Helicobacter pylori infection in bleeding peptic ulcer patients after non-steroidal antiinflammatory drug consumption

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Abstract

AIM: To establish the prevalence of Helicobacter pylori (H. pylori) infection in patients with a bleeding peptic ulcer after consumption of non-steroidal antiinflammatory drugs (NSAIDs).

METHODS: A very early upper endoscopy was performed to find the source of upper gastrointestinal bleeding and to take biopsy specimens for analysis of H. pylori infection by the rapid urease (CLO) test, histological examination, and bacterial culture. IgG anti-CagA were also sought. The gold standard for identifying H. pylori infection was positive culture of biopsy specimens or contemporary positivity of the CLO test and the presence of H. pylori on tissue sections.

RESULTS: Eighty patients, 61 males (76.3%), mean age 61.2 ± 15.9 years, were consecutively enrolled. Forty-seven (58.8%) patients occasionally consumed NSAIDs, while 33 (41.3%) were on chronic treatment with low-dose aspirin (LD ASA). Forty-four (55.0%) patients were considered infected by H. pylori. The infection rate was not different between patients who occasionally or chronically consumed NSAIDs. The culture of biopsy specimens had a sensitivity of 86.4% and a specificity of 100%; corresponding figures for histological analysis were 65.9% and 77.8%, for the CLO test were 68.2% and 75%, for the combined use of histology and the CLO test were 56.8% and 100%, and for IgG anti-CagA were 90% and 98%. The highest accuracy (92.5%) was obtained with the culture of biopsy specimens.

CONCLUSION: Patients with a bleeding peptic ulcer after NSAID/LD ASA consumption frequently have H. pylori infection. Biopsy specimen culture after an early upper gastrointestinal tract endoscopy seems the most efficient test to detect this infection.

Key words: Helicobacter pylori; Helicobacter pylori infection; Low-dose aspirin; Non-steroidal antiinflammatory drugs; Peptic ulcer hemorrhage; Endoscopy

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INTRODUCTION

Acute upper gastrointestinal (GI) bleeding is a life-threatening emergency frequently observed in patients admitted to tertiary care hospitals, with peptic ulcer bleeding accounting for approximately 50% of cases\(^1\). A high number of these emergency admissions for upper GI bleeding are attributable to non-steroidal anti-inflammatory drug (NSAID) use, especially in older patients\(^2,3\).

The fact that peptic ulcers can be effectively treated by acid suppression strongly suggests that it is largely a disease of acid hypersecretion\(^3,5\), although there is much evidence for a role of Helicobacter pylori (H. pylori) infection\(^6\), and NSAID-induced injury\(^3\). Published data about the combined role of H. pylori infection and NSAID use in patients with peptic ulcer bleeding are conflicting. H. pylori infection has been demonstrated in a variety of studies, with a considerable degree of consistency, to increase the risk of NSAID-related GI injury\(^7\). Moreover, a recent meta-analysis indicated that prophylactic H. pylori eradication may help to reduce the risk of both gastric and duodenal ulcers and their complications, including bleeding, in chronic users of non-steroidal anti-inflammatory drugs (NSAIDs)\(^8\). Other studies on the interaction between H. pylori infection and low-dose aspirin (LD ASA) use in patients with a history of upper GI bleeding demonstrated the protective role of H. pylori eradication on rebleeding. In these patients, H. pylori eradication may allow the use of LD ASA instead of other antithrombotic drugs\(^9\). On the other hand, some studies evidenced a lower rate of H. pylori infection in patients with bleeding peptic ulcers than in patients with uncomplicated peptic disease. In particular, a negative interaction between H. pylori infection and NSAID use was postulated, indicating a lower risk of bleeding in ulcer patients taking NSAIDs\(^10\).

H. pylori infection can be diagnosed by invasive techniques requiring endoscopy and biopsy (histological examination, culture, and rapid urease test) and by non-invasive tests (serology, urea breath test, detection of H. pylori antigen in stool specimen). With the exception of the culture test, a single test has not reached acceptable accuracy for the diagnosis of H. pylori infection\(^11\). The rapid urease test and histological examination may indicate the presence of any urease-producing or helix-shaped bacteria, respectively. Moreover, serological tests are markers of exposure to H. pylori but do not indicate whether active infection is ongoing\(^10,11,12\). The results of the urea breath test are influenced by factors related to the patient, the bacteria, and the test itself\(^13\). Rapid gastric emptying, contamination with oral commensals, achlorhydria, and gastric atrophy may cause false positive results, while false negative results can occur through suppression of urease activity if the breath test is performed too soon after antibiotic or acid suppression therapy. The detection of H. pylori antigen in stools has some limitations related to bowel movements: a short transit time could favor elimination of unaltered antigens, while constipation could lead to degradation of the antigens\(^14\). Finally, in patients with recent upper GI bleeding the diagnosis of H. pylori infection can be challenging\(^15\). In fact, the hemorrhage itself (given the pH buffering effect of blood in the GI tract), the use of proton pump inhibitors and antibiotics may influence the results of invasive and non-invasive tests for H. pylori\(^14,17\).

Our study was planned to give further information about the prevalence of H. pylori infection in occasional and chronic NSAID/LD ASA users admitted for peptic ulcer bleeding and submitted to very early upper endoscopy with a concomitant search for H. pylori.

MATERIALS AND METHODS

Ethics

The study was approved by the Institutional Review Board of the A. Cardarelli Hospital of Naples, Italy, and was conducted in compliance with the Declaration of Helsinki (1964 and following amendments), current Good Clinical Practices and the applicable European and local regulatory requirements.

Study design

This was a single-center, observational, prospective, registered study (EMOFANS Study: ACTRN12607000521426) carried out in the A. Cardarelli Hospital, a high volume hospital dedicated to emergencies. This study was planned to obtain information about the prevalence of H. pylori infection in patients regularly or occasionally consuming NSAIDs/LD ASA who were admitted for peptic ulcer disease complicated by hemorrhage. Taking into account our previous data, we calculated that it would be possible to recruit a total of 80 consecutive patients with the characteristics required by the protocol within a period of 12 mo. The primary objective of the study was to establish the prevalence of H. pylori infection in patients consecutively admitted to the emergency unit with upper GI bleeding from complicated peptic ulcer, who had been on chronic treatment or occasionally consumed NSAIDs/LD ASA. A secondary objective was to compare the efficiency of invasive (culture of biopsy specimens, H. pylori on tissue sections, rapid urease test) and non-invasive (IgG anti-CagA) techniques for the detection of H. pylori infection.
Inclusion and exclusion criteria

We recruited patients who fulfilled the following criteria: (1) male or female patients, of any ethnic origin, 18 years or more of age, who provided written informed consent prior to any study-related procedures and who were, in the opinion of the investigator, able to understand and to follow the protocol and likely to comply with all the requirements of the study; (2) patients with peptic ulcer disease complicated by hemorrhage (hematemesis, melaena, hematochezia, or with other clinical signs of blood loss, i.e., hemodynamic instability with hypotension and tachycardia) in the 72 h before admission; (3) patients on chronic treatment with NSAIDs/LD ASA or who had received occasional treatment with NSAIDs in the 30 d before admission. Treatment with LD ASA was defined as the continuous use of up to 300 mg of aspirin per day for prophylaxis against vascular occlusive diseases; and (4) patients with an ulcer, defined as a lesion with loss of mucosal integrity and continuity of ≥ 5 mm with an apparent depth of ≥ 1 mm, as measured using gastric biopsy forceps as standard. The exclusion criteria were: (1) patients who had received treatment with antibiotics or proton pump inhibitors within the 4 wk prior to potential enrolment in order to avoid false negative H. pylori results; (2) a history of previous major upper GI surgery; (3) patients with upper GI neoplastic ulcer; and (4) patients already hospitalized for other reasons.

Data collection

The patients’ details were collected in a database and included: (1) demographic data; (2) comorbidities according to the Charlson Comorbidity Index; (3) clinical and biochemical parameters; (4) occasional or regular use of NSAIDs/LD ASA; (5) other treatments taken; (6) endoscopy findings; (7) histology findings; (8) serological findings; and (9) microbiology results. The American Society of Anesthesiology classification of physical status was calculated for each patient at admission prior to the endoscopy. Lesions were localized and classified according to the Forrest classification (Table 1), and the Rockall risk score was also calculated after endoscopy.

Helicobacter pylori detection

The study plan included very early upper endoscopy, defined as within 6 h of arrival at the hospital, or as soon as possible after hemodynamic stabilization, to evaluate the source of upper GI bleeding and to take biopsies in eligible subjects. Gastric biopsies were obtained during endoscopy after successful hemostasis if needed. Biopsy specimens were taken from the antrum and gastric body to search for H. pylori, according to guidelines, and from all suspicious lesions. In particular, two specimens from the antrum as well as two specimens from the gastric body were used to perform the rapid urea (CLO) test (GASTREX, Warsaw, Poland) with separate kits. Moreover, two biopsy specimens from the antrum as well as two specimens each from the anterior and posterior parts of the gastric body were cultured for H. pylori. Finally, two biopsy specimens from the antrum and two specimens from the body were taken to detect H. pylori infection on tissue sections after modified Giemsa staining. At the same time a blood sample was collected and IgG antibodies against CagA protein (DIA.PRO, Diagnostic Bioprobes Srl, Milan, Italy) were analyzed by enzyme immunoassays. CagA antibody titers (> 5 U/mL) were classified as positive according to the manufacturer’s instructions.

The CLO test was carried out at room temperature, with the sample examined at 24 h, and considered positive when the appropriate color change (yellow to red) occurred. With regards to culture of biopsy specimens, primary isolation was performed on commercial selective Pylori agar (BioMérieux, 43263, Marcy-L’Étoile, France). Following primary selective isolation, H. pylori strains were identified by usual phenotypic tests (Gram stain and by oxidase, catalase and urease tests). To overcome the instability of H. pylori in biopsy material during its transport from the collection site to the laboratory, which is a limiting factor for culture and susceptibility testing, the biopsy specimens for the bacterial culture were immediately placed in an appropriate transport medium (Portagerm-Pylori, BioMérieux, 42041, Marcy-L’Étoile, France). The biopsy histology was interpreted by a GI pathologist blind to the patient’s information and the results of the other H. pylori tests.

The gold standard for identifying H. pylori infection was a positive culture of biopsy specimens or contemporaneous positivity for the CLO test and the presence of H. pylori on tissue sections, in accordance with current guidelines.

Statistical analysis

Continuous data are expressed as means and standard deviations and compared by an independent samples t test. Categorical variables were analyzed by Pearson’s chi-square test or Fisher’s exact test. All tests of significance were two-sided. A P-value less than 0.05 was considered statistically significant. The PASW (Predictive Analytics Software) Statistics for Windows (Release 18.0.0-Jul 30, 2009; SPSS Inc., Chicago, Ill., United States) was used for the statistical analyses. Sensitivity, specificity, positive and negative predictive values, and false positive and false negative rates were determined using StatsDirect statistical software (release 2.7.8, March 15, 2010). The diagnostic accuracy was calculated as follows: Overall Ac-

### Table 1 Forrest classification

| Description               |
|---------------------------|
| 1 Actively bleeding ulcer |
| 1a: Spurtting             |
| 1b: Oozing                |
| 2 Non-actively bleeding ulcer |
| 2a: Non-bleeding visible vessel |
| 2b: Ulcer with surface clot |
| 2c: Ulcer with red or dark blue spots |
| 3 Ulcer with clean base   |

Manguso F et al. Bleeding peptic ulcers and H. pylori infection
**RESULTS**

**Study population**

Eighty consecutive patients (61 male, 19 female; mean age 61.2 ± 15.9 years (range, 21-85)) with upper GI bleeding from complicated peptic ulcer disease and on treatment with NSAIDs/LD ASA before admission, were enrolled. All patients were admitted to the emergency unit of the A. Cardarelli Hospital of Naples between January and December 2008. The characteristics of the study population are summarized in Table 2. No patients had a history of *H. pylori* eradication. In 67 (83.8%) patients endoscopic examinations took place within 6 h of arrival at the hospital, while in the remaining subjects endoscopies were delayed because of the patients’ condition and were performed within 24 h. The site of the ulcers was duodenal in 41 patients (51.3%), gastric in 29 (36.3%), and in both segments in the remaining 10 patients (12.5%). In 14 cases (17.5%) the ulcers were classified as F1, in 23 (28.8%) as F2, and in 43 (53.8%) as F3 (Table 2). Six (7.5%) patients suffered rebleeding. None required surgery for bleeding or died during hospitalization. All patients were given proton-pump inhibitors intravenously in the emergency area and orally thereafter.

**Consumption of non-steroidal antiinflammatory drugs**

Most of the patients had occasionally consumed NSAIDs, particularly for a fever or moderate pain. In detail, 36 patients (45.0%) had occasionally consumed only one NSAID before their bleeding event, with one of these on concomitant chronic treatment with an antiaggregant (ticlopidine). Nine (11.3%) and two (2.5%) patients had consumed two and three NSAIDs in sequence, respectively. Thirty three patients (41.3%) were on chronic treatment with LD ASA for primary or secondary prevention of cardiovascular diseases, 18

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**Table 2** Demographic, clinical and endoscopic characteristics of all patients with bleeding from peptic ulcers after consumption of nonsteroidal anti-inflammatory drugs, grouped according to whether they did or did not have *Helicobacter pylori* infection (n (%))

| Patients | Helicobacter pylori negative | Helicobacter pylori positive | P-value |
|----------|------------------------------|-----------------------------|---------|
|          | (n = 80)                     | (n = 36)                    | (n = 44) |       |
| Males    | 61 (76.3)                    | 28 (77.8)                   | 33 (75.0) | 0.771 |
| Age (yr) (mean ± SD) | 61.2 ± 15.9               | 60.6 ± 18.3                 | 61.7 ± 13.9 | 0.767 |
| Smoker   | 43 (53.8)                    | 21 (58.3)                   | 22 (50.0) | 0.703 |
| Non-smoker | 26 (32.5)                 | 10 (27.8)                   | 16 (36.4) |       |
| Current  | 11 (13.8)                    | 5 (13.9)                    | 6 (13.6)  |       |
| Ex-smoker | 43 (53.8)                 | 17 (47.2)                   | 26 (59.1) |       |
| Symptoms on presentation | 11 (13.8)                  | 4 (11.1)                    | 7 (15.9)  | 0.721 |
| Hematemesis | 58 (72.5)                 | 26 (72.2)                   | 32 (72.7) |       |
| Melena   | 11 (13.8)                    | 6 (16.7)                    | 5 (11.4)  |       |
| Gastritis and melena | 11 (13.8)                  | 6 (16.7)                    | 5 (11.4)  |       |
| Initial mean hemoglobin (g/dL) | 9.2 ± 2.3                  | 8.7 ± 2.2                   | 9.6 ± 2.4  | 0.076 |

American Society of Anesthesiology class

| 1-2                  | 62 (77.5)                | 26 (72.2)                   | 36 (81.8) | 0.600 |
| 3                   | 35 (43.8)                | 8 (22.2)                    | 7 (15.9)  | 0.600 |
| 4                   | 3 (3.8)                  | 2 (5.6)                     | 1 (2.3)   | 0.183 |

Complete Rockall score

| 0-2                  | 39 (48.8)                | 19 (52.8)                   | 20 (45.5) | 0.813 |
| 3-5                 | 34 (42.5)                | 12 (33.3)                   | 22 (50)   |       |
| 6-8                 | 7 (8.8)                  | 5 (13.9)                    | 2 (4.5)   |       |

Comorbidities

| Occasional consumption of NSAIDs | 47 (58.8)                | 19 (52.8)                   | 28 (63.6) | 0.326 |
| Patients on chronic LD ASA | 33 (41.3)                | 17 (47.2)                   | 16 (36.4) | 0.326 |
| Other antiplatelet drugs | 5 (6.3)                  | 2 (5.6)                     | 3 (6.8)   | 0.999 |
| Anticoagulants | 2 (2.5)                  | 2 (5.6)                     | 0 (0)     | 0.199 |
| Other drugs | 39 (48.8)                | 15 (41.7)                   | 24 (54.5) | 0.252 |
| Locations of ulcers | Duodenum alone         | 41 (51.3)                   | 21 (58.3) | 0.515 |
| Stomach alone | 29 (36.3)                | 11 (30.6)                   | 18 (40.9) |       |
| Stomach and duodenum | 10 (12.5)                | 4 (11.1)                    | 6 (13.6)  |       |
| Forrest   | 1a                     | 2 (2.5)                     | 1 (2.3)   | 0.684 |
|           | 1b                     | 12 (15.0)                   | 6 (16.7)  |       |
|           | 2a                     | 4 (5.0)                     | 1 (2.8)   |       |
|           | 2b                     | 3 (3.8)                     | 1 (2.8)   |       |
|           | 2c                     | 16 (20.0)                   | 10 (27.8) |       |
|           | 3                     | 43 (53.8)                   | 17 (47.2) |       |

NSAID: Nonsteroidal anti-inflammatory drug; LD ASA: Low dose aspirin. According to the Charlson Comorbidity Index. Because of rounding, not all percentages total 100.
Among 44 infected, 25 (56.8%) had contemporary positivity of the CLO test and the presence of *H. pylori* on tissue sections, and 38 (86.4%) had a positive culture of biopsy specimens. With regards to IgG anti-CagA, among the infected and non-infected patients 26 (59.1%) and 10 (27.8%), respectively, had antibody titers > 5 U/mL.

Sensitivity, specificity, positive and negative predictive values, false positive rate, false negative rate and accuracy for all techniques are shown in Table 5. Culture of biopsy specimens had a sensitivity of 86.4% and a specificity of 100%. In this analysis, the sensitivities and specificities of the remaining tests were 65.9% and 77.8%, respectively, for histological analysis; 68.2% and 75.0%, respectively, for the CLO test; 56.8% and 100%, respectively, for the combined use of histology and the CLO test; and 90.0% and 98.0%, respectively, for the anti-CagA test. The highest accuracy (92.5%) was obtained with the culture of biopsy specimens.

The 80 patients with bleeding ulcers were divided into two groups for further analysis on the basis of presence or absence of *H. pylori* infection. These two groups were identical with regards demographic, clinical and endoscopic parameters (Table 2).

### DISCUSSION

The reported prevalence of *H. pylori* infection in healthy persons (without GI illness) among many studies ranges from a minimum of 11% to a maximum of 69%, with some of the variability depending on the socioeconomic status of the country of the patients investigated.

The prevalence of *H. pylori* infection in patients with peptic ulcer disease is not well established as yet, especially because the prevalence of non-NSAID non-*H. pylori* ulcer is rising in the West, with current good evidence that 20%-40% of peptic ulcers are not associated with *H. pylori* infection or the use of NSAIDs. In one study it was found that, after excluding NSAID users, only 61% of patients with peptic ulcers had *H. pylori* infection. Data about *H. pylori* infection in patients with peptic ulcer disease complicated by hemorrhage, chronically or occasionally treated with NSAIDs/LD ASA are scarce. Such data might be difficult to collect, especially because of the choice of appropriate tests to detect *H. pylori* infection, and the timing of their performance in patients who bleed.

In our study *H. pylori* infection was found in 55% of patients with peptic ulcer disease complicated by hemorrhage after consumption of NSAIDs/LD ASA, who were not on treatment with antibiotics or proton-pump inhibitors. The Italian National Project for Gastrointestinal Bleeding (PNED) study reported a prevalence of *H. pylori* infection of 44.3% among patients who had bleeding from a non-variceal upper GI source, when the infection was confirmed by any of the accepted methods.

### Table 3 Patients’ distribution on the basis of occasional or chronic consumption of nonsteroidal anti-inflammatory drugs

| Occasional NSAID use                   | Chronic NSAID use                     |
|----------------------------------------|---------------------------------------|
| One NSAID                              | LD ASA alone                           |
| Two NSAIDs (in sequence)               | LD ASA + another NSAID                 |
| Three NSAIDs (in sequence)             | LD ASA + two other NSAIDs (in sequence) |
| NSAID + ticlopidine                     | LD ASA + ticlopidine                   |
| Chronic NSAID use                      | LD ASA + clopidogrel + NSAID           |
| LD ASA + LMWH                          | LD ASA + LMWH + 2 NSAIDs (in sequence) |

Data are expressed as number (percentage). NSAID: Non-steroidal anti-inflammatory drug; LD ASA: Low dose aspirin; LMWH: Low-molecular-weight heparin. *NSAIDs consumed occasionally. Because of rounding, not all percentages total 100.

### Table 4 Frequency of positive diagnostic tests for Helicobacter pylori in culture-positive or culture-negative patients

|                   | Culture-positive (n = 38) | Culture-negative (n = 42) |
|-------------------|---------------------------|---------------------------|
| CLO test + tissue section-positive | 19 (23.8)                | 6 (7.5)                   |
| CLO test positive  | 5 (6.3)                   | 9 (11.3)                  |
| Tissue section-positive | 4 (5.0)                   | 8 (10.0)                  |

Data are expressed as number (percentage).

(22.5%) on treatment with LD ASA alone, eight (10.0%) had occasionally consumed another NSAID, and one (1.3%) had taken two other NSAIDs in sequence. Four (5.0%) patients were on treatment with ticlopidine or clopidogrel (one of whom had occasionally consumed a NSAID), and two (2.5%) were receiving low-molecular-weight heparin (one of whom had consumed two NSAIDs in sequence) (Table 3). Thirty-nine (48.8%) patients were on treatment with other drugs considered not harmful to the intestinal mucosa. Twenty-eight out of 47 (59.6%) patients who occasionally consumed NSAIDs and 16/33 (48.5%) on chronic treatment with LD ASA were considered infected by *H. pylori*, with a statistically significant difference between the two groups (P = 0.326).

### Diagnostic tests for Helicobacter pylori

Among the 80 bleeding patients, 38 (47.5%) had positive cultures of biopsy specimens, 37 (46.3%) had positive histopathological findings, 39 (48.8%) had a positive CLO test, and 36 (45.0%) were positive for IgG anti-CagA. The frequency of positive diagnostic tests for *H. pylori* in culture-positive or culture-negative patients is summarized in Table 4. Contemporaneous positivity of the CLO test and the presence of *H. pylori* on tissue sections were found in 25 (31.3%) patients. In particular, among 42 patients who had a negative culture of biopsy specimens, 6 (14.3%) had contemporary positivity of the CLO test and the presence of *H. pylori* on tissue sections. On the other hand, among 55 patients who did not have contemporary positivity of the CLO test and the presence of *H. pylori* on tissue sections, 19 (34.5%) had a positive culture of biopsy specimens. In accordance with the pre-established gold standard, 44 (55.0%) patients were considered infected by *H. pylori*. Among 44 infected, 25 (56.8%) had contemporary positivity of the CLO test and the presence of *H. pylori* on tissue sections, and 38 (86.4%) had a positive culture of biopsy specimens.
presence of the infection was determined by histological examination performed during the patients’ stay in hospital; 36% of the patients were on treatment with NSAIDs\[31\]. In our study, 46.3% of patients had positive histopathological findings, confirming the results of the PNED study\[31\]. Another study performed in patients who bled from peptic ulcers (57.4% users of NSAID and/or antiplatelet drugs) found, by histological examination and the CLO test, an overall prevalence of H. pylori infection of 53.7%. In detail, the prevalences according to the histological examination and the CLO test were 42.3% and 44.8%, respectively\[32\]. In a study by Schilling et al the CLO test was positive in 50% of patients, while H. pylori infection was detected by the 13C-urea breath test and histological examination (gold standard) in 62% of the cases\[33\]. Our study showed positive CLO test results in 48.8% of cases.

Data about the use of culture of biopsy specimens for the detection of H. pylori in bleeding patients are scarce. Three studies performed between 1998 and 2000 involving a total of 314 patients showed percentages of H. pylori-positive patients from 24.7% to 69.1%\[34\]-\[36\]. In another study involving children with upper GI bleeding, H. pylori infection was considered to be present when histology and/or culture were positive; unfortunately, data about the culture test alone were not presented, but H. pylori infection was detected in 48.8% of patients, with 29.8% of the children on treatment with NSAIDs\[37\]. In our study 47.5% of patients had a positive culture of biopsy specimens.

We used a restrictive gold standard to consider a patient infected by H. pylori. In view of its absolute specificity, if culture alone was positive the patient was considered H. pylori-positive\[38\]. Moreover, because a patient with at least two positive tests should be considered H. pylori-positive\[38\], we used as adjunctive gold standard, the contemporary positivity of the CLO test and the presence of H. pylori on tissue sections. In fact, the concomitant positivity of the rapid urease test and of the histological examination indicates, with the highest probability, the presence of helicase-producing bacteria.

Six patients whose cultures were negative showed contemporary positivity for the CLO test and the presence of H. pylori on tissue sections. This finding demonstrates that culture tests are responsible for a number, albeit low; of false negative results. On the other hand, by using the second gold standard, only 25 (31.3%) patients would have been considered infected by H. pylori, even though 37 (46.3%) had positive histopathological findings and 39 (48.8%) had a positive CLO test. Moreover, among 55 patients who did not have contemporary positivity of the CLO test and the presence of H. pylori on tissue sections, 19 had a positive culture of biopsy specimens. These results indicate that by using the criterion of at least two positive tests to consider patients as infected by H. pylori, many false negative results can be expected.

In our study the combined use of three invasive tests performed during a very early upper endoscopy was adequate for the diagnosis of H. pylori infection. With regards to the non-invasive test, 36 (45%) patients were positive for IgG anti-CagA. This test showed a low sensitivity and specificity confirming that it is a rather inaccurate diagnostic method which cannot be recommended as the first diagnostic test for H. pylori infection.

Patients with complicated peptic ulcer disease are candidates for testing for H. pylori infection. Indeed, accurate and early diagnosis of H. pylori infection is a critical clinical problem in these patients. The discovery of the link between the H. pylori bacterium and peptic ulcer is one of the greatest breakthroughs in medical history, but it is surprising that so far a large bulk of data has led to discordant results in patients with hemorrhagic complicated disease, treated or not with NSAIDs. It has been suggested that the rate of H. pylori infection is lower in patients with bleeding peptic ulcer than in patients with uncomplicated peptic disease, and that there is a negative interaction between H. pylori infection and NSAID use\[38,10\]. Our study demonstrated that many patients with peptic ulcer disease complicated by hemorrhage and consuming NSAIDs/LD ASA are actually infected (55%), and that the infection may be detected with appropriate tests performed during a very early endoscopy. Culture of biopsy specimens appears to be more efficient than other techniques at detecting H. pylori infection (accuracy 92.5%). We believe that the discordant data in the literature are due to the different cohorts of

Table 5

| Test                                      | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | NPV (95% CI) | Accuracy (%) | False positive (%) | False negative (%) |
|-------------------------------------------|----------------------|----------------------|-------------|--------------|--------------|-------------------|-------------------|
| Culture of biopsy specimens               | 38/44 (36/36)        | 38/38 (36/36)        | 38/38 (36/36) | 38/38 (36/38) | 74/80 (72.2-85.8) | 0/36 (0-6.1)       | 6/44 (13.6-13.6)   |
| Helicobacter pylori on tissue sections     | 29/44 (28/28)        | 29/37 (28/28)        | 29/37 (28/28) | 29/37 (28/37) | 57/80 (54.8-85.8) | 8/36 (2.2-13.6)    | 15/44 (31.3-31.3)  |
| Rapid urease test                         | 30/44 (28/28)        | 30/39 (28/28)        | 30/39 (28/39) | 30/39 (28/39) | 57/80 (54.8-85.8) | 9/36 (2.2-13.6)    | 14/44 (28.1-28.1)  |
| Helicobacter pylori on tissue sections and rapid urease test | 25/44 (24/24)        | 25/25 (24/24)        | 25/25 (24/25) | 25/25 (24/25) | 61/80 (58.3-83.8) | 0/36 (0-6.1)       | 19/44 (31.3-31.3)  |
| Anti-CagA                                  | 26/44 (26/26)        | 26/26 (26/26)        | 26/26 (26/26) | 26/26 (26/26) | 52/80 (50.1-83.8) | 10/26 (6.3-13.6)   | 18/44 (31.3-31.3)  |

CI: Confidence interval; NPV: Negative predictive value; PPV: Positive predictive value.

\[34\] Manguso F et al. Bleeding peptic ulcers and H. pylori infection.
patients studied, the kinds of invasive/non-invasive test used, the timing at which the tests were performed, the contemporary use of proton-pump inhibitors or antibiotics, and resources available in the context in which the patient is admitted. We had two ideal conditions for eliminating some of these sources of variability: (1) the presence of a rota of gastroenterologists skilled in diagnostic and therapeutic measures available 24 h a day, 7 d a week (not as a 24-h “on call” service), and able to enroll all consecutive patients; and (2) close collaboration between specialist gastroenterologists and microbiologists, with the possibility of methodologically sound performance of the culture tests, which is a tedious, time-consuming procedure that can be influenced by the transport conditions from the endoscopy room to the laboratory and the speed of processing, because the viability of the organism is reduced by exposure to atmospheric oxygen. Finally, we did not find a statistically significant difference in the percentage of H. pylori infections between patients who occasionally consumed NSAIDs and those on chronic treatment with LD ASA, indicating that chronic consumption does not modify the infection rate.

In conclusion, faced with a person with a bleeding peptic ulcer we suggest that invasive methods should be used to identify H. pylori infection. The accuracy of results of biopsy specimen culture in patients with peptic ulcer bleeding remains very high, and the sensitivity and specificity of this method do not seem to be affected by blood in the stomach or by the use of NSAIDs or LD ASA, when performed after a very early upper endoscopy.

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COMMENTS

Background
Helicobacter pylori (H. pylori) has been considered as a major cause of the development of peptic ulcer disease. Several studies have reported that the prevalence of H. pylori infection may be underestimated in patients with bleeding peptic ulcers. Moreover, knowledge regarding the detection of H. pylori infection in patients with peptic ulcer disease complicated by hemorrhage, chronically or occasionally treated with non-steroidal anti-inflammatory drugs (NSAIDs) is limited. Numerous invasive and non-invasive diagnostic methods are available for the detection of H. pylori. Effectiveness values of these tests may vary depending on the brand of test used, age of the population tested, treatment used and, probably, the bleeding situation.

Research frontiers
In this article, the authors assess the prevalence of H. pylori infection in patients with peptic ulcer disease complicated by hemorrhage after consumption of NSAIDs.

Innovations and breakthroughs
More than 50% of patients with peptic ulcer disease complicated by hemorrhage after consumption of NSAIDs are infected by H. pylori.

Applications
In these patients the authors recommend searching for H. pylori infection by using the culture of biopsy specimens after an early upper gastrointestinal tract endoscopy.

Peer review
This is an interesting and well presented study, in which the authors using an approach that overcomes the previous conditions negatively influencing results presented in the literature, clearly showed that invasive methods should be used to identify H. pylori infection in a patient with a bleeding peptic ulcer.

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