Comparison of invasive and noninvasive tests for detecting *Helicobacter pylori* infection in bleeding peptic ulcers

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Background: **Eradication of *Helicobacter pylori* infection has been shown to prevent recurrent bleeding from peptic ulcers. However, the detection rate for *H pylori* infection seems to be underestimated in this group of patients and has been scarcely investigated.**

Methods: **Eighty patients with bleeding peptic ulcer were studied for evidence of *H pylori* infection. Seventy-seven of these patients were enrolled as having *H pylori* infection after any one of the following 3 tests were positive: culture, histologic study, or any 2 of rapid urease test (CLO test), carbon 13–labeled urea breath test (UBT), and serologic examination. Fresh blood or blood-containing material in the gastric antrum was noted by panendoscopy in 22 patients (group A). In the remaining 55 cases there was no blood in the antrum (group B).**

Results: **The sensitivities of the CLO test, bacterial culture, histologic study, ^{13}C-labeled UBT, and immunoglobulin G serologic test were 45.5%, 36.4%, 77.2%, 95.4%, and 100% in group A, respectively, and 70.9%, 40.0%, 70.9%, 92.7%, and 96.4%, respectively, in group B. There was a statistically significant difference between the sensitivities found for CLO test and ^{13}C-labeled UBT ($p < 0.05$). Of these 5 tests, only the sensitivity of the CLO test showed a statistically significant difference between groups A and B ($p < 0.05$). A delayed positive CLO test result was recorded in 13 patients (3 in group A, 10 in group B).**

Conclusion: **Noninvasive tests seemed to be more sensitive than invasive tests in detecting *H pylori* infection in patients with bleeding peptic ulcers. Blood in the antrum might reduce the sensitivity of the CLO test but have no effect on the other tests. The CLO test should be observed for more than 24 hours because of the possibility of a delayed positive result in some patients with bleeding peptic ulcers. (Gastrointest Endosc 1999;49:302-6)**

Helicobacter pylori has been strongly associated with peptic ulcers since it was first discovered in 1983, and it is now thought to be the main etiologic factor in peptic ulcer disease.[1-3] Consequently, the eradication of *H pylori* is important in the treatment of peptic ulcers and is more beneficial than traditional maintenance antacid therapy.[4-7] Many studies also indicate that eradicating *H pylori* effectively prevents recurrent bleeding in bleeding peptic ulcers.[8-15] Several tests that have similar sensitivities are available to determine whether a patient is infected with *H pylori*.[16,17] However, the prevalence rate of *H pylori* infection in patients with bleeding peptic ulcers has been reported to be lower than the rate in patients with nonbleeding ulcers.[8,18-20] Our study compares the sensitivities of invasive tests and noninvasive tests for determining *H pylori* infection in patients with bleeding peptic ulcers.

Received May 18, 1998. For revision September 10, 1998. Accepted October 21, 1998.

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Supported in part by the Cathay General Hospital Research Grant.

Presented in part at the 10th International Workshop on European Gastroduodenal Pathology and Helicobacter pylori, Lisbon, Portugal, September 1997, and published as an abstract in Gut 1997;41(1 Suppl):A68.

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0016-5107/99/$8.00 + 0 **37/1/95332**

PATIENTS AND METHODS

Patient selection and characteristics

From July 1996 to June 1997, 80 patients who were seen with symptoms of hematemesis or hematochezia within 3 days before panendoscopy were investigated for *H pylori* infection. Among them, 77 were defined as having *H pylori* infection. These patients had not received previous antibiotic treatment for this organism and were not taking nonsteroidal anti-inflammatory drugs (NSAIDs). Age, sex, and medications taken before endoscopy were recorded at baseline. Various GI conditions seen on panendoscopy were also recorded (i.e., the presence of duodenal ulcer, gastric ulcer or both, and whether fresh blood or coffee-ground material was present in the gastric antrum). Each patient underwent multiple tests for *H pylori* infection and gave consent for a carbon 13–labeled urea breath test (UBT). Patients were divided into 2 groups according to the presence or absence of blood in the gastric antrum. This study was approved by the Institutional Human Research Review Committee of our hospital.

A total of 77 patients with a mean age (±SD) of 47.6 ± 14.1 years (46.2 ± 14.4 years in group A, 48.2 ± 14.1 years in group B) were enrolled in the study. Infection with *H pylori* was established according to the above definition in all patients, which included 56 men (16 in group A, 40 in group B) and 21 women (6 in group A, 15 in group B). Blood or blood-containing material was found in the antrum of 22 patients on panendoscopy. Sixteen patients had duodenal ulcers, 4 had gastric ulcers, and 2 had both types of ulcers. Of the 55 patients without blood in the antrum, 41 had duodenal ulcers, 8 had gastric ulcers, and 6 had both.

Diagnostic assays

The presence of *H pylori* was determined by performing a rapid urease test (CLO test; Delta West, Bently, Australia), which was observed for up to 24 hours, histologic examination, bacterial culture from the antral biopsy specimen, ^{13}C-labeled UBT, and serologic examination for the presence of immunoglobulin G (IgG) antibody to *H pylori* (AMRAD, Perations, A.C.N, Melbourne, Australia).

A total of 4 antral biopsy specimens within 3 cm from the pyloric ring were obtained from each patient. The first biopsy specimen was used for the CLO test and was monitored for color change for up to 24 hours at room temperature. The second and third specimens were sent for microbiologic culture, being implanted into blood agar after grinding. These plates were placed in a gas tank that provided environmental conditions of 5% oxygen and 10% carbon dioxide; results were recorded after four days of incubation. The fourth specimen was submitted to an experienced pathologist for histologic examination with hematoxylin and eosin stain.

The ^{13}C-labeled UBT was performed within 1 day of panendoscopy in each patient who agreed to the procedure. The test consisted of a baseline breath sample and a second breath sample collected 15 minutes after oral administration of 100 mg of ^{13}C-labeled urea (INER-Hp ^{13}C-tester, Taiwan) dissolved in tap water. Each patient who underwent the test fasted for at least 6 hours and was then given 100 mL of milk to delay gastric emptying before receiving the ^{13}C-labeled urea. If the value of the carbon dioxide expired in both samples differed by more than 3 per mil, this was considered a positive result. This method has previously been shown to have good sensitivity and specificity.[21]

The blood sample for serologic evaluation was obtained before treatment was begun. An enzyme-linked immunosorbent assay for IgG antibody to *H pylori* was performed with a cutoff value of 30 U/mL, as suggested by the manufacturer.

Criteria for case definition

A positive *H pylori* result was recorded when either of 2 tests (histologic study or culture) were positive or any 2 of the other 3 tests (CLO test, ^{13}C-labeled UBT, and serologic study) were positive. Essentially, 3 positive results were used as the "gold standard" for detecting *H pylori* infection. The CLO test was monitored for color change up to 24 hours. A delayed positive result was defined as a color change at more than 24 hours, but this was still recorded as a negative test result according to our criteria.

Each patient's GI condition was diagnosed by panendoscopy. Patients with bloody material in the antrum were classified as group A; all others were classified as group B.

Statistical analysis

The sensitivity of each test for *H pylori* infection was calculated. To assess whether each test was statistically different in sensitivity between the 2 groups, the 1-tailed Fisher's exact test was used. McNemar's test was performed to evaluate the difference in sensitivity between the CLO test and the ^{13}C-labeled UBT for each group of patients.

RESULTS

Sensitivity of diagnostic tests

Ten patients tested positive for *H pylori* by CLO test in group A and 39 tested positive in group B (Table). A delayed positive result was recorded for 13 patients, 3 in group A and 10 in group B.

With the ^{13}C-labeled UBT, 21 patients in group A were positive for *H pylori* infection and 51 patients were positive in group B. Bacterial culture gave a positive result least frequently, being found in only 8 patients in group A and in 22 in group B. Histologic analysis detected *H pylori* in 17 patients in group A and in 39 patients in group B.

The serology test for IgG was the most sensitive test. All patients in group A tested positive and only 2 patients yielded negative results in group B. The value of any positive serologic result in this study was not less than 68 U/mL. The table lists the sensitivities of all tests for *H pylori* infection used in the study patients.

Comparison of diagnostic test sensitivities

There was a statistically significant difference between the sensitivities of the CLO test and the

Table 1.
Number and percent of patients (n = 77) who were found to be positive for *H pylori* by each of 5 diagnostic tests

Diagnostic test	Group A (n = 22)	Group B (n = 55)
Invasive		
CLO test*	10 (45.5%)	39 (70.9%)
Culture†	8 (36.4%)	22 (40.0%)
Histologic study‡	17 (77.2%)	39 (70.9%)
Noninvasive		
13C-labeled UBT§	21 (95.4%)	51 (92.7%)
IgG serologic study‖	22 (100.0%)	53 (96.4%)

*Rapid urease test conducted on gastric antral biopsy specimen with results ascertained at 24 hours.

†Culture acquired from 2 gastric antral biopsy specimens implanted into blood agar plates and incubated in microaerobic environment.

‡Histologic examination with hematoxylin and eosin stain of gastric antral biopsy specimen.

§UBT performed 15 minutes after oral administration of 100 mg ^{13}C-labeled urea.

‖Detection of serum antibodies to *H pylori.*

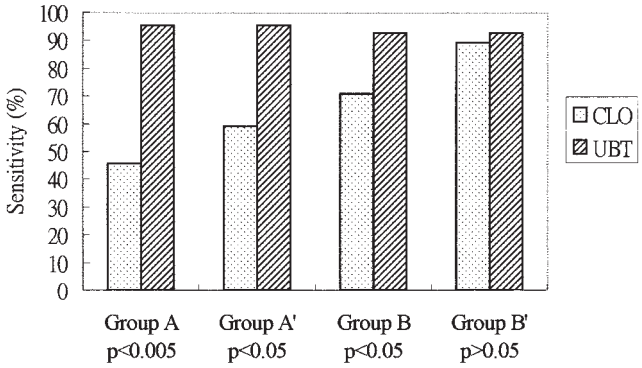

Figure 1. Comparison of sensitivities found for CLO test and ^{13}C-labeled UBT in individual groups with McNemar's test. Group A (blood in antrum) and group B (no blood in antrum): positive CLO test indicates color change within 24 hours. Group A´ and B´: positive CLO test figures include delayed positive results (some color change occurred after 24 hours). Statistically significant difference between both tests was found in patients with blood in the gastric antrum regardless of whether delayed positive results were included.

^{13}C-labeled UBT for diagnosing *H pylori,* regardless of the presence or absence of blood in the antrum (McNemar's test, $p < 0.005$ in group A and $p < 0.05$ in group B, Fig. 1). The sensitivity of every test (CLO test, ^{13}C-labeled UBT, culture, histologic and serologic study) in group A was also compared with that of group B by 1-tailed Fisher's exact test ($p = 0.034$, 0.556, 0.489, 0.396, and 0.508, respectively, Fig. 2). Only the CLO test showed a statistically significant difference between the 2 groups. But if the patients with a delayed positive CLO test result were included in the positive group, the sensitivities of the CLO test in group A and group B increased to 59.1% and 89.1%, respectively; then a statistically significant difference between ^{13}C-labeled UBT and CLO test was only present in group A (McNemar's test, $P < 0.05$, Fig. 1, A´ and B´ group).

The IgG serology test, a noninvasive test, was the most sensitive test used, with nearly 100% sensitivity.

DISCUSSION

There is still no established "gold standard" for the diagnosis of *H pylori* infection. The noninvasive tests seem to be more useful than the invasive tests and more accurately reflect *H pylori* infection status. However, some investigators have not found any statistically significant difference between the 2.[16,17] Although the eradication of *H pylori* has been shown to effectively prevent recurrence of bleeding in patients with bleeding peptic ulcers,[8-15] only a few reports discuss the frequency of *H pylori* in these patients.[18-20] In addition, there are few published studies that compare the sensitivity of the invasive and noninvasive diagnostic tests.

A high frequency of *H* pylori infection was detected in our bleeding patients by noninvasive tests (^{13}C-labeled UBT and IgG serologic studies). Screening for IgG antibodies was the most sensitive of the 5 tests; the sensitivity approached 100%, but it was not a good test for evaluation of *H pylori* eradication.[22] The ^{13}C-labeled UBT also had a sensitivity of more than 90% in both patient groups. Of the remaining 3 invasive tests, histologic examination seemed to have better sensitivity but yielded no statistically significant difference in either group.

The ^{13}C-labeled UBT had a better sensitivity than the CLO test did for *H pylori* infection in patients with bleeding peptic ulcers in this study, especially in patients with blood in the stomach. Nevertheless, 13 patients (3 in group A, 10 in group B) had delayed positive results that were regarded as indicating no *H pylori* infection according to the definition of the CLO test. However, if these patients were considered to be infected, there would not be a statistically significant difference between the CLO test and ^{13}C-labeled UBT in group B, but the difference would still be significant for group A. A false-positive result did not occur when the data were analyzed in this way because these 13 patients were verified to have *H pylori* infection for satisfying any 1 of the 3 criteria. Hence, we believed that the observation time for color change should be more than 24 hours in patients with bleeding peptic ulcers.

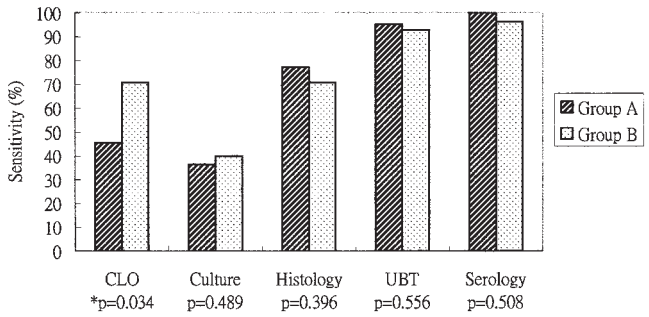

Figure 2. Sensitivities of 5 tests for detecting *H pylori* in patients with bleeding peptic ulcers and *p* values (1-tailed Fisher's exact test) for difference between patients of group A (blood in antrum) and group B (no blood in antrum). *Asterisk,* Statistically significant difference was found for CLO test ($p < 0.05$).

Of the 5 tests used, only the CLO test had a better sensitivity in group B than in group A. This might mean that blood in the gastric antrum can interfere with the diagnosis of *H pylori* and decrease the sensitivity of the test. The presence of blood in the antrum had no effect on the other 4 tests. Our findings are similar to those of 2 recent reports on patients with bleeding peptic ulcers. One study from Greece[23] reported preliminarily that the CLO test is not reliable in patients who are bleeding; the authors incriminated blood as causing the false-negative results. However, half their patients had ingested NSAIDs, whereas ours had not. Another study[24] excluded NSAID use and also found a significant false-negative rate in patients with bleeding duodenal ulcers when the CLO test was used alone. We suspect that blood might affect the pH value of the CLO test medium and do not favor the hypothesis that *H pylori* migrates from the antrum to the body of the stomach when blood is present.

We found the sensitivity of bacterial culture to be low (below 40%). The high rate of false-negative results in our patients might be the result of the presence of some factor that inhibits the growth of *H pylori* that is produced by blood or of the technical difficulty of culturing this organism.

The noninvasive ^{13}C-labeled UBT and IgG serologic tests are more promising methods for diagnosing *H pylori* infection in patients with bleeding peptic ulcers than are the invasive tests (CLO test, bacterial culture, and histologic examination). If only the CLO test is used to detect *H pylori* in these patients, the duration of observation for color change should be longer than 24 hours. We also found that a false-negative result might occur if the biopsy specimen used in the CLO test was acquired from an antrum with blood in it.

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