J Gastroenterol 1996; 31:171–174

Journal of Gastroenterology

Validity of various diagnostic tests to evaluate cure of *Helicobacter pylori* infection

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Abstract: Many diagnostic methods have been developed and used for detecting *Helicobacter pylori* to evaluate the success of treatment of *H. pylori* infection. We investigated and compared the suitability of the rapid urease test (RUT), polymerase chain reaction (PCR), ^{13}C-urea breath test (^{13}C-UBT), and serology with culture for evaluating cure of *H. pylori* infection. Forty-seven *H. pylori*-positive gastric ulcer patients received dual therapy of lansoprazole (30 mg u.i.d.) and clarithromycin (200 mg b.i.d.). Four weeks after the completion of treatment, RUT, PCR, ^{13}C-UBT, and culture were performed and the negative rates of these tests were compared. Anti-*H. pylori* IgG antibodies were measured by enzyme-linked immunosorbent assay (ELISA) before and 4 weeks after completion of the treatment to evaluate changes of titers during the treatment. The negative rate of RUT (55%) was significantly greater than that of culture (27%). Significant declines in titers were seen in the patients who had negative culture results, while the decline in the titer was not significant in the patients who had positive results. PCR assay and ^{13}C-UBT were suitable for the evaluation of *H. pylori* eradication, but RUT was not suitable, because of its sensitivity. By monitoring anti-*H. pylori* IgG antibody titers, therapeutic failure can be detected early after completion of treatment.

Key words: *Helicobacter pylori*, cure, diagnosis

Introduction

Helicobacter pylori has been implicated as the major cause of various gastric diseases.[1,2] The development of reliable diagnostic methods has contributed to our understanding of the role of *H. pylori* in gastric and duodenal ulcers.[2] And the accurate detection of *H. pylori* after therapy has also proved to be of critical importance in evaluating the effects of treatment regimens.[3]

Several diagnostic methods are used for detecting *H. pylori* and various methods are used to evaluate the success of treatment at different institutions. Thus, it would be of value to establish which methods are most suitable for the evaluation of cure of *H. pylori* infection.

Serology is a non-invasive and relatively inexpensive method,[4–7] and, a decline in serum antibody titer is considered to be useful for evaluating the cure of *H. pylori* infection. ^{13}C or ^{14}C-urea breath tests (^{13}C or ^{14}C-UBT) are also non-invasive methods and are considered to be sensitive indicators of cure of *H. pylori* infection. The great advantage of the ^{13}C-UBT is the absence of radiation risk. The rapid urease test (RUT), an invasive method, is simple and inexpensive.[8–10] In Japan, this test is the most commonly used for detecting *H. pylori* and is often adopted for the evaluation of cure of *H. pylori* infection. The polymerase chain reaction (PCR) is considered to be the most sensitive method for detecting *H. pylori* infection,[11–13] but it requires special equipment and trained personnel, and is not a generally agreed method for evaluating cure of *H. pylori* infection.

To evaluate the usefulness of these tests for monitoring the success of treatment, we measured the decline of serum antibodies 4 weeks after the completion of treatment and performed RUT, PCR, ^{13}C-UBT, and culture in patients who had received treatment to cure *H. pylori* infection.

Subjects and methods

In patients in whom active gastric ulcer was detected by endoscopy, three biopsy specimens were taken from the antrum and the body of the stomach to evaluate *H.*

Offprint requests to: T. Shimoyama
(Received for publication on Mar. 31, 1995; accepted on Aug. 18, 1995)

pylori status. Subjects were considered for inclusion in the study if they were healthy and all examinations were positive. All subjects enrolled in the study provided their informed consent. Patients who had previously been treated or who were taking oral anticoagulants were excluded.

Forty-seven gastric ulcer patients, mean age 49 years (range 35–67 years), 26 of whom were men, received treatment to cure *H. pylori* infection: an 8-week course of lansoprazole (30 mg u.i.d.) and a 2-week course of clarithromicin (400 mg b.i.d.). Four weeks after completion of the treatment, RUT (CLO test), PCR, 13C-UBT, and culture were performed to evaluate the success of the treatment. Endoscopy was performed in all patients and three biopsy specimens were taken from the antrum and two from the body of the stomach for RUT, PCR, and culture. The ^{13}C-UBT was also performed 3 h after the endoscopy. In 22 patients, serologic assay for IgG antibodies against *H. pylori*, performed by enzyme-linked immunosorbent assay (ELISA), was performed before and 4 weeks after the treatment. The institutional Ethics Committee gave their permission for the performance of this study.

To compare the value of RUT, PCR, and ^{13}C-UBT with culture in the evaluation of cure of *H. pylori* infection, we compared the negative rates of these tests 4 weeks after completion of the treatment. To investigate anti-*H. pylori* IgG antibody titers after completion of the treatment, the patients were divided into two groups: group A, those who had positive culture results 4 weeks after completion of the treatment ($n = 15$) and group B, those who had negative culture results 4 weeks after completion of the treatment ($n = 7$). In both groups, titers of IgG antibodies against *H. pylori* were measured before and 4 weeks after completion of the treatment. To evaluate the decline of anti-*H. pylori* IgG antibody titers, the titers at both points were compared.

Assessment of RUT (CLO test) was done 20 min, 2 h, and 24 h after the biopsy. If a definite pink color developed, the test was considered to be positive. In this study, all patients showed positive results before the treatment.

PCR was carried out with two primers described by Tonokatsu et al.[14] The PCR assay was considered positive when the product, which was equivalent to the fragment described by Tonokatsu, was found after amplification.

^{13}C-UBT was performed 3 h after the endoscopic examination performed 4 weeks after completion of the treatment. For the test, the patient swallowed ^{13}C-urea (100 mg) in 30 ml distilled water. This was distributed within the stomach by changing the patient's position. After 20 min, 1-liter serial breath samples were collected in a large reservoir bag, from which a single 10-ml aliquot was taken and analyzed by mass spectrometry. A positive ^{13}C-UBT result was defined as excess $\delta\ ^{13}CO_2$ excretion >5‰.[15]

Serologic assay for IgG antibodies against *H. pylori* by ELISA was performed using the kit developed by BML Inc.[16] Tokyo, Japan A positive result was defined as a titer >10 AU (arbitnary units). In this study, all patients tested positive by this kit before the treatment. For the culture, biopsy specimens were cultured in Skirrow's medium for 3–5 days at 37°C at high humidity under strict aerobic conditions (CO_2 15%, O_2 5%).

Statistical analysis χ^2 analysis was used for statistical calculations of RUT, PCR, ^{13}C-UBT, and culture. Wilcoxon's signed-rank test was used for statistical calculations of the decline of serum antibodity titers. Differences were considered significant at $P < 0.05$.

Results

The negative rates of RUT, PCR, ^{13}C-UBT, and culture 4 weeks after completion of the treatment are shown in Table 1. The negative rates of RUT, PCR, ^{13}C-UBT, and culture were 57% (in 27 out of 47 patients), 28% (in 13 out of 47 patients), 32% (in 15 out of 47 patients) and 28% (in 13 out of 47 patients), respectively. The negative rate of RUT was significantly higher than that of culture. In all patients who showed negative culture results, RUT also showed negative results. Negative results for RUT in the patients who showed positive culture results were considered to be false negative. The results for PCR were the same as that for culture in all patients. In two patients with positive culture results, ^{13}C-UBT showed negative results. However, there was no significant difference between the negative rates for ^{13}C-UBT and for culture 4 weeks after the completion of the treatment.

Changes in anti-*H. pylori* IgG antibody titers before and 4 weeks after completion of the treatment are shown in Fig. 1. Before the treatment, there was no significant difference in mean titers between group A and group B patients. In group A, although a decline in anti-*H. pylori* IgG antibody titer was seen in some patients, the difference in titers before and 4 weeks after completion of the treatment was not significant (Fig. 1a). In contrast, in all the group B patients who had

Table 1. Negative rates for four diagnostic methods 4 weeks after completion of treatment for *H. pylori* infection

Method	Rate (Number)
Culture	28% (13/47)
Rapid urease test	57% (27/47)
^{13}C-urea breath test	32% (15/47)
Polymerase chain reaction	28% (13/47)

* $P < 0.05$ (χ^2 analysis)

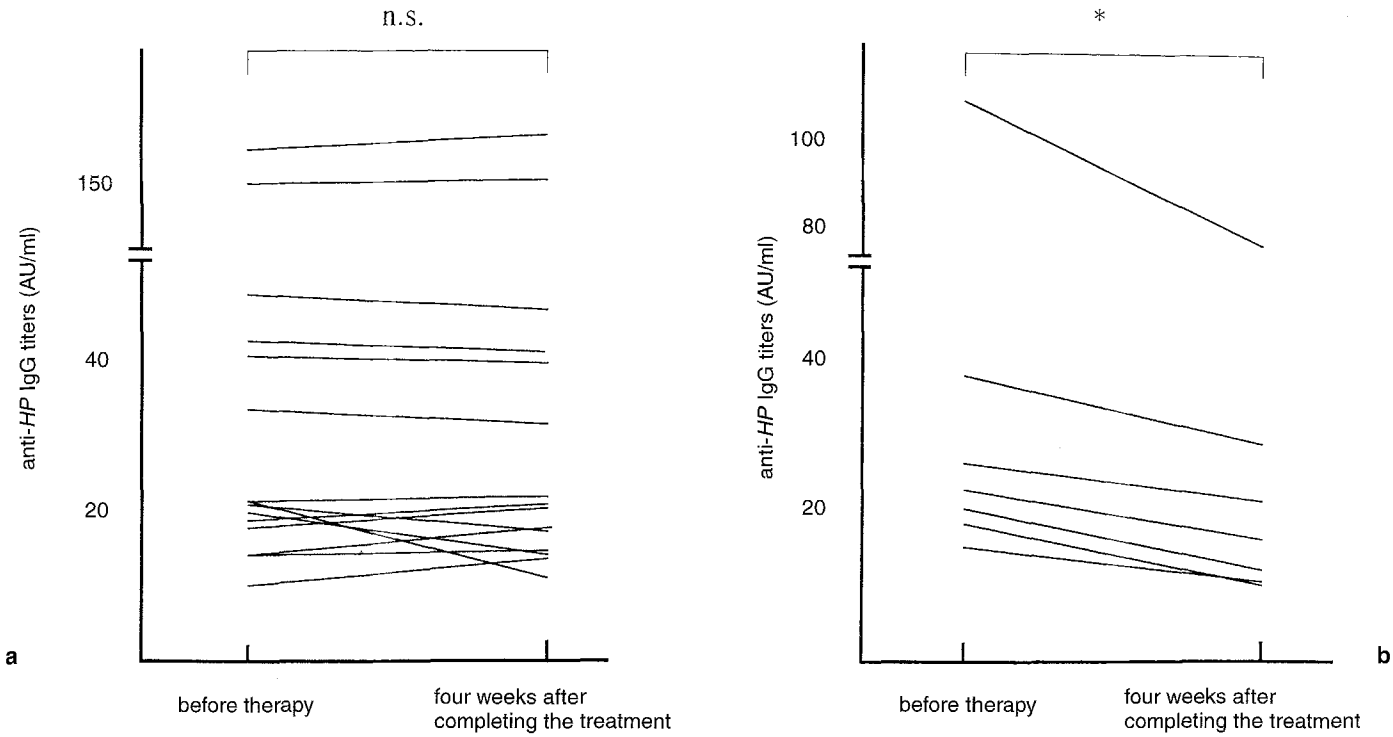

Fig. 1a,b. Changes in anti-*H. pylori* IgG antibody titers before and 4 weeks after completion of the treatment. **a** Titers of patients who showed positive culture results 4 weeks after completion of the treatment ($n = 15$). **b** Titers of patients who showed negative culture results 4 weeks after completion of the treatment. The decline of titer was significant in this group ($n = 7$). $^*P < 0.02$ (Wilcoxon's signed-rank test; one-tailed)

negative culture results, there was a decline in the titers, and the difference in titers before and after treatment was significant (Fig. 1b). These results suggest that a lack of decline in anti-*H. pylori* IgG antibody titers indicates failure of the treatment.

Discussion

We investigated and compared the suitability of RUT, PCR, ^{13}C-UBT, and serology with culture in the evaluation of cure of *H. pylori* infection.

RUT is a cheap and simple method used for detecting *H. pylori* in gastric biopsy specimens.[8–10] RUT can replace culture and histology. However, false-negative results are possible if a low bacterial load is present.[10] Therefore, false-negative results may occur after completion of the treatment. The results of our investigation showed that negative rates of RUT were significantly higher than that of culture 4 weeks after completion of the treatment. If the cure of *H. pylori* infection were to be defined only by RUT 4 weeks after the completion of the treatment, it is possible that the regimen could result in too high a cure rate. Loffeld et al.[17] in their review, noted that all diagnostic techniques currently used had good sensitivity and specificity; however, our results showed that the sensitivity of RUT was insufficient for the evaluation of cure of *H. pylori* infection.

PCR assays are considered the most sensitive method to detect small numbers of *H. pylori*.[11–13] This method would thus be suitable for evaluation of the cure of *H. pylori* infection. Another advantage of PCR is the rapid identification of *H. pylori* compared with culture and histological detection.[13] Because of these advantages, PCR is used in some Japanese institutions to evaluate the cure of *H. pylori* infection. We compared the negative rates of PCR with that of culture after treatment and found no significant difference between the results for the two methods. Fabre et al.[11] also compared PCR with RUT, culture, and histological test. They found that PCR was the most sensitive and specific method. Therefore, we suggest that PCR is also suitable for evaluating cure of *H. pylori* infection.

The major advantages of ^{13}C-UBT for detecting *H. pylori* are its accuracy and its potential for non-invasive assessment.[15,18–20] Slomiansky et al.[21] investigated the utility of ^{13}C-UBT by comparing the results with those of histological examination and CLO test. They suggested that antral biopsy was unnecessary to confirm cure of *H. pylori* infection. We compared the results of ^{13}C-UBT with those of culture and found no significant difference. These results support the suggestion of Slomiansky et al.[21] We suggest that, in the light of these

advantages, ^{13}C-UBT should be approved as a diagnostic method in Japanese institutions.

Serology is considered to be a safe and non-invasive method for the detection of *H. pylori*; serum antibodies are shown to decline after successful treatment.[4–6] Hirschl et al.[7] reported that a breakpoint of 50% reduction in antibody titer indicated bacterial eradication, with a sensitivity of 99.4% 6 weeks after the beginning of the therapy. We found that, 12 weeks after the beginning of the therapy, there were declines in the titers in all patients who had negative culture results. On the other hand, in many of the patients who had positive culture results, antibody titers remained at the baseline level. Consequently, we conclude that therapeutic failures can be detected by the monitoring of anti-*H. pylori* IgG antibody titers 3 months after the beginning of the therapy.

We investigated the suitability of RUT, ^{13}C-UBT, PCR, and serology to evaluate cure of *H. pylori* infection, compared with culture, and conclude that: (i) because of its sensitivity, RUT is not suitable for evaluating cure of *H. pylori* infection; (ii) the PCR assay and ^{13}C-UBT are suitable methods for evaluating cure of *H. pylori* infection; and (iii) by monitoring anti-*H. pylori* IgG antibody titers, therapeutic failures can be detected early after the completion of treatment.

Acknowledgments. This work was supported by the staff of the Department of Gastroenterology of Aomori Prefectural Central Hospital.

The authors thank Dr. Hiroshi Saito and Dr. Hitoshi Kawaguchi for their advice with this study.

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