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Evaluation of Pyloriset Screen, a Rapid Whole-Blood Diagnostic Test for *Helicobacter pylori* Infection

AINO OKSANEN,[1] LEA VEIJOLA,[1] PENTTI SIPPONEN,[2] KNUT-OLOF SCHAUMAN,[1] AND HILPI RAUTELIN[3]*

Herttoniemi Municipal Hospital, FIN-00800 Helsinki,[1] Department of Pathology, Jorvi Hospital, FIN-02740 Espoo,[2] and Department of Bacteriology and Immunology, The Haartman Institute, FIN-00014 University of Helsinki,[3] Finland

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***Helicobacter pylori* infection can be detected by several invasive tests based on gastroscopy and by noninvasive methods such as serologic assays. Noninvasive tests can be used not only in addition to invasive tests but also by themselves to screen for *H. pylori* infection in patients who are not in urgent need of endoscopy. Lately, rapid qualitative serologic tests have been developed. In the present study, the accuracy of a novel rapid whole-blood test, Pyloriset Screen, detecting immunoglobulin G (IgG) and IgA antibodies against *H. pylori* was evaluated. A total of 207 consecutive adult outpatients referred for upper endoscopy were enrolled. Gastric biopsy specimens were taken from the antrum and corpus for histologic examination and rapid urease testing. Cultures were available for 113 patients. Serum samples collected from all patients were tested for *H. pylori* antibodies by two enzyme immunoassays (EIAs) (Pyloriset EIA and an in-house EIA), a rapid latex agglutination test (Pyloriset Dry), and Pyloriset Screen. Patients were considered *H. pylori* positive if helicobacters were seen on histologic examination (77 patients) or, if in combination with histologically verified (although helicobacter-negative) gastritis, their IgG antibody titers were elevated in the two EIAs (five patients). The Pyloriset Screen test had a sensitivity of 95%, a specificity of 94%, a positive predictive value of 91%, and a negative predictive value of 97%. Among 63 patients under the age of 45 years, the Pyloriset Screen test did not miss a single *H. pylori* diagnosis, and only 1 patient had a false-positive result. Pyloriset Screen could be used reliably to screen for *H. pylori* infection.**

Helicobacter pylori is the causative agent of chronic gastritis (8) and the single most important factor in peptic ulcer disease (7, 15, 17); however, the pathogenetic mechanisms underlying *H. pylori* infection are not well understood. Furthermore, there is a strong association between *H. pylori* infection and gastric cancer (3, 12, 18). Various diagnostic methods for detecting *H. pylori* infection are available. These can be divided into invasive methods, requiring endoscopy, and noninvasive tests, mainly urea breath tests (4) and serologic detection of antibodies (9).

It has been recommended that patients with duodenal or gastric ulcers and *H. pylori* infection should receive treatment (11). In the future, the list of indications for treatment may be longer. For instance, eradication therapy for *H. pylori*-positive patients with functional dyspepsia was recently classified as advisable (6). To reduce costs without missing relevant diagnoses, the most recent guidelines recommend gastroscopy only for patients with serious symptoms, with *H. pylori* infection, or over 45 years of age (2, 13, 21). Therefore, a simple, reliable, and noninvasive test for *H. pylori* is needed. For practical purposes, it would be most convenient and economical for both the physician and the patient if the result of the test could be obtained at the initial visit. To fulfill these requirements, rapid tests based on serology have been developed.

In the present study, the accuracy of a novel rapid whole-blood *H. pylori* test, Pyloriset Screen (Orion Diagnostica, Espoo, Finland), was evaluated in a population of unselected adult outpatients undergoing upper gastrointestinal endoscopy for various reasons.

* Corresponding author. Mailing address: Department of Bacteriology and Immunology, P.O. Box 21 (Haartmaninkatu 3), FIN-00014 University of Helsinki, Finland. Phone: 358 9 1911. Fax: 358 9 1912 6382. E-mail: Hilpi.Rautelin@helsinki.fi.

MATERIALS AND METHODS

Patients. A total of 207 consecutive adult outpatients (age range, 19 to 83 years; median age, 55 years; 122 [59%] women) referred to Helsinki Municipal Hospital at Herttoniemi for upper endoscopy between October 1996 and March 1997 were included. Forty-four patients were under 40 years of age, 78 were between 40 and 59 years of age, 82 were between 60 and 80 years of age, and 3 were over 80 years of age. Patients who had not had prior *Helicobacter* eradication therapy and who were willing and able to give written informed consent were included in the study. The study was approved by the ethics committee of the Helsinki City Health Department.

Endoscopy, histology, rapid urease test, and culture. The endoscopies were performed by two of the authors (A.O. and L.V.). Two biopsy specimens for histologic examination were taken from both the antrum and the corpus (both the anterior and posterior walls of each). The biopsy specimens were stained with hematoxylin-eosin, Alcian blue (pH 2.5)-periodic acid-Schiff, and modified Giemsa stains. The specimens were examined in a blinded fashion by a pathologist (P.S.) and scored in accordance with the Sydney System (14), with the antrum and corpus being scored separately. Additional antrum and corpus biopsy specimens were obtained for a rapid urease test (Hut-test; Astra GmbH, Wedel, Germany), which was read at 30 min, 3 h, and 24 h in accordance with the manufacturer's instructions. In some cases, all three readings could not be taken because of working hours. Antrum and corpus biopsy specimens for culture were obtained from 113 patients (those enrolled in 1997). The specimens were cultured on *Brucella* agar plates (BBL, Cockeysville, Md.) supplemented with horse whole blood (7%) and IsoVitalex (1%). In addition, selective *Brucella* agar plates containing vancomycin (6 mg/liter; Eli Lilly, Indianapolis, Ind.), amphotericin (2 mg/liter; Sigma, St. Louis, Mo.), and nalidixic acid (20 mg/liter; Sigma) were used. The plates were incubated at 37°C in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2. The plates were examined every other day from the 3rd to the 12th day of incubation. Isolates were identified on the basis of colony appearance, Gram staining, and positive reactions in biochemical tests (catalase, oxidase, and urease).

Serum samples. Blood was taken from each patient during his or her visit to the endoscopy unit. Separated serum samples were stored at −20°C until analyzed.

EIA. Immunoglobulin G (IgG) and IgA antibodies to *H. pylori* were measured separately, both by an in-house enzyme immunoassay (EIA) (5) and by Pyloriset

TABLE 1. Sensitivities, specificities, positive predictive values, and negative predictive values of culture, histology, biopsy urease test, EIAs, and rapid serologic tests for detection of *H. pylori* infection among 207 adult patients[a]

Method of detection	% Sensitivity	% Specificity	% PPV	% NPV
Culture[b]	77 (85)	100 (100)	100 (100)	87 (92)
Histological examination	94 (100)	100 (100)	100 (100)	96 (100)
Hut-test	88 (94)	97 (97)	95 (95)	92 (96)
In-house EIA-G	99 (99)	97 (93)	95 (89)	99 (99)
Pyloriset EIA-G	99 (99)	98 (94)	96 (90)	99 (99)
In-house EIA-A	61 (64)	99 (98)	98 (96)	79 (82)
Pyloriset EIA-A	94 (95)	94 (92)	92 (87)	96 (97)
In-house EIA[c]	100 (100)	97 (93)	95 (90)	100 (100)
Pyloriset EIA[c]	100 (100)	93 (89)	90 (85)	100 (100)
Pyloriset Dry	99 (100)	94 (91)	91 (87)	99 (100)
Pyloriset Screen	95 (96)	94 (91)	91 (86)	97 (98)

[a] *H. pylori* infection was defined by positive histology alone (results in parentheses) or by the combination of gastritis and elevated IgG antibody titers in both EIAs. Abbreviations: PPV, positive predictive value; NPV, negative predictive value.
[b] Cultures were available for only 113 patients.
[c] Combination of IgG and IgA results.

EIA (Orion Diagnostica), respectively. The antigen used in the in-house EIA was an acid glycine extract from *H. pylori* NCTC 11637. The absorbance readings were converted to reciprocals of the end-point titers. The end-point titers were dilutions of serum at a cutoff level defined on the basis of the optical densities of positive reference serum pools at constant dilutions. Separate reference pools were used for IgG and IgA. Pyloriset EIA-G and EIA-A were performed in accordance with the instructions of the manufacturer.

Rapid tests. Serum samples were tested for total *H. pylori* antibodies by the Pyloriset Dry (Orion Diagnostica) latex agglutination test in accordance with the manufacturer's instructions (10). For Pyloriset Screen, an indirect solid-phase immunoassay, a lancet was used to puncture a fingertip and blood was brought up into a capillary tube with a volume of 25 μl. The blood sample and two drops of test developer solution were placed on the pad at the upper area of a sample well on the test device. The working principle of the test is as follows: by capillary action, the specimen and the developer solution move toward the *H. pylori* antigen band impregnated on the test membrane and mobilize a dye conjugated with anti-human immunoglobulin. In the case of a positive sample, a complex consisting of *H. pylori* antibodies and the conjugated dye binds to the antigen, forming a visible band in the test window. Regardless of the presence of *H. pylori* antibodies, any excess antibody-conjugate complex continues to move along the test membrane, binding to another band (immobilized conjugate-specific antibody) located in the control window and generating a colored band. The test is considered positive if a colored line is seen in both the test window and the control window. In our study, the result was read after 10 min and, if negative, again after 30 min. Even the faintest purple lines were recorded as positive.

RESULTS

All 77 patients with histologically detected *H. pylori* had gastritis, and 74 of them were positive for *H. pylori* antibodies in all four serologic tests. One histologically positive patient showed elevated IgA antibody titers only in the two EIAs and had positive results in the two rapid tests. The remaining three histologically positive patients had positive results in the three previously validated serologic tests. All 34 patients with positive cultures were found to have helicobacters on histological examination.

There is no "gold standard" for the diagnosis of *H. pylori*, and histological examination alone may be too insensitive in some cases. We chose positive histology (and culture) or the combination of gastritis and elevated IgG antibody titers in both EIAs as the criteria for *H. pylori* infection. Thus, a total of 82 patients were considered *H. pylori* positive. Four of the five positive patients with helicobacter-negative gastritis had positive Pyloriset Dry and Screen tests in addition to elevated IgG antibody titers in the two EIAs. Table 1 presents the sensitivities and specificities of all the tests based on the above-described infection criteria and histology alone. For eight patients, the Pyloriset Screen test was positive although our criteria for *H. pylori* infection were not fulfilled. For six of these eight patients, at least one additional serologic test was positive as well. Macroscopic findings for the patients are presented in Table 2. The 12 patients with duodenal ulcers were *H. pylori* positive both histologically and by all four serologic tests.

There were 63 patients younger than 45 years of age in our study population. Twelve of them were considered *H. pylori* positive, and they had helicobacters in their biopsy specimens and positive results in all serologic tests. Of the 51 *H. pylori*-negative patients below the age of 45 years, one had a positive Pyloriset Screen test although the other serologic tests were negative. In all, the Pyloriset Screen test was positive at 10 min for 64 patients and became positive by 30 min for 22 additional patients.

DISCUSSION

The interest in rapid diagnosis of *H. pylori* has increased in recent years. To reduce costs, it has been proposed that endoscopy be reserved for patients with serious symptoms such as the weight loss or anemia suggestive of a bleeding ulcer or cancer, advanced age, or *H. pylori* infection (2, 13, 19, 21). On the other hand, it has been suggested that patients under the age of 45 years without serious symptoms be treated for their *H. pylori* infections and subjected to endoscopy only if still symptomatic after successful eradication therapy (6). Both of these approaches would benefit from a reliable rapid diagnostic test. For screening of dyspeptic patients, a test of high sensitivity is needed to ensure that positive individuals who should have endoscopies will not be missed. However, if treatment decisions are to be made on the basis of the test used, it is extremely important that the test is highly specific so that false-positive results and unnecessary antimicrobial therapy can be avoided.

The novel rapid serologic test Pyloriset Screen was easy to perform; no special equipment was needed, and the results were available generally within 10 min and at least within 30 min. Pyloriset Screen showed both high sensitivity (95%) and high specificity (94%) in our study patients referred for gastroscopy. The validity of the test in a population with less severe symptoms and no need for gastrointestinal endoscopy has yet

TABLE 2. Endoscopic findings for 207 consecutive patients referred for upper endoscopy

Finding	No. of patients who were *H. pylori*:	
	Positive[a]	Negative
Normal	33	61
Hiatus hernia only[b]	13	28
Erosions in the antrum	8	14
Erosive esophagitis	5	11
Duodenal ulcer	12	0
Prepyloric ulcer	3	3
Gastric (angulus) ulcer	2	0
Erosive bulbitis	2	1
Antrum fistula	1	0
Atrophic gastritis	3	7

[a] Infection was defined as either positive histology or the combination of gastritis and elevated IgG antibody titers in both EIAs.
[b] Ten patients with erosive esophagitis and six patients with antrum erosions also had hiatus hernias.

to be determined. In comparison with other rapid serologic tests studied by others, Pyloriset Screen clearly appears to have a higher specificity (1, 2, 16). High specificity is especially important if the test is the only diagnostic method used prior to making decisions on treatment in a population with a low prevalence of *H. pylori* infection. Among the study patients under the age of 45 years, only 19% were infected, and the Pyloriset Screen test did not miss a single patient with *H. pylori* infection. Only one patient in this age group would have received unnecessary antimicrobial treatment if the decision had been made solely on the basis of the Pyloriset Screen test. In the group of patients 45 years of age or older, in whom noninvasive tests would be a useful complement to information obtained with endoscopy, the Pyloriset Screen test revealed *H. pylori* infection in four additional patients who had gastritis but no evidence of helicobacters on histologic examination.

Quantitative serologic tests demonstrate a significant drop in antibody titer after successful antimicrobial therapy. A 50 to 60% fall is already seen at 5 to 6 months posttherapy (5, 20, 22). The higher the initial antibody titer, the longer it usually takes before the antibody titer is within normal range. Therefore, qualitative serologic tests such as Pyloriset Screen cannot be used in the follow-up of *H. pylori* treatment. If a qualitative test is used to screen for the infection, either two additional serum samples, one obtained before and the other obtained 4 to 6 months after treatment, are needed or the follow-up should be carried out with the urea breath test.

In summary, Pyloriset Screen was shown to be a reliable rapid serologic test to screen for *H. pylori* infection. It could well be used in smaller laboratories that do not possess the facilities for quantitative serologic tests or in doctors' offices if there is a need to know the results at the first visit.

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