Short Communication

Applicability of a Monoclonal Antibody-Based Stool Antigen Test to Evaluate the Results of *Helicobacter pylori* Eradication Therapy

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(Received October 2, 2008. Accepted March 26, 2009)

SUMMARY: The 13C-urea breath test (UBT) is widely used to examine the results of eradication therapy for *Helicobacter pylori*. We examined whether a stool antigen test can be used as efficiently as UBT. (i) Ninety-four patients infected with *H. pylori* underwent eradication therapy. Six or eight weeks after the completion of treatment, the results were evaluated by UBT and a stool antigen test (TPAg). In 77 out of 78 patients who had negative UBT results, the TPAg results were also negative. Among the 16 UBT-positive patients, 12 were also positive by TPAg. Agreement of UBT and TPAg was 94.7%. (ii) Twenty-two patients with peptic ulcers in the active stage also received eradication therapy followed by proton pump inhibitor (PPI) administration. TPAg and UBT were performed to examine the results of eradication therapy at the end of PPI administration and at least 7 days after its discontinuation. Of the 22 patients taking PPIs, TPAg evaluated the results of eradication therapy accurately in 21 patients. TPAg appears to be an accurate test for evaluating the results of *H. pylori* eradication therapy, and to be as efficient as 13C-UBT. Use of the stool antigen test should be considered as an alternative, particularly in patients who have to take PPIs in order to avoid the risk of peptic ulcers.

Eradication of *Helicobacter pylori* infection has become increasingly important as the pathogenic role of this bacterium has been implicated in several gastro-duodenal and extra-digestive diseases. The 13C-urea breath test (UBT) has been considered to be the most reliable non-invasive test for the diagnosis of *H. pylori* infection, with an overall accuracy approaching 95% in both untreated and treated patients (1). UBT has been recommended in Japanese guidelines regarding the diagnosis of *H. pylori* infection and is now commonly used to test the results of eradication therapy in Japan (2). UBT does, however, have several limitations. The current cost of UBT is relatively high, and medical staff must be employed to keep patients under observation during the procedure. In addition, patients are required to fast prior to testing. UBT detects urease activity in the gastric mucosa, and this activity of *H. pylori* is modulated by pH changes in the stomach. Hence, UBT can yield false-negative results in patients who have been administered proton pump inhibitors (PPIs) (3). Other non-invasive tests that can help us overcome these limitations will therefore be helpful in evaluating the results of the eradication therapy.

Several stool antigen tests have proved reliable in the diagnosis of *H. pylori* infection. Early enzyme immunoassay (EIA) based on polyclonal antibodies represents a valid method for the detection of *H. pylori* antigens in stool specimens. However, some controversial results in the post-eradication assessment have been reported (4,5). Monoclonal antibody-based techniques generally have higher specificity. Stool antigen tests, which use monoclonal antibodies, have been developed, and they have been found to be more accurate than those using polyclonal antibodies (6,7). The Testmate pylori antigen (TPAg) EIA utilizes monoclonal antibody to native *H. pylori* catalase (8). This test was developed in Japan, and high sensitivity and specificity have been demonstrated in Japanese patients (9). TPAg EIA has been employed by laboratory companies instead of the Western stool antigen test and used to examine clinical stool samples in Japan. However, the accuracy of this test for the evaluation of *H. pylori* eradication has not been fully investigated.

The aim of this study was to evaluate the agreement between the UBT and TPAg EIA results obtained during evaluation of the results of *H. pylori* eradication therapy. We also examined whether stool antigen tests using monoclonal antibodies can be used to evaluate the results of eradication therapy in patients who have been administered PPIs.

Ninety-four patients who underwent *H. pylori* eradication therapy at Hirosaki University Hospital, Toyama City Medical Association Health Care Center, and Oita University Hospital were studied. Eradication therapy consisted of administration of the following PPIs—lansoprazole (30 mg), omeprazole (20 mg), and rabeprazole (10 mg)—and amoxicillin (750 mg) with either clarithromycin (200 mg) or metronidazole (250 mg). The drugs were administered twice a day for 1 week. Six or 8 weeks after the completion of eradication therapy, patients underwent both 13C-UBT and a stool antigen test using monoclonal antibody to *H. pylori* catalase. Breath samples were taken before and 20 min after ingestion of 100 mg 13C-urea. 13CO2 in expired breath was measured using UBit-IR300 (Otsuka Electronics, Osaka, Japan). The UBT results were considered positive if the d-value over baseline (DOB) was >5.0%. DOB < 2.5% was defined as negative, and values between 2.5 and 5.0 were considered to be borderline. Stool samples were collected in the morning.
of the day of UBT by the patients, and the samples were stored at −80°C until measurement. Stool specimens were analyzed by TPAg EIA in accordance with the guidelines provided by the manufacturer (Wakamoto Pharmaceutical Co. Ltd., Kanagawa, Japan & Kyowa Medex, Tokyo, Japan) (9). The results of *H. pylori* eradication were examined at 6 or 8 weeks after finishing the treatment in 31 and 63 patients, respectively. As shown in Table 1, at 6 weeks, 25 patients were negative by both TPAg EIA and 13C-UBT. One patient tested negative by TPAg EIA but positive by 13C-UBT. This patient was reexamined 1 month later, and both tests were negative. Among the 5 patients whose TPAg EIA results were positive, 1 tested negative with the 13C-UBT. At 8 weeks after finishing the treatment, 5 patients tested positive by both TPAg EIA and 13C-UBT. Among the 55 patients who tested negative by TPAg EIA, 3 showed positive results by 13C-UBT. These patients were reexamined at least 1 month later, and among them, 1 tested positive by both tests while another tested negative by both tests. However, once again, there were discrepancies in the results obtained using both the tests in the case of the third patient. Agreement among the results of TPAg EIA and 13C-UBT was 93.5% at 6 weeks and 95.2% at the end of PPI administration. In 15 patients who tested negative by both TPAg EIA and 13C-UBT results were negative after the discontinuation of PPI administration. In 1 patient tested negative with the 13C-UBT. At 8 weeks after finishing the treatment, 5 patients tested positive by both TPAg EIA and 13C-UBT. Among the 5 patients whose TPAg EIA results were positive, 1 tested negative with the 13C-UBT. At 8 weeks after finishing the treatment, 5 patients tested positive by both TPAg EIA and 13C-UBT. Among the 55 patients who tested negative by TPAg EIA, 3 showed positive results by 13C-UBT. These patients were reexamined at least 1 month later, and among them, 1 tested positive by both tests while another tested negative by both tests. However, once again, there were discrepancies in the results obtained using both the tests in the case of the third patient. Agreement among the results of TPAg EIA and 13C-UBT was 93.5% at 6 weeks and 95.2% at 8 weeks after completion of the treatment. Overall agreement of the TPAg EIA and 13C-UBT tests conducted to determine *H. pylori* eradication was 94.7%.

We also studied 22 patients with gastric or duodenal ulcer in the active stage. They underwent eradication therapy for 1 week; PPIs (lansoprazole 30 mg, omeprazole 20 mg, and rabeprazole 10 mg), amoxicillin (750 mg), and clarithromycin (200 mg). All the drugs were administered twice a day. After finishing the eradication therapy, patients continued administration of PPI for 6–8 weeks to treat gastric or duodenal ulcers. Both TPAg EIA and UBT were performed at the end of PPI administration and at least 7 days after its discontinuation. At the end of PPI administration, TPAg EIA showed positive results in 4 patients and negative results in 18. Among the 4 patients who tested positive by TPAg EIA, 3 also tested positive by 13C-UBT, and both tests also showed positive results after the discontinuation of PPI administration. In 1 patient, positive TPAg EIA and negative 13C-UBT results were obtained at the end of and 10 days after the discontinuation of PPI administration. In 18 patients who were negative by TPAg EIA at the end of PPI administration, 13C-UBT results were positive in 2 patients and ambiguous results were obtained for 1 patient. In these 3 patients, both TPAg and 13C-UBT results were negative after the completion of PPI administration. In 15 patients who tested negative by both TPAg EIA and 13C-UBT at the end of PPI administration, both tests also yielded negative results even after the discontinuation of PPI administration. Taken together, the results of eradication therapy were accurately identified by TPAg EIA at the end of PPI administration in 21 of 22 patients.

All subjects provided informed consent before undergoing eradication therapy, and this study was approved by the ethics committee of Hirosaki University. Agreement of both the tests was calculated, and the difference was examined by *χ*² analysis. A *P* value of <0.05 was considered statistically significant.

Stool antigen tests used for the diagnosis of *H. pylori* in earlier studies were based on polyclonal antibodies. The results of UBT and such stool antigen tests performed after the eradication therapy were discordant or indeterminate in nearly 8–10% of patients (4,5). The most common cause for these results was the lower diagnostic accuracy of polyclonal antibody, which often gives false-positive results in stool antigen tests. On the other hand, it has been shown that recent stool antigen tests performed using monoclonal antibodies have an accuracy comparable to that of UBT (10,11). In Japan, previous studies have also examined the usefulness of stool antigen tests for evaluating the results of eradication therapy (4,12,13). However, in these studies, stool antigen tests have been based on polyclonal antibodies or monoclonal antibodies manufactured in the West. TPAg EIA is a Japanese stool antigen test based on the monoclonal antibody specific to *H. pylori* catalase (8). TPAg EIA has high sensitivity and specificity to diagnose *H. pylori* infection (14). In the present study, we examined the usefulness of TPAg EIA for the evaluation of *H. pylori* eradication therapy, and the results were comparable to those of UBT in most patients. Therefore, TPAg EIA can be used to evaluate the results of eradication therapy in place of UBT.

After eradication therapy, the amount of bacteria in the stomach is decreased even if the treatment is not successful. The smaller amounts of bacteria can cause false-negative results in diagnostic tests, and thus the results of eradication therapy should be examined 1 to 2 months after finishing the treatment. In the present study, we compared the results of TPAg EIA and UBT at 6 and 8 weeks after finishing eradication therapy. The results suggest that TPAg EIA can determine the treatment results at both 6 and 8 weeks after finishing the treatment.

UBT is the most popular test to examine the results of *H. pylori* eradication therapy in Japan. However, there are certain cost-related concerns for performing UBT such as the price of 13C-urea and the cost for measuring 13CO₂ (2). In contrast, stool antigen tests, including TPAg EIA, do not require expensive chemical agents or special equipment. Therefore, an inexpensive evaluation procedure can be made available to patients undergoing eradication and thus the total costs for health insurance would also decrease. The other benefit of the test is that the patients are not required to fast in the morning on the day of examination, whereas UBT requires fasting. Furthermore, at some institutions and hospitals, UBT influences the efficacy of the endoscopy unit.

### Table 1. Results of TPAg and UBT to examine the results of eradication therapy

<table>
<thead>
<tr>
<th>TPAg</th>
<th>UBT</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks*</td>
<td>8 weeks*</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>TPAg positive</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>TPAg negative</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

* Duration after finishing the treatment.

1: One patient tested positive by both the tests while 1 patient tested negative by both tests.

There were discrepancies in the results of both the tests in the third patient.

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because doctors or medical staff must be employed to keep patients under observation during the test. Hence, if TPAg EIA is chosen as a treatment plan instead of UBT, more endoscopic examinations can be performed.

In this study, the results of TPAg EIA and UBT did not agree in some cases during the evaluation of eradication therapy. However, in most of these cases, TPAg EIA and UBT produced identical results when they were performed 1 month later. These findings suggest that there could be some false-positive or false-negative results obtained by performing TPAg EIA and UBT at the time of evaluation of eradication therapy. A previous Japanese multicenter study showed that a combination of the stool antigen test and UBT can be useful for accurate evaluation of the eradication therapy, even when a polyclonal antibody-based stool antigen test is used (15). Since no test yields 100% sensitivity and specificity, it would be considered useful to confirm eradication by different tests in patients who have been defined as negative by a diagnostic test.

In this study, we showed that TPAg EIA can be used to evaluate the results of eradication therapy even during PPI administration. Administration of PPIs is the most effective treatment for subjects diagnosed with peptic ulcers. In particular, some patients with peptic ulcers take nonsteroidal anti-inflammatory drugs (NSAIDs) and/or anti-platelet agents, and they are at a high risk of recurrence of bleeding ulcers. In these patients, *H. pylori* infection increases the risk of bleeding from ulcers (16). Therefore, continuation of PPI treatment is desirable until confirmation of *H. pylori* eradication. However, administration of PPIs modulates gastric pH, resulting in lower urease activity of *H. pylori* in the stomach. Since UBT detects urease activity of the gastric mucosa, false-negative results of UBT are seen in patients who have been taking PPIs (3,17). Thus, it is generally recommended that the administration of PPIs be discontinued several days before performing UBT. However, if TPAg EIA could be used to evaluate eradication, patients would be at a lesser risk since they can continue PPI administration until the eradication of infection is confirmed.

In conclusion, TPAg EIA, a monoclonal antibody-based stool antigen test, is a useful diagnostic test for evaluation of the results of *H. pylori* eradication therapy. Since stool antigen tests are not influenced by PPI administration as compared to UBT, TPAg EIA can be a safer alternative for patients who require PPI administration for the treatment of peptic ulcer. Implementation of this test may also lessen the effort required of endoscopists and medical staff, and in turn, may improve the efficacy of the endoscopy unit.

**ACKNOWLEDGMENTS**

This work was supported in part by the Grant-in-Aid for Cancer Research (18-2) from the Ministry of Health, Labour and Welfare, Japan.