Comparison of stool immunoassay with standard methods for detection of *Helicobacter pylori* infection in patients with upper-gastrointestinal bleeding of peptic origin

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Objective **To assess the accuracy of the determination of *Helicobacter pylori* infection by a stool immunoassay in patients with upper-gastrointestinal bleeding (UGB) of peptic origin, in comparison with the routine histological study, serology, rapid urease and ^{13}C-breath tests.**

Methods **Sixty-eight patients with endoscopically proven UGB of peptic origin were included. The presence of *H. pylori* was considered when observed on histology or, if negative, by the positive indications of two of the remaining tests (serology, rapid urease, ^{13}C-breath test). The accuracy of stool immunoassay was estimated according to results obtained with other diagnostic methods.**

Results **Lesions causing gastrointestinal bleeding were 49 duodenal ulcers, 11 gastric ulcers, six pyloric channel ulcers, 13 acute lesions of the gastric mucosa, and 16 erosive duodenitis. *H. pylori* infection was present in 59 (86.76%) patients. Forty-one patients had received nonsteroidal anti-inflammatory drugs. The sensitivity and specificity of the diagnostic methods were 47.5% and 100% for the rapid urease test, 93% and 87.5% for the breath test, 86.4% and 77.7% for serology, 89.4% and 100% for histology, and 96.6% and 33.3% for the stool test.**

Conclusions **The detection of *H. pylori* antigen in stools in patients with UGB of peptic origin has a good sensitivity (96.6%) but a low specificity (33.3%) for the diagnosis of *H. pylori* infection, which probably makes this test an inadequate tool in this setting if utilized alone. *Eur J Gastroenterol Hepatol* 15:525–529 © 2003 Lippincott Williams & Wilkins**

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Introduction

Peptic gastroduodenal lesions are responsible for over 50% of cases of upper-gastrointestinal bleeding (UGB) [1]. Of the aetiological factors that have been shown to be associated with these lesions, the most relevant are infection by *Helicobacter pylori*, which may be isolated in more than 95% of all duodenal ulcers and 70–80% of gastric ulcers [2–4], and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) [5,6].

Previous studies have suggested that the incidence of *H. pylori* infection is lower in patients with UGB of peptic origin [7]. However, if *H. pylori* infection is present, then its accurate identification seems of relevance since its eradication has been shown to reduce the rate of ulcer relapse and associated complications [8–12].

Several tests are currently available for the diagnosis of *H. pylori* infection, some being invasive and requiring endoscopic biopsies, such as the rapid urease test (RUT), histological study and culture, and others being non-invasive, such as the breath test and serology. A new commercially available test allows the detection of *H. pylori* antigen in samples of stools from patients by an enzyme immunoassay (*H. pylori* stool antigen test, HpSA). This is an easy-to-perform test, with a sensitivity and specificity of more than 90% for diagnosis [13] and follow-up [14,15] of patients with *H. pylori* infection.

However, the usefulness of this new diagnostic method has been assessed in a single prospective study in patients with UGB [16]. It seems necessary to confirm its usefulness as compared with the tests generally used before recommending its use in this setting. Therefore, the aim of this investigation is to assess the accuracy of the determination of HpSA in patients with UGB of

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peptic origin, in comparison with the routine histological study, serology, RUT and breath test.

Patients and methods

Between January 1999 and March 2000, 68 consecutively admitted patients with the diagnosis of UGB of peptic origin were included 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, Tokyo, Japan) was performed within the first 24 h of admission. All gastric ulcers were biopsied routinely to rule out malignancy. Patients with bleeding oesophagitis, patients with haemodynamic instability that precluded further investigation, patients with malignant gastric ulcers, and patients in whom endoscopy and other diagnostic tests could not be performed in this period of time were excluded. The use of proton pump inhibitor (PPI) drugs and/or antibiotics in the previous 7 days was not considered an exclusion criterion.

Briefly, the diagnostic tests for identification of *H. pylori* were performed as follows:

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

A histological study was performed in two biopsies obtained from the antrum and two from the 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. The pathologist was not aware of the results of RUT, if available.

Qualitative serological detection of specific immunoglobulin G (IgG) antibodies to *H. pylori* was carried out with a highly sensitive and specific enzyme immunoassay method (*H. pylori* IgG ELISA, Wampole Laboratories, Dist. Cranbury, New Jersey, USA) with an antigen from the strain ATCC 43504. The results were expressed as an index defined as the relation between the value of the sample to be studied and the standard sample. Thus, all samples with a value above 1 were classified as positive and all with a value below 1 were classified as negative.

The breath test was carried out with a commercially available diagnostic method (TAU-KIT, 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 spectrometry. Briefly, patients received a test meal containing 4.2 g of citric acid (Citral pylori) in 200 ml of water. Ten minutes later, a basal sample of expired air was taken, which was 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 to be positive.

For detection of *H. pylori* antigen in stools (Premier Platinum HpSA, Meridian Diagnostics, Cincinnati, OH, USA), the first stools passed after admission (within the first 48 h) were collected, separated into aliquots and frozen to −40°C if they were not processed immediately. The test is based on enzyme immunoassay using anti-*H. pylori* polyclonal antibodies in a microplaque sample dish, which permitted the detection of *H. pylori* directly from the stools of the patient. Stools were diluted and incubated for 1 h after adding the antibodies according to the manufacturer's instructions. The result was read visually, using a spectrophotometer dual wavelength 450/630 nm. The results were considered positive when the values were greater than 0.120 and negative when values were below 0.100.

H. pylori infection was diagnosed if demonstrated by histology (antrum and/or body) or, if this was negative, by positive indications of two of the remaining tests (RUT, serology, breath test). The detection of *H. pylori* in stools was not considered a criterion for infection.

Statistical study

Chi-squared 2×2 contingency tables were used to compare the results obtained with each diagnostic test against a gold standard (histology or two of the other tests apart from HpSA). Yates' correction was applied when needed. We calculated the sensitivity, specificity, positive and negative predictive values, and efficiency of each diagnostic test, as well as the 95% confidence intervals. Quantitative variables were expressed as means and standard deviations. Qualitative variables were expressed as absolute values and percentages.

Results

Sixty-eight consecutively admitted patients with UGB of peptic origin were included in the study. The clinical characteristics of patients together with the characteristics of the bleeding episode and the endoscopic findings at admission are shown in Table 1. In some cases, two or more of these diagnoses were found.

Table 1 **Patients' characteristics (percentages expressed in parentheses)**

Characteristic	*N* (*n* = 68)
Age (years; range)	63.6 (30–88)
Gender (males/females)	56/12
Antecedents of ulcer or UGB	28 (41.2)
Associated pathologies	51 (75)
Smokers	19 (27.9)
Previous NSAIDs	41 (60.3)
Previous PPIs	7 (10.3)
Previous antibiotics	4 (5.8)
Melaena	64 (94.1)
Coffee-ground vomiting	19 (27.9)
Haematemesis	7 (10.3)
Duodenal ulcer	49 (72.1)
Gastric ulcer	11 (16.2)
Pyloric channel ulcer	6 (8.8)
Acute lesions of gastric mucosa	13 (19.1)
Erosive duodenitis	16 (23.5)

NSAIDs, nonsteroidal anti-inflammatory drugs; PPIs, proton-pump inhibitors; UGB, upper-gastrointestinal bleeding.

Table 2 **Results obtained with the application of the diagnostic tests**

Test	*H. pylori*-positive*	*H. pylori*-negative
Rapid urease test		
Positive	28	0
Negative	31	9
^{13}C-breath test		
Positive	54	1
Negative	4	7
Serology		
Positive	51	2
Negative	8	7
H. pylori *stool antigen test*		
Positive	57	6
Negative	2	3
Histology		
Positive	51	0
Negative	6	9

* *H. pylori* was considered positive if evident on histology or positive in two tests other than *Helicobacter pylori* stool antigen test.

In 13 (19.2%) patients, the diagnostic endoscopy was associated with adrenaline injection because of active bleeding or risk of rebleeding. In spite of this, six (9.1%) patients rebled, and two of them finally required surgery 3 and 4 days, respectively, after admission. None of our patients died as a consequence of the bleeding episode.

According to the above-mentioned diagnostic criteria, *H. pylori* infection was present in 59 (86.76%) patients. Of these, 35 (59.3%) had received NSAIDs in the days before admission. Three of nine patients without *H. pylori* infection had not received NSAIDs. Therefore, 24/27 (88.9%) patients not receiving NSAIDs showed *H. pylori* infection. In three (4.4%) patients, no identifiable aetiological factors for UGB were found. All patients with positive histology had at least two other positive tests.

Table 2 shows the results of the breath test, RUT, serology and histology together with HpSA in all patients included in the study. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy, together with the 95% confidence intervals, are shown in Table 3. In our series, HpSA was associated with a low specificity (33.3%) and a high sensitivity (96.6%). These findings did not show significant variations after excluding those patients who had received NSAIDs or PPIs previous to hospital admission (data not shown).

Discussion

In this study, we have shown that the detection of *H. pylori* antigen in the stools of patients admitted due to UGB of peptic origin, as a diagnostic method of *H. pylori* infection, shows a good sensitivity (96.6%) but a low specificity (33.3%). Almost 87% of these patients show *H. pylori* infection, a figure slightly higher than that described previously in this setting.

Peptic-ulcer-induced UGB is a frequent cause of hospital admissions and constitutes about 50% of all episodes of UGB [1,17]. Although *H. pylori* has been isolated in almost 100% of patients with duodenal ulcers and in 80% of patients with gastric ulcers [2–4], there is no consensus in the literature regarding its real prevalence in cases of peptic-ulcer-induced UGB [18–22]. However, it seems important to know the real prevalence of *H. pylori* in this setting, since its eradication is associated with a marked decrease in the rate of ulcer and bleeding recidivism [8–12].

Table 3 **Diagnostic value of the tests employed in the overall group of patients (*n* = 68) (values presented as percentages with 95% confidence intervals in parentheses)**

	Sensitivity	Specificity	PPV	NPV	Accuracy
RUT	47.5 (34.5–60.8)	100 (62.9–100)	100 (85–100)	22.5 (11.4–38.9)	54.4
^{13}C-breath test	93.1 (82.5–97.8)	87.5 (46.7–99.3)	98.2 (89–99.9)	63.6 (31.6–87.6)	92.4
Serology	86.4 (74.5–93.6)	77.7 (40.2–96.1)	96.2 (85.9–99.3)	46.6 (22.3–72.6)	85.3
HpSA	96.6 (87.3–99.4)	33.3 (9–69.1)	90.4 (79.8–96.1)	60 (17–92.7)	88.6
Histology	89.4 (77.8–95.6)	100 (62.9–100)	100 (91.3–100)	60 (32.9–82.5)	90.9

HpSA, *Helicobacter pylori* stool antigen test; NPV, negative predictive value; PPV, positive predictive value; RUT, rapid urease test.

We have observed a prevalence of 86.76% of *H. pylori* infection in the patients included in this study. This figure is slightly lower than that reported in patients with non-bleeding peptic ulcer disease in our geographical area [18] but higher than that commonly reported in patients with UGB of peptic origin [20–22]. According to the above-mentioned diagnostic criteria for diagnosis of *H. pylori* infection in this study, it is not likely that we have misdiagnosed patients with real *H. pylori* infection.

The detection of HpSA has been proposed recently as a valuable tool for the detection and follow-up of patients with *H. pylori* infection [13–15]. It has the advantage of being non-invasive, easy, quick and cheaper than the breath test [14], and recent studies have reported a sensitivity and specificity above 90% in patients with peptic ulcer disease [13–15,23], which might make it convenient for the diagnosis and follow-up of *H. pylori* eradication in these patients. However, these results may not be transposable to patients with UGB, since the presence of blood in the stomach has been seen to modify the results of other commonly used diagnostic tests [24–26]. To our knowledge, only one study has investigated its usefulness in patients with UGB [16]. In that study, the authors report a sensitivity of 95.6% and a specificity of 33.3% in this setting to detect *H. pylori* infection. We have investigated the accuracy of this method in patients with UGB in comparison with the routine histological study, serology, RUT and urea breath test for diagnosis of *H. pylori* infection with surprisingly similar results. In our large series of patients with UGB, we found a sensitivity of 96.6% and a specificity of 33%. A possible explanation for these poor results in patients with UGB may be the spectrophotometric cut-off point for positivity recommended by the manufacturer (>0.120). Although already validated [23,27,28], it might be too low in patients with UGB, and a different cut-off point may be utilized instead, as suggested by Ohkura *et al.* [29]. Whatever the cause, according to our data and other previously reported data, this test does not seem to be a useful tool for the diagnosis of *H. pylori* infection in patients with peptic-ulcer-related UGB if utilized alone. However, given its high sensitivity, it may be useful if utilized in combination with other, more specific tests. These findings were similar after excluding in the statistical analysis those patients who had received PPIs and/or antibiotics previous to the bleeding episode.

In summary, the detection of *H. pylori* antigen (HpSA) in stools of these patients has a good sensitivity (96.6%) but a low specificity (33.3%) for the diagnosis of *H. pylori* infection, which probably makes this test an inadequate tool if utilized alone. Also, a high prevalence of *H. pylori* infection may be expected in patients with peptic-ulcer-related UGB, including patients with previous intake of NSAIDs.

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