DETECTION OF *HELICOBACTER PYLORI* BY PCR ON GASTRIC BIOPSY SPECIMENS TAKEN FOR CP TEST: COMPARISON WITH HISTOPATHOLOGICAL ANALYSIS

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The aims of the present study were: (i) to assess whether *H. pylori* could be succesfully detected by PCR from the same biopsy sample used for CPtest; and ii) to evaluate CPtest comparatively to both PCR and histology for detection of *H. pylori* infection in dyspeptic patients. Three antral gastric biopsies were collected from each of 80 consecutive dyspeptic patients undergoing oesophago-gastroduodenoscopy. Two biopsies were for histology (gold standard), one for CPtest, scored at 20min, 1h and 24h for the presence of urease activity. Gastric biopsy was then removed from CPtest and used for *ureC*-targeted PCR. Fifty-five (68.7%) patients were positive for *H. pylori* infection by histology. CPtest yielded an overall diagnostic accuracy of 93.8% (95% CI: 91-96.4%), regardless of observation period. No erroneous categorization of *H. pylori* status occurred using PCR, yielding sensitivity, specificity, positive and negative predictive values, and overall diagnostic accuracy of 100%. Our results suggest that *H. pylori* can be detected by PCR in gastric biopsies previously taken for CPtest, so reducing the workload of the endoscopist by saving additional biopsies for culture analysis and susceptibility tests.

Helicobacter pylori (*H. pylori*) infection is associated with up to 95% of duodenal and gastric ulcers and with the development of gastric cancer and gastric MALT lymphoma (1). There is a wide variety of tests available for diagnosing *H. pylori* infection. The desire to diagnose *H. pylori* infection rapidly led to the development of several non-invasive tests, including ^{13}C or ^{14}C urea breath tests, serologic tests and, more recently, stool antigen test (1-2). However, as long as we do not have general treatment recommendations for *H. pylori* infection, the reference method for the diagnosis of active *H. pylori* infection is oesophago-gastroduodenoscopy (EGDS) with multiple gastric biopsies for histologic examination, rapid urease testing (RUT), and culture.

Rapid urease testing of gastric biopsy specimens - because of its low cost, rapid availability of results, simplicity and accuracy - remains the main practical tool for the endoscopist in the decision as to whether therapy should be prescribed. Worldwide, a number of RUTs have been developed to serve this purpouse (3-5). CPtest is a commercially available rapid urease test (RUT) whose liquid medium confirms the

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presence of *H. pylori* by changing the colour of an indicator dye from yellow to red. The manufacturer recommends to evaluate the test within 20 minutes or, if the patient has been previously treated or a very low presence of bacteria is found, up to 1 h. To our knowledge, few studies have been conducted to compare CPtest with other methods (6-7), and there are no reports on the comparison between CPtest and PCR in detecting *H. pylori* infection.

In order to minimize the workload of the endoscopist, the present study was undertaken to assess whether *H. pylori* could be succesfully detected by PCR from the same biopsy sample used for CPtest. Further, we comparatively evaluate CPtest with PCR for detection of *H. pylori* infection in gastric biopsy specimens from dyspeptic patients. Histology was considered as the gold standard.

MATERIALS AND METHODS

A total of 80 consecutive dyspeptic patients (40 M; range: 20-55 yrs) undergoing EGDS were included in this study. Exclusion criteria for the study included patients who were treated with antibiotics or proton pump inhibitors in the previous 4 weeks. Other exclusion criteria were malignancy and prior gastric surgery. All patients gave informed consent for the study. All examinations were performed blind.

EGDS was done after the patient had fasted overnight. The endoscope and biopsy equipment were cleaned and disinfected with 2% glutaraldehyde before use. In each endoscopic analysis, three antral gastric biopsies were taken for evaluation. Two biopsies were processed in formalin and sent for routine histopathological examination by Genta stain. The last biopsy specimen was immediately processed for CPtest (Yamanouchi Pharma S.p.A., Carugate, Milan, Italy): after reconstituting of the lyophilized reagent with 1 ml diluent, biopsy specimen was placed into the vial and examined at three intervals: 20 minutes and 1 h (as recommended by the manufacturer), and 24 h. The result was scored positive when the reagent changed from yellow to red. If no color change in the reagent occurred within 24 h, the CP-test result was scored negative.

Gastric biopsy sample was then taken from CPtest by a sterile loop and transferred in sterile saline and sent to the microbiological laboratory within 1 hour. During transport the specimen was maintained at + 4°C. Biopsy sample was finely dissected and the suspension was washed with sterile saline and centrifuged at 12,000 x g for 3 min. The pellet was resuspended in 200 ml of helix fast DNA lysis buffer (DiaTech; Jesi, Ancona, Italy). Incubation was carried out at 56°C overnight; the sample was then incubated for 8 minutes at 95°C to inactivate protease K.

A PCR assay targeted at the *ureC* gene of *H. pylori* was performed, using primers 5'-AAGCTTTTAGGGGTGTTAGGGGTTT-3' and 5'-AGCTTACTTTCTAACACTAACGC-3', corresponding to nucleotides 1293 to 1318 and 1587 to 1563, respectively, derived from the sequenced urease genes (8). PCR reactions were performed in a 100 ml volume in a Omn-E thermocycler (Hybaid, Teddington, UK). Reaction mixtures containing 0.4 mM (each) primer, 0.2 mM (each) deoxynucleoside triphosphate, 2.5 U of *Taq* DNA polymerase, and reaction buffer (100 mM Tris-HCl [pH 8.2], 500 mM KCl, 15 mM $MgCl_2$) before addition of 10 ml of sample DNA. For *ureC* amplification, cycles comprising 94°C/2 min, 55°C/2 min, and 72°C/2 min were used. After 35 cycles, a final extension of 55°C/1 min for 1 cycle was performed. The final PCR product was subsequently analyzed by electrophoresis with (>6%) polyacrilammide gel. Samples were scored as positive when a band of 294 bp could be detected on the gel. Negative reagent controls consisted of tubes containing distilled water instead of the DNA sample. Positive controls were examined with each batch of amplified product.

Helicobacter pylori status was documented by histopathological examination. Sensitivity, specificity, predictive values, and overall diagnostic accuracy were calculated for CPtest and PCR, compared to the gold standard. Qualitative data were assessed by the chi-square test (C^2). A p value of < 0.05 was considered significant.

RESULTS

Using histopathological examination as gold standard, 55 (68.7%) patients were positive for *H. pylori* infection. From the endoscopic diagnosis, histological gastritis was found in 60 (75%) patients, of whom 50 (83.3%) were positive for *H. pylori*. Normal mucosa was found in 20 (25%) patients, of whom 4 (20%) were posi-

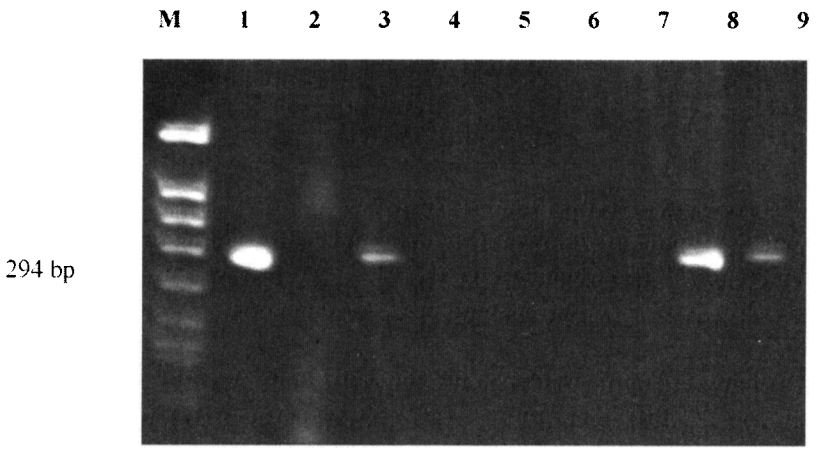

Fig. 1. *Detection of* H. pylori *in DNA extracts of seven gastric biopsy specimens by PCR assay. Lanes: M, molecular size marker; 1, positive control of* H. pylori*; 2, negative control (distilled water); 3 to 9, patients no. 1 to 7, respectively. Lanes 3, 8, and 9 showed positive results.*

tive for *H. pylori*. The accuracy of CPtest and PCR for diagnosing *H. pylori* infection, compared to the gold standard, is summarized in the Tab. I. No erroneous categorization of *H. pylori* status occurred using PCR (Fig. 1), performing a 100% of sensitivity, specificity, positive and negative predictive values, and overall diagnostic accuracy. CPtest scored at 20 min and 1h gave the same performance, resulting in 90.9%, 100%, 100%, and 83.3% for sensitivity, specificity, predictive positive and negative values, respectively. Scoring CPtest at 24h improved sensitivity and negative predictive value of the test to 100% ($c^2 = 9.534$, $p = 0.002$; $c^2 = 18.236$, $p < 0.001$, respectively), but decreased its specificity and positive predictive values to 80% ($c^2 = 22.113$, $p < 0.001$) and 91.6% ($c^2 = 8.768$, $p = 0.003$), respectively. CPtest yielded an overall diagnostic accuracy of 93.8% regardless of observation period: 5 false negative results and 5 false positive results when CPtest was scored at 20 min/1h and 24h, respectively. CPtest and PCR performed on the same biopsy specimen showed concordant results for 75 of the 80

Tab. I. *Results of CPtest and PCR for diagnosis of Helicobacter pylori infection in 80 dyspeptic patients. Histology was considered as the gold standard.*

	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	ODA (95% CI)
CPtest (20 min)	90.9 (84.5-97.3)	100	100	83.3 (79.2-87.4)	93.8 (91-96.4)
CPtest (1 h)	90.9 (84.5-97.3)	100	100	83.3 (79.2-87.4)	93.8 (91-96.4)
CPtest (24h)	100	80 (75.6-84.4)	91.6 (85.4-97.8)	100	93.8 (91-96.4)
PCR	100	100	100	100	100

CPtest (20 min), CPtest (1 h), CPtest (24 h): CPtest scored at 20 min, 1 h, and 24 h, respectively.
95% CI: 95% confidence interval; PPV: positive predictive value; NPV: negative predictive value; ODA: overall diagnostic accuracy.

(93.8%) patients. When CPtest was scored at 20min/1h, 50 patients were positive for both tests, whereas 25 were negative for both tests; an erroneous categorization of *H. pylori* status occurred in 5 of 80 (5%) patients: CPtest was positive while PCR was negative. When CPtest was scored at 24h, 55 patients were positive for both tests, whereas 20 were negative for both tests; an erroneous categorization of *H. pylori* status occurred in 5 of 80 (5%) patients: CPtest was negative while PCR was positive.

DISCUSSION

RUTs are commonly used during EGDS for the diagnosis of *H. pylori* infection. Although CLOtest has been used most commonly since it is considered a reference standard for *H. pylori*, other RUTs are commercially available (3-5). Few data exhist concerning diagnostic accuracy of CPtest (6-7). To our knowledge, similar studies comparing CPtest with PCR for detecting *H. pylori* infection have not been performed. In the present study, we found an excellent concordance (93.8%, overall diagnostic accuracy) between CPtest and both PCR and histology. CPtest showed sensitivity at 20 min of 90.9 %, significantly better than 71% ($c^2 = 12.841$, $p < 0.001$) and 66% ($c^2 = 18.377$, $p < 0.001$) sensitivities at 1h found by Laine *et al.* for CLOtest and Hpfast, respectively (3). CPtest resulted in an erroneous categorization of *H. pylori* status in only 6.2% (95% CI: 3.5-8.8) of cases, regardless of reading-time. In fact, CPtest scored at 20 min or 1h, as recommended by the manufacturer, yielded 5 false negative results, probably due to the low bacterial load or unappropriate processing of samples. When CPtest was scored up to 24h the overall diagnostic accuracy did not change: in fact, sensitivity improved significantly ($p = 0.002$) to 100% but, at the same time, specificity decreased significantly ($p < 0.001$) to 80%, resulting in 5 false positive results. None of these patients had been treated with H_2-blockers in the previous 4 weeks. Thus, CPtest scored at 24h improved sensitivity up to 100%, but this may negate the benefit from examining the test earlier at 20 min. This speculation needs further studies to be resolved.

The Maastricht II-2000 Consensus Report has suggested that urea breath and stool antigen tests be used to screen untreated young (under 45 years old) dyspeptic patients for *H. pylori* infection in primary care settings (9). However, the increased detection of antibiotic-resistant strains of *H. pylori* (10), added to the evidence that the failure in eradication of infection is associated to a greater incidence of secondary resistances (11), justifies the importance of routine pre-treatment susceptibility testing. Although the application of this "test and treat" strategy is founded on economic analyses, because it results in a significant reduction of endoscopic workload, recent studies showed that this approach is not necessarily associated with a cost benefit (12, 13).

In order to search for both accurate and cost-effective protocols for the diagnosis of *H. pylori* infection, several studies showed the feasibility to perform culture directly on the biopsy used for liquid- or agar-based RUT (14-16). Rautelin *et al.* suggested that savings may be possible only if urease-positive biopsies are sent to the microbiological laboratory for isolation of *H. pylori* and sensitivity studies (17).

Culture of gastric biopsies allows further *H. pylori* strain analysis, including determination of antibiotic susceptibility; however, tests can take up to 2 weeks to complete and endoscopists need to collect at least five gastric biopsies for detecting at least 95% of infections (4). Molecular biology techniques, such as PCR, are highly specific and sensitive for detecting *H. pylori* infection and they provide genetic information of the infecting strains, such as the presence of genes linked to virulence or mutations conferring antibiotic resistance (18-22) In contrast to culture techniques (23), PCR detects the non viable coccoid forms of the microorganism (20), probably involved in the transmission of infection and in relapses following antimicrobial therapy (24).

The results of the present study demonstrated the feasibility of detecting *H. pylori* infection by PCR directly from biopsy specimen previously taken for CPtest. PCR yielded 100% of sensitivity and specificity, compared to the gold standard (histology), in detecting *H. pylori* infection. Lin *et al.* showed that gastric biopsies used for

CLOtests were reliable to detect *H. pylori* by PCR obtaining 87.6% of concordance rate (25). However, CLOtest needs to be monitored over a 24h-period, as recommended by the manufacturer. In this regard, the results of the present study showed that CPtest offered a reliable result at 20 min thereby allowing a same-day diagnosis of infection. Further, unlike the CLOtest, CPtest does not need incubation so it requires less use of personnel and incubators.

In conclusion, the present study showed that CPtest scored at 20 min was comparable to PCR and histology for detection of *H. pylori* in gastric biopsies from dyspeptic patients. On the other hand, we found that CPtest is reliable for detecting *H. pylori* by PCR. From a practical point of view, this PCR-based approach not only allows same-day diagnosis of *H. pylori* infection, but it also could give the clinician rapid enhanced information concerning antibiotic susceptibility as well as pathogenicity markers. As a result, the PCR testing may have a significant impact on patient management in terms of time, appropriate antibiotic prescription, and treatment outcome. Further, culture analysis and additional biopsies could be avoided, reducing the laboratory costs as well as the risk of complications.

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