

## Immunoglobulin A Antibodies to *Helicobacter pylori*

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**Serological testing for immunoglobulin G (IgG) antibodies to *Helicobacter pylori* has proven useful in supporting the diagnosis of infection with this organism, but the clinical value of IgA antibodies in *H. pylori*-related gastritis remains controversial. The purpose of our study was to determine the frequency of IgA-positive IgG-negative patients with symptoms of gastrointestinal (GI) disorders, thus assessing the clinical utility of IgA testing for *H. pylori*-related gastritis. It was found previously that the frequency of infected individuals in this category (IgA positive and IgG negative) is about 2%, but a large number of IgG-negative patients with GI disorders suggestive of *H. pylori* infection have not been investigated until now.**

In the early 1980s, it was found that *Helicobacter pylori* is associated with gastritis and peptic and duodenal ulcers and more recently, with gastric carcinoma (2, 4, 5, 10, 11, 15). Serological testing is often relied upon to determine the presence or absence of infection with this organism. Moreover, serology may be useful in monitoring the effectiveness of treatment in infected individuals (3, 7, 13). Immunoglobulin A (IgA) antibodies may appear earlier than IgG antibodies in patients who become reinfected after unsuccessful treatment with antibiotics (8, 14). Studies supporting the clinical utility of IgA serology have appeared. In the presence of IgG, IgA has been shown to correlate with active infection in 95 and 74% of cases of duodenal and gastric ulcers, respectively (6). IgA antibodies to *H. pylori* and low levels of pepsinogen I in patients' sera increase the risk of gastric carcinoma (1). Yamamoto et al. have shown the IgA antibody to be 100% specific for *H. pylori* infection compared to *Campylobacter*-like organism test, culture, and histology (16). Two studies have noted few patients (2%) with confirmed *H. pylori* infection and with only IgA antibodies (6, 7). We have determined a more realistic frequency of IgA-positive IgG-negative patients with gastrointestinal (GI) disorders suggestive of infection, thus further assessing the clinical utility of IgA testing for *H. pylori*.

Sera collected from 824 patients and submitted to our reference laboratory for *H. pylori* IgG testing were included in the study. Patients' sera were collected over a 3-week period and consisted of 526 negative, 290 positive, and 8 equivocal results for IgG antibody to *H. pylori*. Patients' sera were from 453 females ranging from 3 to 95 years of age (mean, 50 years) and 371 males ranging from 1 to 90 years of age (mean, 47 years). All sera were stored at  $-20^{\circ}\text{C}$  until all testing was completed. Patients with sera giving IgA-positive IgG-negative results ( $n = 38$ ) were followed up to obtain additional information from the clinician that would support a diagnosis of infection with *H. pylori*.

Testing for IgA antibody against *H. pylori* was accomplished with an enzyme immunoassay (EIA) kit provided by HYCOR Biomedical Inc. (Irvine, Calif.). This EIA detects IgA antibodies against *H. pylori*-associated antigens (14 to 120 kDa). The performance of this EIA was validated against endoscopy (culture and histology) results from 396 patients with symptoms of

GI disorders. One hundred fourteen patients were negative and 282 were positive for *H. pylori* by endoscopy. Compared with endoscopy, this *H. pylori* IgA EIA had a sensitivity of 90.2%, a specificity of 99.0%, and an accuracy of 92.8%.

Qualitative IgA values for patients' sera were determined from the optical densities (ODs) obtained from four calibrator serum samples, and results were reported as negative, equivocal, moderately positive, or highly positive. Sera with ODs greater than that of the moderate control cutoff OD were considered as being positive for IgA antibody to *H. pylori*. All sera with equivocal results were retested. All assay procedures were followed as stated in the product insert.

All IgG testing was completed with EIA kits purchased from Enteric Products Inc. (Stony Brook, N.Y.). This EIA detects IgG antibodies directed against high-molecular-weight cell-associated proteins of *H. pylori*. The performance of this EIA kit was validated against the [ $^{13}\text{C}$ ]urea breath test (UBT) with 556 serum samples from patients with symptoms of GI disorders and from nonsymptomatic volunteers. Compared with the UBT, this *H. pylori* IgG EIA was 97.6% sensitive and 94.1% specific.

Semiquantitative values were calculated for each patient, and the assay results were categorized as follows:  $\leq 1.7$ , negative; 1.8 to 2.2, indeterminate; and  $\geq 2.3$ , positive. All sera with indeterminate results were retested. All assay procedures were followed as stated in the product insert.

Other than the IgA EIA kits, no funds were derived from the manufacturers for these experiments. Washing steps for all EIAs were accomplished with a Wellwash 4 automated EIA plate washer from Denley Instruments, Inc. (Durham, N.C.). ODs for EIAs were measured with a Thermomax bichromatic microplate reader from Molecular Devices Corp. (Menlo Park, Calif.).

Of the 526 serum samples that were negative for IgG antibody to *H. pylori*, 38 were positive for IgA, giving a frequency of 7.2% of IgA-positive IgG-negative sera (Table 1). Follow-up on these 38 patients revealed that all had symptoms of GI disorders which prompted the initial testing for IgG antibody to *H. pylori*. The possibility of infection with *H. pylori* was excluded by the clinician for the majority (30 patients [78.9%]) of these 38 patients based on a negative IgG result. No further testing or procedures were utilized (i.e., additional serology, UBT, endoscopy, *Campylobacter*-like organism test, histological examination, and culture) to further rule out infection with *H. pylori*.

Of the 38 patients, endoscopy was performed on 6 and ulcers

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TABLE 1. *H. pylori* IgA results compared to IgG results for serum samples from 824 patients<sup>a</sup>

IgA result	No. of serum samples with the following IgG result:		
	Positive	Negative	Equivocal
Positive	121	38	1
Negative	131	458	5
Equivocal	38	30	2
Total	290	526	8

<sup>a</sup> Frequency of IgA-positive IgG-negative sera, 7.2%.

were noted for 5, while 1 patient was diagnosed as having Barrett's esophagus. Of the five patients with observed ulcers (two duodenal, one antral, one peptic, and one site unknown), two had biopsy specimens taken for histological examination in which both were negative for *H. pylori*. No patients were reported as having a history of nonsteroidal anti-inflammatory drug use.

Two of the 38 patients had been tested for IgA antibody per the clinician's request in an attempt to further rule out infection with *H. pylori* after negative results for IgG were obtained. Both patients were positive for IgA antibody to *H. pylori* by another reference laboratory, which offered IgA testing. Each patient was treated with antibiotics. In the first, treatment was successful and the patient had no further symptoms of a GI disorder posttreatment. Symptoms in the second patient subsided during and shortly after treatment but reappeared 3 weeks later.

Our data show that the frequency of IgA-positive IgG-negative patients (7.2%) with GI disorders suggestive of *H. pylori* infection is higher than the previously mentioned 2% (6, 7). All 38 patients in this category (IgA positive and IgG negative) were symptomatic for GI disorders compatible with *H. pylori*-related gastritis. Infection with *H. pylori* was excluded by the clinician in the majority (78.9%) of these 38 patients solely on the basis of a negative IgG serology result. Although repeat testing for *H. pylori* IgA and IgG antibodies was offered to these clinicians at no charge, only one clinician submitted a second serum sample for which similar results were obtained. We assume that symptoms continued in these patients, since infection with *H. pylori* had been excluded and no treatment was prescribed.

Only 8 of these 38 patients had additional testing to confirm or rule out infection with *H. pylori*. Endoscopy had been performed on six of the eight patients, of which five had ulcers and one patient was diagnosed as having Barrett's esophagus. Histology gave a negative result for *H. pylori* in two patients in which biopsy specimens were taken, but histology occasionally will fail to detect *H. pylori* in patients with infection that were confirmed positive by other methods (9, 12). These two patients had no history of nonsteroidal anti-inflammatory drug use.

Two of the 38 patients were tested for IgA antibody to *H. pylori*; positive IgA results were obtained for both by another reference laboratory. Symptoms in both patients subsided posttreatment with antibiotics, but one patient relapsed 3 weeks later. Two studies have shown that each of their patients ( $n = 2$ ;  $n = 3$ ) who were IgA positive and IgG negative was confirmed as *H. pylori* positive by culture and histology (6, 7). Moreover, IgA antibody may be specific for GI infection with *H. pylori* (16). Thus far, we have not found any patients reported in the literature in this category (IgA positive and IgG negative) that were not confirmed as being *H. pylori* positive. Therefore, a positive finding of IgA antibody to *H. pylori* in patients who are symptomatic may be of significant clinical value in supporting a diagnosis of infection, especially if IgG serology is negative.

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