

Serodiagnosis of *Helicobacter pylori*: Comparison of Enzyme-Linked Immunosorbent Assays

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Enzyme-linked immunosorbent assays (ELISAs) have been developed to diagnose *Helicobacter pylori* infection. However, the methods are not standardized. We therefore prospectively evaluated the sensitivities and specificities of ELISAs developed in the United States and the United Kingdom in a study population comprising 41 consecutive symptomatic outpatients and 35 volunteers. At endoscopy, multiple biopsies were obtained for histology and culture and stained sections were graded for chronic gastritis, active chronic gastritis, and density of *H. pylori*. Serum samples were analyzed for *H. pylori* by ELISA. The first set of assays for immunoglobulin G (IgG) and IgA used a pool of sonicated isolates of *H. pylori* from five patients in the United States (antigen A). The second set of assays, developed in the United Kingdom, used three different antigens: antigen 1, an acid-extractable surface antigen; antigen 2, an acid-extractable antigen from an aflagellate variant; and antigen 3, a urease-containing fraction. Cutoff scores for positive results were determined a priori on the basis of previous serological studies. There was close agreement between histology and culture. In the study population, 36% of the individuals were *H. pylori* positive. The diagnostic value of the different ELISAs were highly comparable, and the crude antigens performed as well as the more purified antigens. The antigen A IgG had a sensitivity and specificity of 96 and 94%, respectively; the values for antigen 1 were 93 and 96%, respectively. The antigen A IgA and antigen 3 assays were the least sensitive tests. All of the serological assays were significantly associated with active chronic gastritis scores, but the IgA assay provided additional independent information for discriminating mild active gastritis from more severe gastritis. Despite the antigenic variation that exists between *H. pylori* strains, the diagnostic characteristics of these ELISAs from the United States and the United Kingdom are similarly highly sensitive and specific.

Helicobacter pylori is strongly associated with chronic diffuse superficial histological gastritis, in which there is infiltration of mononuclear and, frequently, polymorphonuclear cells into the lamina propria (1, 3, 22). *H. pylori*-associated gastritis is present in virtually all patients with idiopathic chronic duodenal ulceration (30), and there is convincing evidence that *H. pylori* is the cause of the associated gastritis (1, 20). Indeed, this inflammatory response may represent in part the host's immune response to infection (16), as the local antibody produced by mature B cells consists primarily of immunoglobulin A (IgA) and IgG (26).

Direct diagnosis of *H. pylori* gastritis can be made by obtaining samples of the gastric mucosa at endoscopy; however, endoscopy is an invasive procedure, and sampling error can result in failure to detect infection, as it is often patchy (20). Moreover, endoscopy is not suitable for screening of populations. Serological tests have, therefore, been developed to diagnose *H. pylori* infection; techniques for serodiagnosis include bacterial agglutination (12), passive hemagglutination (15), indirect immunofluorescence (8), complement fixation (11, 12), and enzyme-linked immunosorbent assays (ELISAs) (6, 18, 25). The technique of choice is currently ELISA because it is a simple, quick, low-cost technique that permits immunoglobulin class-specific determinations (19, 24). ELISAs, however, are not standardized; different antigen preparations have been used and definitions

of what constitutes a positive result have varied from study to study (19, 24). Very little work has been done in directly comparing the sensitivities and specificities of different ELISAs in the same study population (10). We therefore prospectively evaluated different ELISAs developed in the United States and the United Kingdom by using as a "gold standard" the results from histology and culture of gastric biopsies.

MATERIALS AND METHODS

Patient population. The study population comprised 76 consecutive patients. There were 41 patients with various gastrointestinal symptoms (83% had abdominal pain); their mean age was 50 years (20 males and 21 females). In addition, 35 volunteers agreed to participate in the study (mean age, 50 years; 16 males and 19 females); these included outpatients attending the gastroenterology clinic and healthy volunteers who had responded to an advertisement. All gave informed consent, and the studies were approved by the Institutional Review Board of Mayo Clinic. Before endoscopy, all study subjects had 10 ml of blood withdrawn for serological testing. The blood was centrifuged and the serum was stored in aliquots at -20°C . Endoscopy was routinely performed, and biopsies were obtained from the gastric antrum; in addition, biopsies were obtained from the gastric body in 65% of the cases. Of the 76 patients, 9 had chronic gastric ulceration while 18 had gastroduodenal erosive changes at endoscopy.

Histology. Biopsies were evaluated in all cases by a single

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histopathologist (H.A.C.) who was unaware of the patient's clinical status, culture results, and serological findings. Multiple sections of each biopsy were prepared and stained with hematoxylin and eosin and with Giemsa or Warthin-Starry silver stain (22).

Each biopsy was assigned chronic and active chronic gastritis scores after review of the hematoxylin-and-eosin-stained preparation. A chronic gastritis score of 0 indicated that no mononuclear cells were present, 1 indicated that occasional mononuclear cells were present in a patchy distribution, 2 was intermediate between grades 1 and 3, and 3 indicated very dense infiltration of mononuclear cells throughout the entire section. Active chronic gastritis scores were similarly graded from 0 to 3 on the basis of the additional presence and degree of infiltration by polymorphonuclear cells (21, 22).

H. pylori was identified, a semiquantitative score was assigned after examination of the hematoxylin-and-eosin-stained slides, and the findings were confirmed on Giemsa- or Warthin-Starry-stained biopsy sections. Density per high-power field was graded as 0, 1 (fewer than 10 organisms per section), 2 (10 to 100 organisms per section) or 3 (more than 100 organisms per section) (21, 22).

Culture. Culture was also performed for 27 of the 76 patients; the technique has been described elsewhere (21). In brief, additional biopsy specimens taken for culture were well minced with a sterile scalpel blade in the endoscopy suite and immediately plated on a thin layer of medium consisting of brucella agar supplemented with 5% fresh horse blood and containing vancomycin (3 mg/liter) and trimethoprim (5 mg/liter). After the medium was inoculated, the plates were placed in Bio-Bags and transported to the microbiology laboratory within 30 min. The cultures were incubated in microaerobic conditions at 35°C. On day 3, the plates were removed from the Bio-Bags and inspected for growth. They were then transferred to a Campy-pak Plus system with 100% humidity and inspected three times weekly for up to 30 days. Isolates of *H. pylori* were identified when highly curved, gram-negative bacilli that were urease, oxidase, and catalase positive were found. One patient had overgrowth of other microflora; results were thus available in 26 cases (13 symptomatic patients and 13 volunteers).

The remaining 49 patients did not have culture performed, as this technique was not available at the beginning of the study. However, a high degree of concordance between histology and culture has subsequently been confirmed in our laboratory; 49 consecutive patients with rheumatoid arthritis from the local community had both histology and culture performed at endoscopy. The tests agreed in 94% of the cases; culture was negative in three patients who had organisms on histology, while culture was not positive alone in any case (13).

Serological testing. Coded serum samples were analyzed for *H. pylori* by ELISA; the investigators were unaware of the clinical or *H. pylori* status of the patients from whom the sera were obtained. The first assay was developed by Perez-Perez et al. and has been described in detail elsewhere (25). The antigen used (antigen A) was derived from a pool of sonicated *H. pylori* isolates from five patients in the United States; serum samples were diluted 1:800 for the IgG assay and 1:100 for the IgA assay. The second antibody was peroxidase-conjugated goat anti-human IgG or IgA. The cutoff score for seropositivity was based on previously determined values (4, 25).

The second set of assays, developed in the United Kingdom, used three different antigens. For all three assays, the

TABLE 1. Comparison of *H. pylori*-positive and -negative patients

<i>H. pylori</i> status (no. of patients)	Median age (range)	% of males	% with a chronic gastritis grade of:				% with a chronic active gastritis grade of:			
			0	1	2	3	0	1	2	3
Positive ^a (27)	58 (33-79)	52	0	26	63	11	19	48	30	3
Negative (49)	48 (15-78)	45	78	22	0	0	98	2	0	0
<i>P</i> value ^b	<0.05	NS ^c	<0.01				<0.01			

^aPositive by histology and/or culture.

^bBased on logistic regression with *H. pylori* status as the dependent (binary) variable.

^cNS, no significant difference.

human sera were diluted at 1 in 200 and the concentration of specific IgG was calculated as previously described (28). Antigen 1 comprised acid-extractable surface antigens from *H. pylori* NCTC 11638 (18). The cutoff score for a positive result was set a priori at 10 µg/ml on the basis of a previous study of 47 patients (27). Antigen 2 used the acid-extractable antigens from an aflagellate variant of *H. pylori* NCTC 11638. Antigen 3 used a urease-containing fraction isolated from the sonicated antigen preparation of *H. pylori* NCTC 11638 by size exclusion fast protein liquid chromatography in a Superose 6 column (9). The cutoff scores for antigens 2 and 3 were set a priori at 5.0 and 2.5 µg/ml, respectively, on the basis of the same set of sera as for antigen 1.

Determination of *H. pylori* status. A patient was classified *H. pylori* positive if either culture or histology reflected the presence of the organisms. A patient was classified *H. pylori* negative if histology and culture (when available) were negative (21).

Statistical analysis. Sensitivity, specificity, and predictive value were calculated for each ELISA. The association between the antibody scores and the gastritis scores was determined on the basis of ordinal logistic regression analysis. A receiver operator characteristic (ROC) curve was constructed to summarize the sensitivity and specificity estimates (21). Agreement between culture and histology for the subjects with both tests was summarized on the basis of estimated kappa coefficients.

RESULTS

Association among culture, histology, and gastritis score. The percent agreement between culture and histology for detection of *H. pylori* was 81% (kappa = 0.43). Culture was negative for two patients for whom histology was positive and positive in three cases in which histology was negative, but in all cases of disagreement histological gastritis was present. Of the 36 patients with histologically proven chronic gastritis, 27 (75%) were *H. pylori* positive; no patient had *H. pylori* in the absence of chronic gastritis (Table 1). In only one patient was chronic gastritis present in the gastric body and not in the antrum when biopsies were obtained from both areas, indicating that antral biopsies provided reliable data. Of the 23 patients with active chronic gastritis, 22 (96%) were *H. pylori* positive; in only one patient with active chronic gastritis was *H. pylori* not detected. *H. pylori* was significantly associated with chronic gastritis ($P < 0.01$) and active chronic gastritis ($P < 0.01$). Those with gastritis and *H. pylori* had significantly higher chronic and active chronic gastritis scores than those without *H. pylori*. Seventeen

TABLE 2. Diagnosis of *H. pylori* in 76 subjects who underwent endoscopy: comparison of serological methods with a priori cutoff scores

Parameter(s) tested	% Sensitivity	% Specificity	Positive predictive value (%)	Negative predictive value (%)
Antigen A IgG	96	94	90	98
Antigen A IgA	70	86	73	84
IgG and IgA	96	80	72	98
Antigen 1	93	96	93	96
Antigen 2	89	96	92	94
Antigen 3	85	96	92	92
Antigen 1, 2, and 3	93	96	93	96

(42%) of the 41 symptomatic patients and 10 (29%) of the 35 volunteers were infected with *H. pylori* (no significant difference).

Serological tests. On the basis of a priori preset cutoff scores, the diagnostic values of the serological tests are presented in Table 2, and the ROC curves for antigen A IgG, antigen A IgA, and antigen 1 are presented in Fig. 1. There was almost perfect discrimination of *H. pylori* status on the basis of ROC curves for antigen A IgG and antigen 1 but not antigen A IgA. The antigen A IgG and antigen 1 assays disagreed in only three cases (4%).

A subject was classified as having false-positive serology if

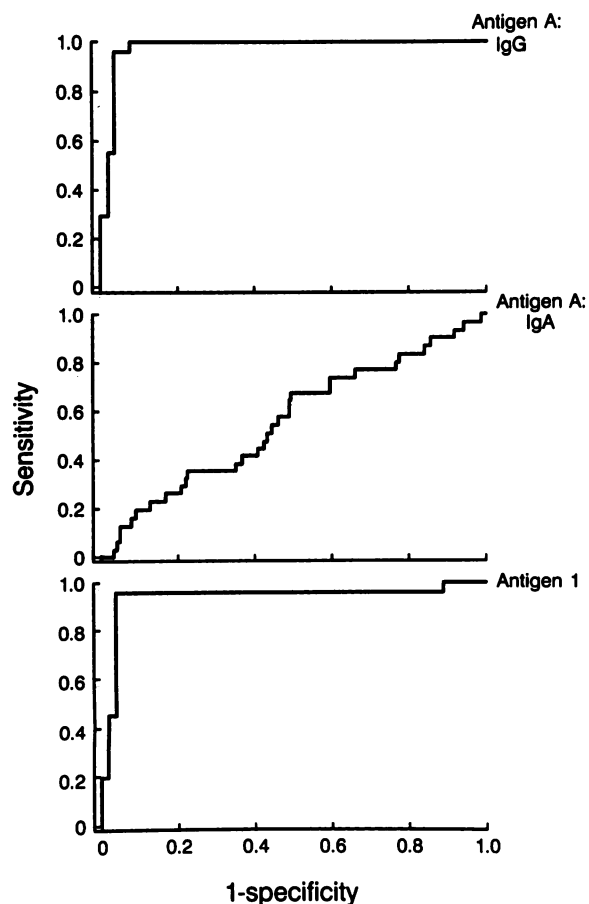


FIG. 1. ROC curves for antigen A IgG, antigen A IgA, and antigen 1.

any of the serological tests was positive but examination of the biopsy material was negative for *H. pylori*; 10 subjects fell into this group. Of these, three had chronic gastritis and one also had active chronic gastritis. Of the seven subjects without histological gastritis, six were positive only for antigen A IgA, and one was positive only for antigen A IgG. Eight subjects were *H. pylori* positive on examination of the biopsy material but negative on one or more of the serological tests. Only one subject was negative on all of the serological tests. Of the remainder, one had false-negative results on four of the serological tests (only antigen A IgG was positive) and six had false-negative results on three or fewer of the other tests.

All of the serological tests were significantly associated with the active chronic gastritis score (all $P < 0.01$). However, antigen A IgA provided additional independent information ($P < 0.01$) for discrimination between mild and more severe active chronic gastritis; the greater the antigen A IgA response, the more likely it was that severe active chronic gastritis was present.

DISCUSSION

Our results indicate that ELISA can be a highly accurate method by which *H. pylori* status can be determined, confirming previous reports (6, 18, 25). Moreover, we found that despite the antigenic variation that exists between *H. pylori* strains (23), the sensitivities and specificities of ELISAs from the United States and the United Kingdom gave remarkably similar results in the population evaluated, indicating that most patients produce antibodies that react against common antigens.

Evaluation of a diagnostic test depends on knowledge of whether the disease is truly present or not. While histology and culture remain the best available standards, there is no absolute "gold standard" for the diagnosis of *H. pylori*. For many other infectious diseases, culture is the "gold standard" for diagnosis because of exquisite sensitivity as well as complete specificity (by definition). However, for *H. pylori* infection, culture is problematic because of the fastidious nature of the organism and, in some cases, overgrowth of competing microflora, especially in the presence of hypochlorhydria; moreover, sampling error and other technical factors can reduce the diagnostic yield from infected patients (1, 20, 29). We have confirmed that histology and culture usually yielded comparable results (13, 21). Although cultures were not obtained for a number of our patients, in previous comparisons with the serology of Perez-Perez et al., culture has been found to be inferior to identification of *H. pylori* by examination of stained histologic sections (3, 7, 29), and we have confirmed these observations (13). We therefore believe that additional culture findings are not likely to have altered our results significantly. Indeed, on the basis of available data, virtually all authorities recognize that histology or a combination of histology and culture represents the "gold standard" for diagnosis (2, 14). However, it should be appreciated that serology can appear to perform no better than the "gold standard" selected and will seem to be inferior as it approaches the truth more closely. Thus, the three subjects with histological gastritis who were positive on serology but *H. pylori* negative by histology and culture are likely either to have had their *H. pylori* infection missed on biopsy or to have had an infection in the past (19, 24).

By using arbitrary cutoff scores that were determined a priori on the basis of previous studies (4, 18, 25), we found that our serological methods were highly sensitive and

specific. It is interesting that the crude antigens did as well as the more purified ones in determining *H. pylori* status. The lower sensitivity of antigen 3 confirmed other results when a similar urease-enriched fraction was used (5). Although the serological tests also had high positive and negative predictive values, it should be noted that these calculations depend not only on sensitivity and specificity but also on the prevalence of *H. pylori* in the population being tested and will thus vary in different settings. One of the ways to express the relationship between the sensitivity and specificity of a test is to construct an ROC curve; tests that discriminate the best are placed toward the upper left corner of the curve, and the overall accuracy can be described by the area under the curve. While there is a trade-off between sensitivity and specificity, it can be seen that the United States and United Kingdom serological tests are both accurate over a range of cutoff points (Fig. 1).

Others have shown that IgG and IgA levels remain elevated for prolonged periods (11, 24), and therefore, it is likely that patients retain the capacity to respond to chronic *H. pylori* infection. While some studies suggest that IgA levels, in combination with IgG levels, improve diagnostic accuracy (11, 25), we could not confirm these observations. Wyatt et al. (31) have reported that elevated IgA levels may reflect active chronic gastritis in some patients; similarly, we found that the IgA level reflected the activity of active chronic gastritis. While we have confirmed that IgG is the best marker of *H. pylori* infection, the value of analyzing IgG subclasses was not explored; the major subclasses that are consistently elevated are IgG1 and IgG4, while IgG2 is frequently raised (28). IgM antibody levels have not been found to discriminate between chronically infected and uninfected patients (11, 25) and so were not measured in this study; however, IgM may reflect acute rather than chronic *H. pylori* infection (17).

The different serodiagnostic ELISAs evaluated in this study demonstrated comparable sensitivities and specificities and were highly associated with histology and culture. We conclude that serological screening is accurate; in time, it may be recognized that serology is the closest to a "gold standard" for *H. pylori* infection, paralleling the role serology plays in syphilis and human immunodeficiency virus infections. Thus, serology may in the future be useful in the management of dyspepsia, as testing may detect patients at increased risk for chronic peptic ulceration. However, the use of serological tests as a screening procedure in clinical practice requires evaluation in controlled trials before general recommendations can be made.

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