Polymerase chain reaction: A sensitive method for detecting *Helicobacter pylori* infection in bleeding peptic ulcers

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CONCLUSION: PCR is the most accurate method among the biopsy-based tests to detect *H pylori* infection in patients with bleeding peptic ulcers. Blood may reduce the sensitivities of all biopsy-based tests.

INTRODUCTION

Bleeding is a common and serious complication of peptic ulcer diseases. It is estimated that peptic ulcer bleeding accounts for approximately 150 000 hospitalizations per year in the USA[1,2]. The prevalence of *Helicobacter pylori* (*H pylori*) in bleeding peptic ulcers has not been definitely determined, but it is estimated to be 70%[3-5]. The accurate diagnosis of *H pylori* infection is crucial in the short-term and long-term management of patients with bleeding peptic ulcers[6]. If a patient with a bleeding ulcer requires surgical intervention, knowledge of his or her *H pylori* status may guide the selection of procedures for the patient (i.e., a simple closure vs full-blown ulcer surgery)[6]. Patients whose bleeding episodes cease in the short term, one-third of those who do not receive maintenance therapy, surgery or anti-*H pylori* therapy will experience recurrent bleeding within the next 1-2 years[7]. However, numerous studies have demonstrated that eradicating *H pylori* can drastically reduce the incidence of rebleeding in patients with bleeding peptic ulcers, preventing the need for long-term antisecretory therapy or surgical intervention[8-10]. Therefore, the *H pylori* status in a patient with bleeding peptic ulcers must be documented.

Currently, *H pylori* infection can be diagnosed by invasive assays, i.e., those requiring esophagogastroduodenoscopy (EGD), or by non-invasive assays in which EGD is not necessary. Invasive diagnostic tests include culture, histology, rapid urease test (RUT) and polymerase chain reaction (PCR). Non-invasive tests comprise serology, stool antigen test and urea breath test (UBT). The choice of a diagnostic test should depend on the clinical circumstances, sensitivity...
and specificity of the tests, and the cost effectiveness of
the testing strategy. Because of its simplicity, accuracy and
rapid determination of \( H \) pylori status, RUT is generally
considered to be the initial endoscopic test of choice for
uncomplicated peptic ulcers\(^{[13]}\). However, many studies have
demonstrated that RUT lacks sensitivity in \( H \) pylori diagnosis
when peptic ulcer diseases are presented with bleeding\(^{[13,12]}\).
Moreover, a recent study by Colin et al.\(^{[13]}\), indicated that all
direct tests on \( H \) pylori including RUT, culture and histology
reduced the sensitivity in the setting of ulcer bleeding. The
sensitivities of aforementioned three tests were 31%, 25%
and 26%, respectively.

PCR can diagnose \( H \) pylori infection under non-bleeding
conditions much more accurately than histology or
culture\(^{[14-18]}\). The level of sensitivity of this test is extremely
high and has a threshold of 10 to 100 \( H \) pylori strains per
specimen\(^{[14-16]}\). An accurate diagnosis of \( H \) pylori at the time of
bleeding episode is essential, but few studies have
addressed the application of PCR to bleeding ulcers. We
performed this prospective study to evaluate the sensitivity,
specificity and accuracy of PCR assay for detecting \( H \) pylori
infection in patients with bleeding peptic ulcers and to
compare its diagnostic efficacy with that of other invasive
and non-invasive tests.

**MATERIALS AND METHODS**

**Patients**

From April to September 2002, 60 consecutive patients
with hematemesis, melena, or both due to gastroduodenal
ulcer bleeding, who underwent an EGD, were enrolled in
this study. Exclusion criteria included: age <15 or >80 years;
history of coagulopathy or other disorders contraindicated
for EGD or biopsy sampling; previous history of anti-
\( H \) pylori therapy. Data regarding age, sex, medical history,
drug history, presenting symptoms, gastroduodenal lesions
and presence or absence of blood in the stomach were
recorded. Written informed consent was obtained from each
subject. This study was approved by the Human Medical
Research Committee of the Kaohsiung Veterans General
Hospital, Kaohsiung, Taiwan.

**Endoscopy and biopsy sampling**

During endoscopy, gastric biopsy specimens were taken
from the lesser curvature of the antrum and corpus for
RUT (one antrum biopsy specimen), histology (one antrum
and one corpus biopsy specimen), culture (one antrum
biopsy specimen) and PCR (one antrum biopsy specimen).
Endoscopes were cleaned by a mechanical wash and then
washed in an Olympus washing machine. They were then
air-dried and cleaned with 70% ethanol.

**RUT**

RUT was performed according to our previous study\(^{[17,18]}\).
A biopsy specimen from antrum was immediately placed
in 1 mL of a 10% solution of urea in deionized water (pH
6.8) to which two drops of 1% phenol red solution was
added and incubated at 37 °C for up to 24 h. If the yellowish
color around the area of inserted specimen changed to bright
pink within the 24-h limit, the urease test was considered
positive. In our laboratory, the sensitivity and specificity of
RUT were 96% and 91%, respectively\(^{[17]}\).

**Histological examination**

Biopsy specimens were fixed in 10% buffered formalin,
embedded in paraffin, and sectioned. One 4-μm-thick
section was cut and stained with hematoxylin-eosin to
observe the presence of curved rod shaped bacteria on the
mucosal surface\(^{[19,20]}\). The specimens were interpreted by a histopathologist (H-H Tseng) blinded to the patient
status and the results of other laboratory tests.

**Culture**

The specimen for culture was transferred with brain heart
infusion on ice for microbiological examination and
inoculated onto the CDC anaerobic blood agar (Becton
Dickinson Microbiology System, Cockeysville, MD)
according to our previous studies\(^{[21,25]}\). The agar was
incubated at 35 °C for two days in a micro-aerophilic gas
mixture containing 5% O\(_2\), 100 mL/L CO\(_2\), and 85% N\(_2\).
Culture-positive patients were those with bacterial colonies
grown in culture within 7 d. The organisms were identified
as \( H \) pylori by Gram staining, colony morphology and positive
oxidase, catalase and urease reaction.

**PCR amplification**

DNA extraction was performed using a commercially
available kit (QIAamp Tissue kit, QiAGEN Inc., Valencia,
CA) according to the manufacturer’s instructions\(^{[23]}\). The
primers used were derived from the internal 411-bp fragment
of the \( ureA \) gene as described by Clayton et al.\(^{[24]}\): HPU1
(5’GCCAATGGTAAATTAGTT3’) and HPU2 (5’CTCCT-
TAATTGTGTTTAC3’). Reactions were performed in a 25 µL.
volume in a thermal cycler 480 (Perkin Elmer Applied
Biosystems, Foster City, CA). A reaction mixture contained
2.5 µL of extracted DNA, 0.5 µmol/L of each primer,
2.5 µmol/L of MgCl\(_2\), 2.5 µL of 10× PCR buffer, 1 U of
AmpliTaq DNA polymerase (Perkin-Elmer Corp., Foster
City, CA) and 100 µmol/L of each of dATP, dCTP, dGTP
dTTP. The amplification cycle consisted of an initial
denaturation at 94 °C for 1 min, primer annealing at 45 °C
for 1 min, and extension for 5 min at 72 °C to ensure a full
extension of the products. Samples were amplified in 35
consecutive cycles. The final cycle included a 7-min extension
step to ensure a full extension of the PCR products. PCR
products were analyzed on a 2% agarose electrophoresis
gel stained with ethidium bromide.

**UBT**

UBT was performed according to our previous studies\(^{[21,25]}\)
within 1 d of EGD. The patients were fasted for at least
6 h. Fresh milk (1 000 mL) was taken to delay gastric
emptying. The test consisted of a baseline breath sample
and a second breath sample collected 15 min after oral
administration of 100 mg of \(^{13}\)C-labeled urea (INER-Hp
C-tester, Taiwan) dissolved in 50 mL sterile water. Values
were expressed as an excess \( \delta^{13} \)CO\(_2\)% excretion. The
\( \delta^{13} \)CO\(_2\) was the ratio of \(^{13}\)C to \(^{12}\)C in the sample compared
to the Pee Dee Belemnite (PDB) standard. The equation
was given as: \( \delta^{13} \)CO\(_2\) = (R samp - R std )/ R std ×1 000. R samp
and R std
represented the ratio of $^{12}$C to $^{13}$C in samples and standard, respectively. If the value of $\delta^{13}$CO$_2$ was more than 4.8‰, this was considered as a positive result.

**Serosology**

Blood samples for serological evaluation were obtained before EGD. A serological assay for IgG antibodies against *H. pylori* was performed by an indirect solid-phase immunochromatographic assay using the ASSURE™ *H pylori* rapid test kit (Genelabs Diagnostics, Cavendish Singapore Science Park, Singapore). The sensitivity and specificity of the assay were 96% and 92%, respectively according to the manufacturer’s instructions.

**Gold standard definition**

A patient was classified as being *H pylori*-positive on the basis of at least three positive results of RUT, histology, UBT and serological tests.

**Statistical analysis**

Statistical tests were performed using the SPSS system. Sensitivity, specificity, accuracy, predictive values of positive and negative results were calculated in accordance with standard methods. $\chi^2$ test and 95%CI were used to compare the sensitivity, specificity and accuracy of different diagnostic methods. Two-sample $t$-tests were used to compare the excess $\delta^{13}$CO$_2$ values of UBT between the true-positive and false-negative groups of various invasive tests. $P<0.05$ was considered statistically significant.

**RESULTS**

Of the 60 patients initially enrolled in this study, five did not complete all the tests and were excluded from the statistical analysis. Table 1 presents the demographic data of the remaining 55 patients (37 males, 18 females; mean age: 62.2±14.5 years) who finished all of the invasive and non-invasive assays. EGD of these subjects showed a gastric ulcer in 19 patients (35%), a duodenal ulcer in 13 (24%) and both gastric and duodenal ulcers in 23 (42%). According to the gold standard definition, 35 (63.6%) were *H pylori*-positive and 20 (36.4%) were *H pylori*-negative.

**Comparison of various tests for detecting *H pylori* infection in bleeding peptic ulcers**

Table 2 presents the sensitivity, specificity and accuracy of various tests in diagnosing *H pylori* infection. The sensitivities of RUT, histology and culture were low (66%, 43% and 57%, respectively) although these assays exhibited a high specificity (95-100%). Their overall accuracies were 76%, 62% and 60%, respectively. Among the four biopsy-based methods, only PCR exhibited the satisfactory sensitivity (91%), specificity (100%), and accuracy (94%). Its sensitivity and accuracy were significantly higher than those of RUT, histology and culture (sensitivity: $P<0.05$, $P<0.001$ and $P<0.001$, respectively; accuracy: $P<0.05$, $P<0.001$, $P<0.001$, respectively).

Of the non-invasive assays, the serological test had a high sensitivity (95%) but its specificity (65%) was lower than that of PCR ($P<0.05$). The sensitivity (94%), specificity (85%) and accuracy (91%) of UBT were similar to those of PCR. Among all of the tests investigated herein, PCR and UBT were the most accurate methods for diagnosing *H pylori* infection in patients with bleeding peptic ulcers.

**Relationship between intragastric blood and sensitivity of various tests in diagnosis of *H pylori* infection**

The patients were divided into two groups according to the presence or absence of blood in the stomach, to investigate the relationships between intragastric blood and sensitivities.

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**Table 1** Baseline characteristics of patients with bleeding peptic ulcers (n, %)

| Number of patients (n = 55) |
|-----------------------------|
| Age (mean±SD)                |
| 62.2 (14.5)                 |
| Sex                         |
| Male                        |
| 37 (67)                     |
| Female                      |
| 18 (33)                     |
| Smoking                     |
| Alcohol consumption         |
| 13 (24)                     |
| Coffee consumption          |
| 5 (9)                       |
| Ingestion of tea            |
| 18 (33)                     |
| Ingestion of NSAID$^1$      |
| 26 (47)                     |
| Sites of ulcer              |
| Stomach                     |
| 19 (35)                     |
| Duodenum                    |
| 13 (24)                     |
| Stomach and duodenum        |
| 23 (42)                     |
| Ulcer lesions               |
| Bleeding visible vessel     |
| 2 (4)                       |
| Non-bleeding visible vessel |
| 3 (6)                       |
| Adherent clot               |
| 15 (27)                     |
| Red or black spot           |
| 21 (38)                     |
| Clean base                  |
| 14 (26)                     |

$^1$NSAID: non-steroidal anti-inflammatory drugs.

**Table 2** Sensitivity, specificity, and accuracy of various tests for diagnosis of *H pylori* infection in bleeding peptic ulcers (n = 55) [%/(95%CI)]

|                      | Sensitivity | Specificity | Accuracy | PPV       | NPV        |
|----------------------|-------------|-------------|----------|-----------|------------|
| RUT                  | 66 (49–82)$^a$ | 95 (84-100) | 76 (64–88)$^a$ | 96 (87–100) | 61 (43–79)$^a$ |
| Histology            | 43 (26–60)$^a$ | 95 (84-100) | 62 (48–75)$^a$ | 100       | 48 (31–64)$^a$ |
| Culture              | 37 (20–54)$^a$ | 100         | 60 (46–73)$^a$ | 100       | 48 (32–63)$^a$ |
| PCR                  | 91 (82–100)  | 100         | 95 (88–100) | 100       | 87 (72–100)  |
| Serology             | 94 (86–100)  | 65 (42–88)$^a$ | 84 (73–93) | 83 (70–95)$^a$ | 87 (67–100) |
| UBT                  | 94 (86–100)  | 85 (68–100) | 91 (83–98) | 92 (82–100) | 90 (74–100)  |

$^aP<0.05$ vs PCR; $^bP<0.01$ vs PCR; $^cP<0.001$ vs PCR.
of biopsy-based tests for *H. pylori* infection. The sensitivities of RUT, histology, culture, PCR, serology and UBT were 29%, 21%, 21%, 79%, 93% and 100% in the patients with intragastric blood, respectively, and 90%, 57%, 48%, 100%, 95% and 91% in the patients without intragastric blood, respectively. There were statistically significant differences in the sensitivities of RUT, histology and PCR between the patients with and without blood in the stomach (Figure 1; \(P<0.01\), \(P<0.05\) and \(P<0.05\), respectively). Additionally, there was a trend towards decreased sensitivity of culture in the patients with blood in the stomach \((P = 0.09)\).

**DISCUSSION**

The *H. pylori* status in a patient presenting with a bleeding ulcer must be documented to determine the method of further management. However, many studies have disclosed that biopsy-based tests including RUT, histology and culture have low sensitivities in detecting *H. pylori* in bleeding peptic ulcers\(^5,12,13\). Additionally, two recent reports\(^{20,27}\) also revealed a lack of accuracy in *H. pylori* stool antigen (HpSA) tests in patients with ulcer bleeding. In this study, we also demonstrated that the sensitivities of RUT, histology and culture in bleeding peptic ulcer were only 66%, 43% and 37%, respectively. However, PCR could reach a diagnostic sensitivity of 91%, much higher than that of RUT, histology and culture. Additionally, the test had 100% specificity in diagnosing *H. pylori* infection under bleeding conditions. This, therefore, is the most accurate biopsy-based method for determining *H. pylori* status in bleeding peptic ulcers.

UBT is one of the non-invasive methods for diagnosing *H. pylori* infection. Both its sensitivity and specificity are greater than 90% in patients with bleeding peptic ulcers. Its accuracy is comparable to that of PCR. Although the serological test has a high sensitivity in patients with bleeding peptic ulcers, it may not be a good choice for diagnosing *H. pylori* infection in patients with a complicated ulcer because antibody tests lack a good specificity (only 65%). The presence of anti-*H. pylori* IgG antibody implies prior exposure to the organisms, but does not imply the presence of a current infection. If a patient presenting with ulcer bleeding has a false-positive antibody test and is treated by eradication therapy, the physician may mistakenly believe that the cause of bleeding has been removed and will then not provide further preventive therapy to the patient. In such a case, the patient would have a high risk of recurrent bleeding\(^{10}\). Therefore, establishing the *H. pylori* status with certainty at the time of bleeding episode is quite important.

Following this work, we recommend that an endoscopist may initially perform a RUT to detect *H. pylori* infection in a patient with bleeding peptic ulcer because of its simplicity, low cost, moderate sensitivity and excellent specificity. If the RUT is negative, either PCR or UBT can be used for the definite diagnosis of *H. pylori* status. The choice of the final diagnostic modality may depend on the availability of tests in the hospital. Nonetheless, the aim of PCR is to detect specific DNA sequences rather than the whole viable bacterium. No special requirements pertain to the treatment, transport, or storage of the biopsy specimens for PCR\(^{20-29}\). Several laboratories have reported the successful detection of *H. pylori* by PCR from a biopsy specimen placed and transported by mail in the RUT\(^{36,40,41}\). This capability is particularly useful for a gastroenterologist who does not have access to laboratory facilities and requires a confirmation of the RUT\(^{18}\).

In this study, the sensitivities of RUT, histology, culture and PCR were found to be 29%, 21%, 21% and 79% in patients with intragastric blood, and 90%, 57%, 48% and 100% in patients without intragastric blood. These data imply that blood may reduce the diagnostic yield of all endoscopic biopsy tests in patients with bleeding peptic ulcers. Additionally, we also demonstrated that the bacterial load in the false-negative group of culture was significantly lower.
than that in the true-positive group. Furthermore, there was also a trend toward decreased bacterial load in the false-negative group of histological examination and PCR assay. The decreased bacterial density therefore, may be the major cause for decreased sensitivities of all biopsy-based assays. There are several possible reasons for the aforementioned findings. The decreased bacterial load in bleeding ulcer patients may be related to a direct suppression effect of intraluminal blood on H pylori, the administration of antisecretory drugs, or the removal of some H pylori from the gastric epithelium or mucus by gastric lavage before EGD[32]. Recently, Leung et al[33] reported that a false-negative result of RUT in bleeding ulcer might be caused by the buffered effects of blood. An in vitro study[34] also showed that sheep’s blood inhibited the growth of H pylori in broth media. The exact reason concerning the association between bleeding ulcer and intragastric bacterial density merits further investigations.

In conclusion, PCR is the most accurate biopsy-based method for determining H pylori status in patients with bleeding peptic ulcers. RUT, histology and culture have a poor sensitivity under bleeding conditions. A decline in the intragastric bacterial density during bleeding ulcer may be a major cause of the reduced sensitivity of biopsy-based assays.

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