ORIGINAL ARTICLE

Comparison of Diagnostic Methods for *Helicobacter pylori* Infection in Patients with Upper Gastrointestinal Bleeding

P. Griñó, S. Pascual, J. Such, J. A. Casellas, M. Niveiro, M. Andreu, J. Sáez, E. Griñó, J. M. Palazón, F. Carnicer & M. Pérez-Mateo

Gastroenterology and Liver Units, Dept. of Internal Medicine; Digestive Endoscopy Unit; Dept. of Pathology; Dept. of Microbiology; all at University General Hospital, Universidad Miguel Hernández, Alicante, Spain

Griñó P, Pascual S, Such J, Casellas JA, Niveiro M, Andreu M, Sáez J, Griñó E, Palazón JM, Carnicer F, Pérez-Mateo M. Comparison of diagnostic methods for *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding. Scand J Gastroenterol 2001;36:1254–1258.

Background: Accuracy of the most frequently used tests for diagnosing *Helicobacter pylori* infection in patients with upper gastrointestinal bleeding of peptic origin is determined. **Methods:** Seventy-eight patients with endoscopically-proven upper gastrointestinal bleeding of peptic origin were included. The presence of *H. pylori* was considered when observed from the histology or, if negative, when serology and breath test were both positive. Accuracy of the rapid urease test was estimated in accordance with results obtained with other diagnostic methods. **Results:** Lesions causing gastrointestinal bleeding were 56 duodenal ulcers, 13 gastric ulcers, 7 pyloric channel ulcers, 13 acute lesions of the gastric mucosa and 16 erosive duodenitis. *H. pylori* infection was present in 68 patients (87.2%). Forty-four patients had received non-steroidal anti-inflammatory drugs. The sensitivity/specificity (%) of the diagnostic methods was 48.5/100 for the rapid urease test, 91/77.8 for the breath test, 89.5/80 for serology and 86.3/100 for histology. The prior consumption of proton-pump inhibitors and antibiotics induced false-negative results in the rapid urease test and breath test, with no effect on serology and histology. **Conclusions:** The prevalence of *H. pylori* infection in patients with upper gastrointestinal bleeding from peptic lesions is high. Sensitivity of the rapid urease test for diagnosing *H. pylori* is low in this setting. Cases with negative rapid urease test need the combination of two or more additional tests if diagnosis is to be achieved. Cases with positive rapid urease test do not need further investigation for diagnosis.

Key words: Bleeding; C^{13} urea breath test; diagnostic test; *Helicobacter pylori* ; histology; non-steroidal anti-inflammatory drugs; peptic ulcer; rapid urease test; serology

Miguel Pérez-Mateo, M.D., Servicio de Medicina Interna, Hospital General Universitario, C/Pintor Baeza s/n., ES-03010 Alicante, Spain (fax. +34 965 93 83 55, e-mail. perezmateo_mig@gva.es)

Gastroduodenal peptic lesions are the most frequent cause of upper gastrointestinal bleeding (UGB) and are responsible for more than 50% of cases (1). Several etiological factors of peptic ulcer disease are known, the principal one being *Helicobacter pylori* infection, which has been identified in more than 95% of duodenal ulcers and in 70%–80% of gastric ulcers (2–4), and use of non-steroidal anti-inflammatory drugs (NSAIDs) (5–7). It has been suggested that the incidence of *H. pylori* is lower in patients who present with UGB of peptic origin (8).

Since *H. pylori* eradication has been shown to decrease the rate of recurrence of peptic ulcer disease, it is of interest to determine the real incidence of *H. pylori* infection in patients with UGB of peptic origin (9–13). Several tests are currently available for the diagnosis of *H. pylori* infection. Invasive tests include the rapid urease test (RUT), histological study and culture of gastric mucosa; non-invasive tests include serology and the urea breath test. RUT is the most commonly used test but it shows a high rate of false-negative results (up to 20%) in patients with UGB (14–16).

The aim of the present study was to assess the diagnostic accuracy of the different diagnostic tests for *H. pylori* infection in patients with UGB of peptic origin.

Patients and Methods

Between January 1999 and March 2000, 78 consecutively admitted patients with UGB of peptic origin were enrolled in the study. The Institution's Ethics Committee approved the protocol and informed consent was obtained from all patients. A diagnostic endoscopy (Olympus GIF V2) was performed within the first 24 h of admission. During oral endoscopy, we took a sample from the antrum for RUT and two samples from the antrum and corpus of the stomach for histological study. Patients in whom the endoscopy and other diagnostic tests could not be performed in this period of time, patients with

bleeding oesophagitis and those with haemodynamic instability that precluded further investigation, were excluded. The use of proton-pump inhibitor (PPI) drugs and/or antibiotics in the previous 7 days was not considered an exclusion criterion, but these patients were evaluated separately. Diagnostic tests for identifying *H. pylori* were performed as follows:

(a) A sample of mucosa from gastric antrum obtained during endoscopy was immediately introduced into a commercially available diagnostic media for RUT (CLOtest®, Ballard, USA) that had been previously heated to room temperature, and incubation was maintained for 24 h. According to the manufacturer's instructions, a change of colour from the initial yellow to pink or orange was considered positive.

(b) Histological study was performed in two biopsies obtained from antrum and two from gastric body. All biopsies were stained with haematoxylin-eosin. Doubtful specimens were re-evaluated with Giemsa staining. A sample was considered positive for *H. pylori* when the pathologist observed the presence of bacillus of helicoidal morphology adhering to the surface epithelium of the gastric mucosa.

(c) Qualitative serological detection of specific IgG antibodies to *H. pylori* was done with a highly sensitive and specific enzyme immunoassay method (*H. pylori* IgG ELISA), Wampole Laboratories R, Dist; USA) with an antigen from the strain ATCC 43504. The results were expressed as indices, which were defined as the relation between the value of the sample to be studied and the standard sample. All samples with a value over 1 were therefore classified as positive and those below 1 as negative.

(d) The breath test was done using a commercially available diagnostic method (TAU-KIT R, Isomed, SL. Madrid, Spain) with ^{13}C labelled urea to detect urease activity, indicating the presence of *H. pylori* . The ^{13}C-urea in the presence of the enzyme urease is hydrolysed, liberating $^{13}CO_2$, which is detected by Continuous Flow Mass Relation Isotopic Spectometry (CF_IRMS). Briefly, patients received a test meal containing 4.2 g of citric acid (Citral pylori R) in 200 ml of water. Ten minutes later a basal sample of expired air was taken, followed immediately by the ingestion of 100 mg of ^{13}C-urea dissolved in 50 ml of water. Thirty minutes later a second sample of expired air was collected. The result was expressed as 'Delta Over Baseline'. An excess of ^{13}C above 5‰ in the second expired sample was considered as positive.

H. pylori infection was diagnosed if demonstrated by histology (antrum and/or body) and, if this was negative, by the positive indications of the combined serology and breath tests. A positive RUT was not considered a criterion of infection.

Statistical Analyses

Chi-squared 2×2 contingency tables were used to compare the results obtained with each diagnostic test and with the 'gold standard' (positive histology or the combination of breath test and serology). Yate's correction was applied when necessary. The sensitivity, specificity, positive predictive value, negative predictive value, efficiency and the 95% confidence interval were calculated for each diagnostic test. The results of the quantitative variables are expressed as mean and standard deviation (*s*) and, the qualitative variable, as the absolute values and percentages.

Table I. Endoscopic findings in patients with bleeding peptic ulcer disease

Endoscopic findings	*n*
Duodenal ulcer	56
Gastric ulcer	13
Pyloric channel ulcer	7
Acute lesions of the gastric mucosa	13
Erosive duodenitis	16

Results

Seventy-eight patients (64 men) were included. The mean age was 63.2 (range 26–87), and among them 33 patients (42.3%) had a previous history of peptic ulcer disease. Seventy-three patients presented with maelena, 23 with coffee-ground vomiting, and 7 with frank haematemesis. Four and 10 patients had received antibiotics or PPI, respectively, in the previous 7 days. Previous NSAID use was recorded in 44 patients (56.4%).

Endoscopic diagnoses are given in Table I. Some of the patients presented with two or more of these conditions. In 17 patients (21.8%) the diagnostic endoscopy was associated with epinephrine injection because of the risk of rebleeding.

Recurrence of UGB occurred in six patients (7.7%), two of whom required surgery. Endoscopic treatment was effective in the other four patients. No patient died as a consequence of UGB.

H. pylori infection was present in 68 patients (87.2%), and, of these, 37 had received NSAIDs prior to hospital admission (54.4%). Importantly, 31 of the 34 patients without previous use of NSAIDs showed *H. pylori* infection (91.2%).

Seven out of the 10 patients without *H. pylori* infection had received NSAIDs prior to hospital admission. No identifiable etiological factors for UGB were found in the remaining three patients.

The corresponding figures for sensitivity, specificity, positive and negative predictive values, as well as diagnostic accuracy (with the 95% confidence interval) are given in Table II. The higher accuracy was observed with the urea breath test (89.4%).

Table III gives this same information after excluding the patients who had previously received PPI or antibiotics. Overall, two out of four patients who had received previous treatment with antibiotics showed simultaneous false-negatives in RUT and the breath test. Similarly, among the nine patients who had received PPI before admission, we observed

Table II. Diagnostic value of the different tests employed for *Helicobacter pylori* infection

	Sensitivity	Specificity	PPV	NPV	Accuracy
RUT	48.5 (36.4–60.9)	100 (65.5–100)	100 (87–100)	22.2 (11.7–37.5)	55.12
Breath	91 (80.9–96.3)	77.8 (40.2–96.1)	96.8 (88–99.4)	53.8 (26.1–79.6)	89.4
Serology	89.5 (79.1–95.3)	80 (44.2–96.5)	96.8 (87.8–99.4)	53 (27.4–77.4)	88.3
Histology	86.3 (74.8–93.1)	100 (65.5–100)	100 (92–100)	53.5 (29.5–74.8)	88.1

Values are presented as percentages, and the 95% confidence intervals in parentheses. PPV = positive predictive value. NPV = negative predictive value. RUT: rapid urease test. Breath: ^{13}C breath test.

false-negatives in RUT (2/9), breath test (2/9), serology (1/9) and histological study (1/9). After excluding these patients, accuracy of the breath test and serology increased slightly, but no major statistical changes were observed.

Discussion

Patients with UGB constitute a considerable proportion of hospital admissions in a Gastrointestinal Department, and gastroduodenal peptic ulcer disease is responsible for about 50% of cases of UGB (1, 7). Although *H. pylori* has been isolated in almost 100% of patients with duodenal ulcers and in 80% of patients with gastric ulcers, there is no consensus in the literature regarding its real prevalence in cases of peptic ulcer-induced UGB. Gisbert et al. (18) found 97.5% of patients showing *H. pylori* infection—a figure similar to that described in patients without UGB (2–4). In other studies, however, a marked reduction in prevalence compared to patients with peptic ulcer disease not-associated with UGB has been reported ranging between 20% and 70% (9, 19–21). NSAIDs-related peptic ulcer disease is also a common cause of UGB (5, 6).

Since *H. pylori* eradication is associated with a marked reduction in peptic-ulcer disease recidivism and accompanying complications, such as UGB (10–12), it is of relevance to assess the real prevalence of *H. pylori* infection in patients with UGB.

The main aim of the present study was therefore to determine the accuracy of the different available diagnostic tests for *H. pylori* infection in patients with peptic ulcer disease-related UGB.

In the patients investigated here, we found an 87.2% prevalence of *H. pylori* infection, a figure slightly inferior to that previously reported in patients with non-bleeding peptic ulcer disease (22) but higher than that reported in patients with UGB (19–21). This discrepancy seems not to be due to the existence of false-positives, given the diagnostic criteria used in the present study, but either by either direct visualization (56 out of 68 cases) or the simultaneous positivity of two different diagnostic tests in the remaining 12 cases (serology plus breath test). Moreover, all cases with positive histological identification showed a combined positivity of serology and breath test.

There may be several reasons for the lower reported prevalence of *H. pylori* in patients with peptic-ulcer-related UGB. An unadvertised ingestion of NSAIDs has been reported in up to 12% of patients with gastrointestinal bleeding, by means of the platelet cyclo-oxygenase activity test (23), which can explain, at least in part, a significant number of cases with previously unidentified cause of UGB. Also, false-negative results can be obtained with certain diagnostic methods in cases with active bleeding (24–26), or if the patient had been receiving either PPI or antibiotics (27–30). The most frequently used test for identification of *H. pylori* infection is the RUT, which is based on the ability of *H. pylori* to convert urea into ammonia (31). The presence of blood in the stomach can promote false-negatives, probably because of the buffer effect of serum albumin that interferes with the chemical reaction (32). Other reasons may be technical, such as collection of an insufficient number of good quality biopsies, or cases in which the samples have not been properly processed (33).

The reason for the high prevalence of *H. pylori* infection observed in our series may be related to the fact that we have not utilized RUT as diagnostic criteria, and to the prospective combination of at least two diagnostic methods to consider a patient as infected. In our opinion this approach probably reduces the number of false-negative cases for *H. pylori* infection in patients with UGB.

In our series, we observed a 48.5% sensitivity by means of

Table III. Diagnostic value of the tests employed excluding patients with PPI and ATB previous intake

	Sensitivity	Specificity	PPV	NPV	Accuracy
RUT	48.3 (35.1–61.7)	100 (59.8–100)	100 (85–100)	21 (10.1–37.8)	54.5
Breath	94.7 (84.5–98.6)	71.4 (30.2–94.9)	96.4 (86.6–99.4)	62.5 (25.9–89.8)	92.2
Serology	91.4 (80.3–96.8)	87.5 (46.7–99.3)	98.1 (88.8–99.9)	58.3 (28.6–83.5)	90.9
Histology	85 (73.2–93.2)	100 (59.8–100)	100 (90.8–100)	50 (25.5–74.5)	87.5

Values are presented as percentages, and the 95% confidence intervals in parentheses. PPV = positive predictive value; NPV = negative predictive value; RUT: rapid urease test; Breath: ^{13}C breath test.

the RUT (Table II), a figure similar to that described previously by other investigators (15, 16, 24–26), which seems to confirm that RUT is not a good diagnostic test of *H. pylori* infection in patients with UGB. However, the high specificity appreciated with this test (100%) indicates that a positive RUT result probably does not require confirmation by any other additional diagnostic test.

Both the breath test and serology show a reasonable sensitivity in our series, being in each case above 85% (Table II), and either could be used in diagnosis. However, the existence of a lower specificity (77.8% and 80%, respectively) recommends combining two available tests to achieve a correct diagnosis.

From a different point of view, a reduced number of active bacteria could induce false-negative results. Hence, the use of PPI can transform *H. pylori* into a non-reactive bacilar form for RUT or the breath test (27–29), and the previous use of antibiotics can also reduce the number of viable bacteria (27, 30, 31). We have included, deliberately, patients with previous use of both drugs in the present study so as to reflect the real conditions in which the patients with UGB are usually admitted. If we exclude this group of patients (Table III) the statistical results are similar. This is probably because we are using four tests for the diagnosis of *H. pylori* infection. So, our results confirm that the prior ingestion of antibiotics or PPI may induce false-negative results in the RUT and urea breath test. This fact, however, does not seem to affect the results obtained with serology and histological observations, as has been described already (27–31). This fact reinforces the need for a carefully detailed anamnesis in patients with UGB, with special attention to previous intake of PPI and/or antibiotics. This is relevant, since this fact allows the selection of the most adequate diagnostic test to achieve a real diagnosis of *H. pylori* infection.

In conclusion, the present study shows that the use of RUT in patients with UGB is not a good diagnostic method to be used alone because of its low sensitivity. Patients with UGB of peptic origin and negative RUT should be investigated with other additional diagnostic methods, such as serology, breath test and histological observation of gastric biopsies. Contrary, in patients presenting with a positive RUT no other confirmatory tests seem to be needed. Also, we have observed a high prevalence of *H. pylori* infection in patients with UGB of peptic origin, being higher than that reported previously, including patients with previous use of NSAIDs.

References

1. Mignon M, Penston JG, Deltenre M, Ruszniewski P, Dobrill G. Natural history of duodenal ulcer disease: are we at a turning point? Gastroenterol Int 1994;7:95–113.
2. Tytgat GNJ, Rauws EAJ. *Campylobacter pylori* and its role in peptic ulcer disease. Gastroenterol Clin North Am 1990;19:183–96.
3. Kuipers EJ, Thijs JC, Festan HP. The prevalence of *Helicobacter pylori* in peptic ulcer disease. Aliment Pharmacol Ther 1995;9:59–69.
4. O'Connor HJ. Eradication of *Helicobacter pylori* : therapies and clinical implications. Postgrad Med J 1992;68:549–55.
5. Soll AH, Weinstein WM, Kurata JH, McCarthy D. Non-steroidal anti-inflammatory drugs and peptic ulcer disease. Ann Intern Med 1991;114:307–19.
6. Grahan DY. The relationship between non-steroidal anti-inflammatory drug use and peptic ulcer disease. Gastroenterol Clin North Am 1990;19:171–2.
7. Maton PN, Gardner JD, Jensen RB. Recent advances in the management of gastric acid hypersecretion in patients with Zollinger–Ellison syndrome. Gastroenterol Clin N Am 1989;18: 847–64.
8. Lee CL, Tu TC, Yang RN, Lin HJ, Ma VL, Wu CHCH. Does blood in the stomach influence the diagnosis of *H. pylori* infection in patients with bleeding peptic ulcer? [Abstract]. Gut 1997;41 Suppl 1:76.
9. Neil GA and the *ad hoc* Committee on FDA-Related Matters. Do ulcers burn out or burn on? Managing duodenal ulcer diathesis in the *Helicobacter pylori* era. Am J Gastroenterol 1997;92:387–93.
10. Wilhemsen I. Quality of life and *Helicobacter pylori* eradication. Scand J Gastroenterol 1996;31 Suppl 221:18–20.
11. Labenz J, Börsch G. Role of *H. pylori* eradication in the prevention of peptic ulcer bleeding release. Digestion 1994;55:19–23.
12. RokkasT, Karameris A, Mavrogeorgis A, Rallis E, Giannikos N. Eradication of *Helicobacter pylori* reduces the possibility of rebleeding in peptic ulcer disease. Gastrointest Endosc 1995; 41:1–4.
13. Grahan DY, Hepps KS, Ramirez FC, Lew GM, Saeed ZA. Treatment of *Helicobacter pylori* reduces the rate of rebleeding in peptic ulcer disease. Scand J Gastroenterol 1993;28:938–42.
14. Romero Gómez M, Vargas J, Utrilla MC, Rufo MC, Otero MA, Chavez M, et al. Estudio prospectivo sobre la influencia de la hemorragia por ulcus gastroduodenal en los métodos diagnósticos de infección por *Helicobacter pylori*. Gastroenterol Hepatol 1998;21:267–71.
15. Lai KC, Hui WM, Lam SK. Bleeding ulcers have high false-negative rates for antral *Helicobacter pylori* when tested with ureasa test [Abstract]. Gastroenterology 1996;110:A167.
16. Archimandritis A, Tzivras M, Souyioultzis S, Papaparaskevas P, Apostolopoulos P, Delladetsima I, et al. High rates of false-negative rapid urease test (CLO) in patients with upper gastrointestinal bleeding (UGB) [Abstract]. Gut 1997;41 Suppl 1:76.
17. Laine L, Peterson WL. Bleeding peptic ulcer. N Engl J Med 1994;331:717–27.
18. Gisbert JP, Boixeda D, Aller R, De la Serna C, Sanz E, Martin de Argila C, et al. *Helicobacter pylori* y hemorragia digestiva por úlcera duodenal. Prevalencia de la infección, eficacia de tres terapias triples y papel de la erradicación en la prevención de la recidiva hemorrágica. Med Clín (Barc) 1999;112:161–5.
19. Hosking SW, Yung MY, Chung SC, Li AKC. Differing prevalence of *Helicobacter pylori* in bleeding and non-bleeding ulcers [Abstract]. Gastroenterology 1992;102:85.
20. Sondergard L, Lassen A, Schaffalitzky de Muckadell OB. Prevalence of *Helicobacter pylori* and ASA/NSAID use in patients with bleeding peptic ulcer. Gut 1996;39 Suppl 2:28.
21. Lee JM, Breslin NP, Fallon C, O'Morain CA. Rapid urease tests lack sensitivity in *Helicobacter pylori* diagnosis when peptic ulcer disease presents with bleeding. Am J Gastroenterol 2000; 95:1166–70.
22. Gisbert JP, Blanco M, Mateos JM, Fernandez-Salazar L, Fernandez-Bermejo M, Cantero J, et al. *H. pylori* -negative duodenal ulcer prevalence and causes in 774 patients. Dig Dis Sci 1999;44:2295–302.
23. Lanas A, Serrano P, Bajador E, Esteva F, Benito R, Sáinz R. Evidence of aspirin use in both upper and lower gastrointestinal perforation. Gastroenterology 1997;112:683–9.
24. Colin R, Czernichow P, Baty V, Touzé I, Brazier F, Bretagne JF, et al. Low sensitivity of invasive tests for the detection of *Helicobacter pylori* infection in patients with bleeding ulcer. Gastroenterol Clin Biol 2000;24:31–5.

25. Tu TC, Lee CL, Wu CH, Chen TK, Chan CC Huang SH, et al. Comparison of invasive and noninvasive tests for detecting *Helicobacter pylori* infection in bleeding peptic ulcer. Gastrointest Endosc 1999;49:302–6.
26. Archimandritis A, Tzivras M, Sougioultzis S, Papaparasdevas I, Apostolopoulos P, Avlami A, et al. Rapid urease test is less sensitive than histology in diagnosing *Helicobacter pylori* infection in patients with non-variceal upper gastrointestinal bleeding. J Gastroenterol Hepatol 2000;15:369–73.
27. Kalantar J, Xia HHX, Wyayy JM, Rose D, Talley NJ. Determination of optimal biopsy sites for detection of *H. pylori* in patients treated or not treated with antibiotics and anti-secretory drugs [Abstract]. Gastroenterology 1997;112:A165.
28. Chey WD, Spybrook M, Carpenter S, Nostrant TT, Elta GH, Scheiman JM. Prolonged effect of omeprazole on the ^{14}C urea breath test. Am J Gastroenterol 1996;91:89.
29. Vaira D, Holton J, Menegatti M, Ricci C, Gatta L, Geminiani A, et al. Review article: invasive and non-invasive tests for *Helicobacter pylori* infection. Aliment Pharmacol Ther 2000;14 Suppl 3:13–22.
30. Rollan A, Giancaspero R, Arrese M, Figueroa C, Vollreth V, Schultz M, et al. Accuracy of invasive and non-invasive tests to diagnose *Helicobacter pylori* infection after antibiotic treatment. Am J Gastroenterol 1997;92:1268–74.
31. Working party of the European *Helicobacter pylori* Study Group. Guidelines for clinical trials in *Helicobacter pylori* infection. Gut 1997;41 Suppl 2:S1–9.
32. Leung WK, Sung JJ, Siu KL, Chan FK, Ling TK, Cheng AF. False-negative biopsy urease test in bleeding ulcers caused by the buffering effects of blood. Am J Gastroenterol 1998;93:1914–8.
33. Laine L, Chun D, Stein C, El-Bablawi I, Sharma V, Chandrasoma P. The influence of size or number of biopsies in rapid urease test results: a prospective evaluation. Gastrointest Endosc 1996;43:49–53.

Received 19 March 2001
Accepted 29 May 2001