given a ^{13}C-urea breath test, endoscopy for culture of *H. pylori*, and assessment of clarithromycin resistance. The patients who had failed triple therapy then received 1 week of quadruple therapy to eradicate residual *H. pylori*.

RESULTS: The posttreatment value of the ^{13}C-urea breath test expressed as excessive $\delta^{13}CO_2$ per ml (ECR) was higher in patients with residual *H. pylori* with clarithromycin resistance than in those without (23.8 vs 10.6; $P<.0001$). With a cutoff of ECR $>$ or ≤ 15, the ^{13}C-urea breath test was 88.6% sensitive and 88.9% specific in predicting clarithromycin resistance of residual *H. pylori*. The *H. pylori* eradication rate of the rescue regimen was higher for patients with a posttreatment ECR of the ^{13}C-urea breath test ≤ 15 than for those with a value >15 (93.8% vs 73.3%; $P<.05$). In contrast, in treatment-naive *H. pylori*-infected patients, the pretreatment value of the ^{13}C-urea breath test did not differ between patients infected with clarithromycin-resistant or-sensitive isolates ($P>.05$).

CONCLUSION: The posttreatment value of the ^{13}C-urea breath test is predictive of clarithromycin resistance in residual *H. pylori* after failed triple therapy and predicts efficacy of the rescue regimen. The value of the noninvasive test is promising for primary care physicians who need to select a rescue regimen without invasive *H. pylori* culture.

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Triple therapy, combining a proton pump inhibitor with two antibiotics, is the current standard of therapy for eradicating *Helicobacter pylori* (*H. pylori*).[1–5] Amoxicillin and clarithromycin plus a proton pump inhibitor is the first-line therapy recommended by the Maastricht-2 Consensus group,[1] despite there being *H. pylori* which are resistant to clarithromycin and amoxicillin. This first-line regimen has a 10% to 23% failure rate.[3–8] Evaluation of the effectiveness of the eradication regimen with a reliable tool is important. Except for patients with gastric ulcers at risk of gastric malignancy, the ^{13}C-urea breath test is confirmed as a reliable noninvasive test to assess the treatment outcome of triple therapy.[9–12]

A positive result on the ^{13}C-urea breath test confirms the failure of the anti-*H. pylori* eradication therapy. However, the posttreatment value of the ^{13}C-urea breath test can range widely from less than 10 excessive $\delta^{13}CO_2$ per ml (ECR) to nearly 40 to 50. The clinical significance of such a wide range of values for the posttreatment ^{13}C-urea breath test remains uncertain for patients with failed triple therapy. Because clarithromycin resistance of *H. pylori* is one of the major causes of the failure of triple therapy,[4,6,8] it is of clinical interest to test whether the posttreatment of the ^{13}C-urea breath test can be predictive of clarithromycin resistance of *H. pylori* in a noninvasive manner. If such a posttreatment value of the ^{13}C-urea breath test is highly predictive of clarithromycin resistance, it may eliminate the need for follow-up endoscopy in order to take *H. pylori* culture. Moreover, it may improve the selection of the rescue regimen in primary clinics where routine *H. pylori* culture is not available. As patients with failed triple therapy need an effective rescue regimen,[13–15] this study tests whether the posttreatment value of the ^{13}C-urea breath test can allow physicians to choose a rescue regimen without using an invasive biopsy method. As a secondary aim, we evaluated, in a second sample of patients, the value of the pretreatment ^{13}C-urea breath test results for predicting clarithromycin resistance.

MATERIALS AND METHODS

Patients and Study Design

Seventy dyspeptic patients, with an initial diagnosis of duodenal ulcer or gastritis only, were consecutively enrolled when 1 week of triple therapy (amoxicillin 1 g, clarithromycin 500 mg, omeprazole 20 mg, twice daily) had failed to eradicate *H. pylori*. The enrolled patients had at least 5 days of compliance in the previous 1 week of triple therapy. Failure of triple therapy was defined as a positive ^{13}C-urea breath test with a uniform protocol having an ECR value more than 2.5.[9] The protocol of our breath test for each patient was uniformly applied with 50 mg ^{13}C-labeled urea (INER-Hp^{13}C-tester, Institute of Nuclear Energy Research, Taoyuan County, Taiwan) to collect the baseline and 15-minute gas samples for analyzing the excessive $\delta^{13}CO_2$ per ml value by continuous flow isotope ratio mass spectrometry (Atomatic Breath Carbon Analyzer, Europa Scientific, Crewe, UK) as in our previous study.[12] The same breath test was performed for each patient during the fifth week after the cessation of triple therapy. Before each urea breath test, medications including proton pump inhibitors, bismuth salt, and antimicrobial agents were withheld for at least the 4 preceding weeks.

In addition to these 70 patients who had failed triple therapy, 108 patients with naive *H. pylori* infection were selected from our database for analysis as the pretreatment group. These 108 patients had no past history of anti-*H. pylori* therapy and were known to have refrained from exposure to antibiotics, bismuth salts, and proton pump inhibitors before

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endoscopy and the urea breath test. In the course of usual care, a specimen from each of these patients had demonstrated *H. pylori* and was tested for resistant organisms. The same protocol for the ^{13}C-urea breath test was used in these patients as had been used in the patients with failed triple therapy.

After obtaining informed consent, each of the 70 patients who had failed triple therapy had endoscopy for *H. pylori* culture as previously published.[15,16] The successfully collected *H. pylori* isolates were then checked for the presence of antimicrobial resistance, defined by the MIC level of an E-test.[15] Each patient had a gastric biopsy for histology to reconfirm *H. pylori* status, regardless of a positive ^{13}C-urea breath test. When negative results for both histology and culture were obtained during this follow-up endoscopy, patients were excluded from rescue therapy. All included patients received 1 week of rescue quadruple therapy, including amoxicillin 1 g twice daily, metronidazole 500 mg twice daily, omeprazole 20 mg twice daily, with bismuth subcitrate 120 mg thrice daily. The drug compliance and side effects of rescue therapy were recorded at the next week's visit. The degree of drug compliance was categorized as "good" (the 7-day quadruple therapy was completely ingested), "modest" (ingested at least 5 days), and "poor" (ingested less than 5 days) as used before.[15] A little more than 6 weeks after the rescue regimen, the ^{13}C-urea breath test was repeated to check for *H. pylori* eradication. For those with a negative result for the ^{13}C-urea breath test after quadruple therapy, a repeat ^{13}C-urea breath test was done during the third month to prevent a false negative result. Thus, both negative results on the ^{13}C-urea breath test during the sixth week and third month were needed to define successful *H. pylori* eradication by rescue therapy. The proton pump inhibitors and antibiotics were withheld until the follow-up test. Patients known to be allergic to bismuth or metronidazole were not included. Those patients with gastric malignancy were also excluded.

Endoscopy to Take a Gastric Biopsy for *H. pylori* Culture and Histology

For each patient, two pairs of gastric biopsy samples (each pair included one from the antrum and the other from the lower body) were obtained during endoscopy. Each pair of biopsies was sent for *H. pylori* culture and histological staining with hematoxylin and eosin.[15] In cases where gastric ulceration was found during follow-up endoscopy, these cases were excluded. These two bits of gastric biopsy were taken for *H. pylori* culture, as done in our previous study.[15,16] For each *H. pylori* isolate therapy, we analyzed the clarithromycin resistance by an E-test, defined by an MIC >1 μg/ml for clarithromycin resistance.[15] Furthermore, we checked for metronidazole resistance and amoxicillin resistance by an E-test, defined by an MIC >1 and >8 μg/ml, respectively.[15]

Statistics

The Student's t test, Pearson's χ^2 test, and Fisher's exact test were used to determine the parametric difference and nonparametric proportions between the 2 study groups. All tests of significance were two-tailed with a P value less than .05. Data from all of the enrolled patients for rescue therapy were analyzed using intention-to-treat analyses. Patients who stopped medication or had poor drug compliance, or were lost to follow-up after rescue therapy, were excluded from the per-protocol analysis.

RESULTS

Efficacy of the ^{13}C-Urea Breath Test to Predict Clarithromycin Resistance of *H. pylori*

There were 53 *H. pylori* isolates obtained from the 70 patients who had failed triple therapy. Clarithromycin resistance was found in nearly 66% (35/53) of the *H. pylori* isolates collected after triple therapy. None of these 53 isolates was proven to have amoxicillin resistance. The mean posttreatment ECR of the ^{13}C-urea breath test performed after triple therapy was higher in patients with residual *H. pylori* having clarithromycin resistance than in those without (23.8 vs 10.6; $P<.0001$). In Table 1, we list the test characteristics of the ^{13}C-urea breath test at several cutoffs for predicting clarithromycin resistance after failed triple therapy. Setting the posttreatment ECR at >15 yielded a sensitivity of 88.6% (95% confidence interval [CI], 78.1 to 99.1) and a specificity of 88.9% (95% CI, 71 to 100) for predicting clarithromycin resistance in residual *H. pylori*. Furthermore, in our sample, this cutoff point resulted in a positive predictive value of 93.9% (95% CI, 83.6 to 100).

There were no differences in the demographic features, endoscopic diagnoses, side effects, and compliance with rescue therapy between patients with a posttreatment ECR of ^{13}C-urea breath test ≤ 15 and >15 (Table 2). The distribution of metronidazole resistance was also not different

Table 1. Diagnostic Efficacy of the Posttreatment Value of Urea Breath Test to Predict the Clarithromycin Resistance of *H. pylori* After Failed Triple Therapy and Before Treatment

Cutoff Point of Urea Breath Test	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
After failed triple therapy				
>10	94.2 (84.6 to 100)	50 (39.2 to 61.8)	78.6 (66.2 to 91.4)	81.8 (54.4 to 100)
>15	88.6 (78.1 to 99.1)	88.9 (71 to 100)	93.9 (83.6 to 100)	80 (62.5 to 97.5)
>20	65.7 (47.7 to 80.9)	100 (81 to 100)	100 (85 to 100)	60 (42.5 to 77.5)
Pretreatment				
>10	100 (63 to 100)	5 (0.7 to 9.3)	7.8 (2.6 to 13)	100 (48 to 100)
>15	100 (63 to 100)	10 (4.1 to 15.9)	8.3 (2.8 to 13.8)	100 (69 to 100)
>20	87.5 (54.2 to 100)	31 (22 to 40)	9.2 (2.7 to 15.7)	96.9 (88 to 100)
>25	75 (40 to 100)	60 (50.4 to 69.6)	14.3 (3.7 to 24.9)	90.9 (84 to 97.6)
>30	37.5 (4 to 71)	85 (78 to 92)	16.7 (0 to 34.4)	85 (78 to 92)

Pretreatment value indicates the value of urea breath test collected before triple therapy. Posttreatment value indicates the value of urea breath test collected after the failed triple therapy.
CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 2. Demographic Characteristics, Side Effects, and Drug Compliance in Two Groups Divided by a Cutoff Point of 15 of the Posttreatment Value of the Urea Breath Test After Failed Triple Therapy

Variables	Posttreatment Value ≤ 15 (n = 36)	Posttreatment Value > 15 (n = 34)	P Value
Mean age, y	44.4	43.5	NS
Female, % (*n*)	50 (18)	50 (17)	NS
Endoscopic findings, (*n*)			
DU:NUD	25:11	24:10	NS
Compliance with triple therapy, % (*n*)			
Good (7-day)	55.6 (20)	55.9 (19)	NS
Modest (>5 &<7 days)	33.3 (12)	32.3 (11)	NS
Poor (<5 days) & dropout	11.1 (4)	11.8 (4)	NS
Side effects, *n*			
Nausea/vomiting	5	5	NS
Constipation	5	6	NS
Diarrhea	6	6	NS
Metallic taste	6	9	NS
H. pylori isolates,* % (*n*)			
Metronidazole Resistance (MR)	45 (9)	42.4 (14)	NS
Clarithromycin Resistance (CR)	20 (4)	93.9 (31)	<.05
Both MR & CR	10 (2)	39.3 (13)	<.05

**Fifty-three* H. pylori *isolates (20 from patients with a posttreatment value less than 15/ml, 33 from patients with a value more than 15/ml) collected before rescue therapy.*
DU, duodenal ulcer; NUD, nonulcer gastritis.

between the 2 study groups ($P>.05$; Table 2). The prevalence of clarithromycin resistance was higher in the patients with a posttreatment ECR of ^{13}C-urea breath test >15 than in those with ECR ≤15 ($P<.05$). The prevalence of residual *H. pylori* with concurrent clarithromycin resistance and metronidazole resistance was higher in patients with a posttreatment ECR of ^{13}C-urea breath test >15 than in those with ECR≤15 ($P<.05$).

Among the 108 patients who had not yet received treatment, there were 8 patients infected by *H. pylori* with culture-proven clarithromycin resistance. The mean pretreatment ECR of the ^{13}C-urea breath test was not significantly different between the patients infected by *H. pylori* with and without primary clarithromycin resistance (28.9 vs 23.8; $P>.05$). Moreover, as shown in Table 1, there was no cutoff value of ECR to make the ^{13}C-urea breath test very sensitive or specific for predicting clarithromycin resistance.

Eradication Rates of Quadruple Therapy to Residual *H. pylori* After Triple Therapy

All of the 70 patients with histology that supported residual *H. pylori* infection received 1 week of quadruple therapy. Excluded from the per-protocol analysis were 4 patients with a posttreatment ECR of ^{13}C-urea breath test ≤15 (3 dropped out of follow-up, and 1 showed poor drug compliance) and 4 patients with ECR >15 (2 dropped out of follow-up, and 2 showed poor drug compliance). Accordingly, 62 patients completed the rescue therapy protocol. By per-protocol analysis, the patients with a posttreatment ECR of ^{13}C-urea breath test ≤15 had a significantly higher *H. pylori* eradication rate than that of patients with ECR value >15 (93.8% vs 73.3%; $P<.05$; Table 3).

DISCUSSION

In this study, the mean posttreatment value of the ^{13}C-urea breath test after triple therapy was higher in the patients with residual *H. pylori* having clarithromycin resistance than in those without. These data indicate that a higher posttreatment value for the ^{13}C-urea breath test may be useful in diagnosing clarithromycin resistance in residual *H. pylori* after failed triple therapy, which had included clarithromycin. Setting the ECR value of the ^{13}C-urea breath test at >15 after triple therapy had a sensitivity of 88.6% and specificity of 88.9% for predicting the presence of clarithromycin resistance in residual *H. pylori*. This suggests that the posttreatment value of the ^{13}C-urea breath test may not only indicate the presence of residual *H. pylori*, but may predict the existence of clarithromycin resistance in these residual bacteria. From a clinical standpoint, this use of a noninvasive test may be very valuable, especially for primary care physicians who need to select rescue regimens without use of endoscopy and culture. For example, if patients have a posttreatment ECR of the ^{13}C-urea breath test<15, suggesting a low prevalence of clarithromycin resistance, triple therapy with clarithromycin might be repeated.

The reason why residual *H. pylori* with clarithromycin resistance has a higher posttreatment value for the ^{13}C-urea breath test remains uncertain. The posttreatment value of the ^{13}C-urea breath test can be correlated with the urease activity of *H. pylori*, and thus may be correlated with the bacterial loads of the stomach.[9] We speculate that there is a higher residual *H. pylori* load due to the poor bacteriocidal effect of clarithromycin on isolates with clarithromycin resistance. It is of research interest to determine whether *H. pylori* isolates with clarithromycin resistance have higher urease activity or any special urease genomic features.

In contrast to the posttreatment value after failed triple therapy, the pretreatment value of the ^{13}C-urea breath test was not significantly different between patients infected with ($n=100$) and without ($n=8$) primary clarithromycin-resistant *H. pylori* isolates. Before treatment, patients may have abundant bacterial colonization dampening the difference in urease activity between clarithromycin-resistant and-sensitive *H. pylori* infections.

In this study, only 53 out of 70 patients with histological evidence of infection had a positive *H. pylori* culture, which may be due to the low residual bacterial load after triple ther-

Table 3. Eradication Rates of Rescue Therapy for *H. pylori* Infection

Eradication Rate, % (*n*) (95% Confidence Interval)	Per-protocol* Analysis	Intention-to-treat Analysis
Patients with posttreatment value of urea breath test<&=15	93.8 (30/32) (83.2 to 100)	83.3 (30/36) (71.2 to 95.4)
Patients with posttreatment value of urea breath test>15	73.3 (22/30) (57.8 to 89.1)	64.7 (22/34) (48.7 to 80.7)

**This indicates that there was a significant difference of H. pylori eradication rates between the 2 study groups by both per-protocol analysis and intention-to-treat analysis ($P<.05$).*

apy. Thus, 24.3% (17/70) of patients after failed triple therapy could not be analyzed for antimicrobial resistance, despite the use of invasive endoscopy for culture. This demonstrates the poor sensitivity of culture of *H. pylori*, especially after therapy. Our study once again illustrates a hazard in clinical practice, and thus further supports the clinical demand for an addition tool to evaluate posttreatment antimicrobial resistance.

As quadruple therapy is the common rescue regimen for failed triple therapy,[13–15] it is clinically indicated to test whether the posttreatment value of the urea breath test is related to the outcome of rescue therapy. As shown in Table 3, 1-week quadruple therapy, serving as a rescue regimen, had a 93.8% eradication rate by per-protocol analysis for residual *H. pylori*, when the posttreatment ECR of the urea breath test was not more than 15, indicating a scanty bacteria load in the stomach. In contrast, for those with an ECR more than 15, the per-protocol eradication rate for residual *H. pylori* was significantly lower, suggesting that a more potent regimen or a longer duration of the same quadruple therapy should be selected for those patients with a higher posttreatment value after failed triple therapy. Accordingly, the posttreatment value of the urea breath test can provide promising clinical guidance in selecting an optimal regimen to eradicate residual *H. pylori*, especially in primary clinical service without routine availability of *H. pylori* culture.

In summary, the posttreatment value of the ^{13}C-urea breath test may be predictive of clarithromycin resistance of residual *H. pylori* after failed triple therapy, although these results need to be validated in other patient populations.

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