Journal of Clinical Laboratory Analysis 13:194–198 (1999)

Serum Antibodies Anti-*H. pylori* and Anti-CagA: A Comparison Between Four Different Assays

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The authors compare efficacy of two ELISA assays (one supplied by DIAMEDIX [Delta Biological s.r.l.], and the other by RADIM [RADIM l]) in detecting total anti-*H. pylori* antibodies, and of two further ELISA methods (one supplied by EUROSPITAL [Helori CTX IgG] and the other by RADIM [RADIM 2]) in identifying anti-CagA antibodies, using sera from 69 controls (20 adults and 49 children) and from 96 patients, obtained before endoscopy. Seventy-three of the patients had *H. pylori* infection, while the remaining 23 were *H. pylori* negative (histology and polymerase chain reaction [PCR]). Fifty-two of the *H. pylori* positive patients, had cagA-positive strain infection, identified by PCR. The DIAMEDIX assay was found to be more sensitive (92%) than RADIM 1 (79%) in identifying *H. pylori* positive patients, irrespective of the infecting strain. On the other hand, the DIAMEDIX assay was less specific than RADIM 1 for *H. pylori*-negative patients (43% vs. 83%). However, when patients already treated for *H. pylori* infection were excluded from the group of *H. pylori*-negative patients, the DIAMEDIX assay had a specificity of 89%. In identifying anti-CagA antibodies, the kit supplied by RADIM (RADIM 2) had a sensitivity of 90% and a specificity of 94%, whereas that supplied by EUROSPITAL had a sensitivity of 100% and a specificity of 76%. The performances of the two methods in the identification of anti-CagA antibodies were found to be similar. The authors conclude that, in view of its high sensitivity, the DIAMEDIX assay may be useful in screening for *H. pylori* infection. J. Clin. Lab. Anal. 13:194–198, 1999. © 1999 Wiley-Liss, Inc.

Key words: serodiagnosis; vacuolating cytotoxin; polymerse chain reaction; peptic ulcer; gastritis

INTRODUCTION

The CagA antigen is expressed only by *H. pylori* strains with the *cagA* gene, which is located within the PAI (pathogenicity island), present in the more virulent strains (1). Besides *cagA*, PAI comprises several genes, including *picB*, which encodes protein-inducing gastric epithelial cells to produce IL-8, a chemotactic factor for PMN (1–3). The *cagA* gene, which is frequently detected in *H. pylori* strains producing a vacuolating cytotoxin (VacA), is encoded by the *vacA* gene (4–6). Located in a genomic region different to and distant from the PAI, *vacA* is polymorphic. The differences between cytotoxic and noncytotoxic strains depend mainly upon the mid-portion (m) and the region encoding for the signal peptide (s) of *vacA* (7,8). Two different alleles have been described for the mid-region (m1 and m2) and for the signal sequence (s1 and s2); *H. pylori* strains with s1 and m1 *vacA* alleles produce active cytotoxin, while those with s2 and m2 alleles produce an inactive protein (7,8).

Although the biological role of the *cagA* gene and its product, the CagA protein, is not yet known, their presence allows the characterization of *H. pylori* strains (4–9):

1. *cagA* gene: when identified using molecular techniques, it may discriminate between cytotoxic and noncytotoxic strains.
2. As the CagA protein is highly immunogenic, the identification of antibodies against it provides indirect indication on the infecting strain.

Data recently reported suggest that cytotoxic *H. pylori* strains (*cagA*-positive) are associated with more severe gastrointestinal disease (10–15), and therefore the description of the *H. pylori* genotype should be useful in patient management. However, the gastric mucosa specimens required in order to genotype this bacterium can only be obtained by means of invasive endoscopy. Alternatively, indirect information on the infecting strain may be obtained by detecting serum antibodies against not only the whole *H. pylori*, but

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Received 14 December 1998; Accepted 2 April 1999

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also against CagA (16,17). Serum antibodies against a 55-kDa protein seem to have a very high sensitivity (100%) in identifying *H. pylori*-infected subjects, and are therefore promising for diagnostic purposes (10). The procedure for the determination of serum antibodies is noninvasive, easier to perform, and more cost effective than that used for *H. pylori* genotyping.

The aims of our study were therefore to compare the efficacy of anti-*H. pylori* antibody measurement with the following ELISA assays:

1. DIAMEDIX (Delta Biologicals s.r.l.), which detects anti-58-KDa protein and anti-CagA.
2. EUROSPITAL, which identifies anti-CagA antibodies.
3. RADIM 1, which detects total anti-*H. pylori* antibodies.
4. RADIM 2, which detects anti-CagA antibodies.

MATERIALS AND METHODS

We studied a total of 165 subjects. Gold standards for the diagnosis of *H. pylori* infection were the histological identification of the bacterium in gastric mucosal samples and/or the ^{13}C urea breath test. The gold standard procedure for the diagnosis of an *H. pylori* infecting strain was the polymerase chain reaction (PCR). The healthy control group consisted of 20 asymptomatic blood donors (11 males, 9 females, age 25–40 yrs) and 49 asymptomatic children (23 males, 26 females, age 2–14 yrs), in whom *H. pylori* infection had been ruled out on the basis of a negative ^{13}C urea breath test. Ninety-six subjects were symptomatic patients (52 males, 44 females, age 19–79 yrs) who consecutively underwent upper gastrointestinal endoscopy. The endoscopic diagnoses were active duodenal ulcer (6 cases), healed duodenal ulcer (7 cases), active gastric ulcer (1 case), healed gastric ulcer (1 case), duodenitis (22 cases), antral gastritis (56 cases), and no evident endoscopic lesions (3 cases).

Of the six antral biopsies obtained from each patient at endoscopy, two were used for gastritis evaluation (H&E staining), two for histological assessment of *H. pylori* infection (Giemsa and/or Wartin-Starry), and the remaining two for *H. pylori* genotyping, which was performed as we described elsewhere (18,19); the *ureA* and *cagA* genes in particular were identified. At histology, *H. pylori* infection was diagnosed in 73 of the adults who underwent upper gastrointestinal endoscopy, and was ruled out in the remaining 23. Findings for *ureA* were also positive in all the 73 *H. pylori*-positive patients, but negative in the 23 *H. pylori*-negative patients. Of the *H. pylori*-positive patients, 52 were *cagA* positive and 21 *cagA* negative. In 14 of the 23 *H. pylori* negative subjects, the bacterium had been eradicated prior to the study period. From each fasting subject, a serum sample was obtained and stored at –20°C until biochemical determinations were made. Anti-*H. pylori* antibodies were measured in all subjects using two ELISA methods (DIAMEDIX, Delta Biologicals s.r.l., Pomezia, Italy, and RADIM 1, Radim, Pomezia, Italy), which identify a pool of *H. pylori* antigens (RADIM 1), or the two antigens CagA and the 58-kDa protein (DIAMEDIX). Anti-CagA antibodies were measured in all subjects using the ELISA kit purchased from RADIM (Pomezia, Italy), and in 142 subjects using the ELISA method purchased from EUROSPITAL (Helori CTX IgG, Trieste, Italy). The latter was not performed in the 23 *H. pylori*-negative patients, as no further sera were available in these cases.

For the statistical analysis of data we used analysis of variance (Anova one-way), Bonferroni's test for pairwise comparisons, linear regression analysis, and receiver operating characteristic (ROC) curves.

RESULTS

As no differences were found between the 20 healthy adults and the 49 healthy children for the mean values of the four assays (Student's *t*-test: t = 1.04, p:ns for DIAMEDIX, t = 0.99, p:ns for RADIM 1, t = 1.2, p:ns for RADIM 2 and t = 0.67, p:ns for EUROSPITAL), we used these subjects together as the healthy control group. Three other patient groups were identified: (1) *H. pylori* positive, *cagA* negative (52 cases); (2) *H. pylori* positive, *cagA* negative (21 cases); and (3) *H. pylori* negative (23 cases).

Figure 1 reports the findings following DIAMEDIX assay and the statistical analysis of data.

Table 1 shows mean values, standard deviations and the statistical analysis of data of the other three methods considered. As findings of analysis of variance were statistically significant for all the three assays, Bonferroni's test was then applied for the pairwise comparison of the means.

For the DIAMEDIX assay, the mean value + 3 SD of the healthy control group gave a cut-off value of 22 U/mL, with which the sensitivity in identifying *H. pylori*-positive patients was 92%. The low specificity towards *H. pylori*-negative patients (43%) increased significantly (89%) when patients already treated for *H. pylori* infection were excluded from the analysis. Using the same procedure, the RADIM 1 assay had a sensitivity of 79% and a specificity of 83%, at a cut-off of 10 U/mL (mean + 3 SD of our healthy controls). A 95% specificity towards *H. pylori*-negative patients was then fixed for DIAMEDIX and RADIM 1 assays; with this specificity, the cut-offs were 180 U/mL and 17 U/mL, respectively. Table 2 shows the sensitivity of DIAMEDIX and RADIM 1 assays in diagnosing *H. pylori*-positive/*cagA*-positive or *H. pylori*-positive/*cagA*-negative patients when 180 U/mL and 17 U/mL respectively were considered as cut-off values.

Table 3 shows the linear correlations found between the results of the four ELISA methods used; the patients were considered overall, and then considered as *H. pylori*-positive/*cagA*-negative or *H. pylori*-positive/*cagA*-negative, or *H. pylori*-negative groups.

Figure 2 shows the results of the receiver operating characteristic (ROC) curves in discriminating between: *H. pylori*-

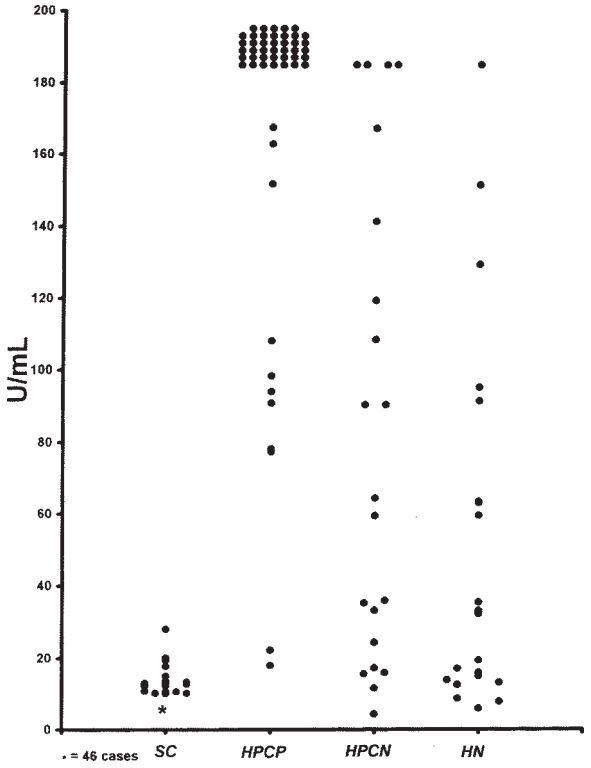

Fig. 1. Individual values for serum levels of anti-*H. pylori* antibodies detected with DIAMEDIX assay. CS, control subjects; HPCP, patients *H. pylori*-positive/*cagA*-positive; HPCN, patients *H. pylori*-positive/*cagA*-negative; HN, patients *H. pylori* negative. Anova one-way: F = 146.7, $P < 0.001$. Mean values of HPCP patients were significantly higher than that of all the other patient groups; mean values of HPCN and HN patients were significantly higher than that of CS.

TABLE 1. Mean values, standard deviations, and statistical analysis of the results obtained using three different ELISA methods for the detection of Anti-CagA or anti-*H. pylori* antibodies[a]

	EUROSPITAL U/mL		RADIUM 2 U/mL		RADIM 1 U/mL	
	Mean	SD	Mean	SD	Mean	SD
CS n = 69	0.60	0.59	1.90	0.64	3.85	2.96
HPCP n = 52	27.30*	12.04	97.21*	94.30	88.54*	114.96
HPCN n = 21	2.06	2.61	1.73	1.81	25.66	25.77
HN n = 23			9.47	16.42	5.90	5.26
	F = 92.38 $P < 0.001$		F = 18.16 $P < 0.001$		F = 9.17 $P < 0.001$	

Bonferroni's test for pairwise comparisons: * = $P < 0.001$ in comparison with the other groups.

[a]EUROSPITAL, anti-CagA; RADIM 2, anti-CagA; RADIM 1, anti-*H. pylori* total antibodies; CS, control subjects; HPCP, *H. pylori*-positive/*cagA*-positive patients; HPCN, *H. pylori*-positive/*cagA*-negative patients; HN-*H. pylori*-negative patients.

TABLE 2. Sensitivity and cut-offs of the DIAMEDIX and RADIM 1 assays in identifying *H. pylori*-positive/*CagA*-positive (HPCP) or *H. pylori*-positive/*CagA*-negative (HPCN) when a specificity of 95% towards *H. pylori*-negative patients was fixed

	HPCP	Cut-off	HPCN	Cut-off
DIAMEDIX	81%	180 U/mL	19%	180 U/mL
RADIM 1	77%	17 U/mL	52%	17 U/mL

positive/*cagA*-positive and *H. pylori*-negative patients; *H. pylori*-positive/*cagA*-negative and *H. pylori*-negative patients; *H. pylori*-positive/*cagA*-positive and *H. pylori*-positive/*cagA*-negative patients.

DISCUSSION

The mean values of anti-*H. pylori* antibodies elicited against a pool of *H. pylori* antigens (RADIM 1 assay) were significantly higher in *H. pylori*-positive/*cagA*-positive patients than in controls, *H. pylori*-negative patients, and *H. pylori*-positive/*cagA*-negative patients, whose mean values did not significantly differ from those of controls or *H. pylori*-negative patients. On the other hand, the mean values for anti-*H. pylori* identified by the DIAMEDIX assays were significantly higher in *H. pylori*-positive/*cagA*-positive, and *H. pylori*-positive/*cagA*-negative patients than in controls. DIAMEDIX assay, however, did not discriminate between *H. pylori*-positive/*cagA*-negative and *H. pylori*-negative patients. When a specificity of 95% towards *H. pylori*-negative patients was fixed, the sensitivity of DIAMEDIX assay was higher than that of RADIM 1 in identifying *H. pylori*-positive/*cagA*-positive patients, whereas the inverse was observed when sensitivity was calculated for *H. pylori*-positive/*cagA*-negative patients. The different behavior of the anti-*H. pylori* antibodies identified by RADIM 1 and DIAMEDIX in *H. pylori*-negative patients, may depend on the fact that the antibodies identified by RADIM 1 have a shorter half life than those detected with DIAMEDIX. Furthermore, as confirmed by ROC curves, the DIAMEDIX assay has a higher sensitivity than RADIM 1 in diagnosing *H. pylori*-positive/*cagA*-negative patients; this may depend on this assay's ability to identify antibodies elicited against the 58-kDa antigen, which are probably the same as those directed against a 55-kDa protein (described by us elsewhere using western blot (10)), and found in almost all *H. pylori*-infected patients.

Depending on the infecting strain, *H. pylori* infection can cause diseases, some of which become severe (6,11–15). The identification of the *cagA* gene, which is frequently present in the more virulent strains, might therefore provide information on the infecting bacterium (10,15). However, the molecular characterization of *H. pylori* calls for biopsy and is therefore time consuming and costly. These drawbacks might, in selected cases, be overcome by serology, with the identification of anti-CagA antibodies, since the CagA antigen al-

TABLE 3. Linear correlations between the four ELISA methods evaluated

	DIAMEDIX	EUROSPITAL	RADIM 1
All patients			
EUROSPITAL	r = 0.75 $P < 0.001$		
RADIM 1	r = 0.42 $P < 0.001$	r = 0.46 $P < 0.001$	
RADIM 2	r = 0.56 $P < 0.001$	r = 0.77 $P < 0.001$	r = 0.33 $P < 0.001$
H. pylori-positive/*CagA*-positive patients			
EUROSPITAL	r = 0.49 $P < 0.001$		
RADIM 1	r = 0.21 *P*:ns	r = 0.24 *P*:ns	
RADIM 2	r = 0.35 $P < 0.05$	r = 0.66 $P < 0.001$	r = 0.10 *P*:ns
H. pylori-positive/*CagA*-negative patients			
EUROSPITAL	r = 0.44 *P*:ns		
RADIM 1	r = 0.16 *P*:ns	r = 0.30 *P*:ns	
RADIM 2	r = 0.36 *P*:ns	r = 0.67 $P < 0.01$	r = 0.36 *P*:ns
H. pylori-negative patients			
RADIM 1	r = 0.64 $P < 0.01$		
RADIM 2	r = 0.78 $P < 0.001$		r = 0.56 $P < 0.05$

most always triggers a serological response due to its high immunogenicity (5,6,10,15). On comparing two ELISA methods designed for the identification of anti-CagA, we found that both gave mean values that were significantly higher in *H. pylori*-positive/*cagA*-positive patients than in controls or *H. pylori*-positive/*cagA*-negative patients, and their overall capacity to discriminate *cagA*-positive from *cagA*-negative infecting strains was similar (ROC curves). However, the method provided by RADIM (RADIM 2) seems preferable to that provided by EUROSPITAL, since it had a sensitivity of 90% and a specificity of 94% (cut-off = 5 U/mL), whereas the latter had a sensitivity of 100% but a specificity of 76% (cut-off = 2.4 U/mL) in discriminating *cagA*-positive from *cagA*-negative *H. pylori*-infected patients.

In conclusion, the high sensitivity of the DIAMEDIX assay in identifying *H. pylori* infected patients suggests that it

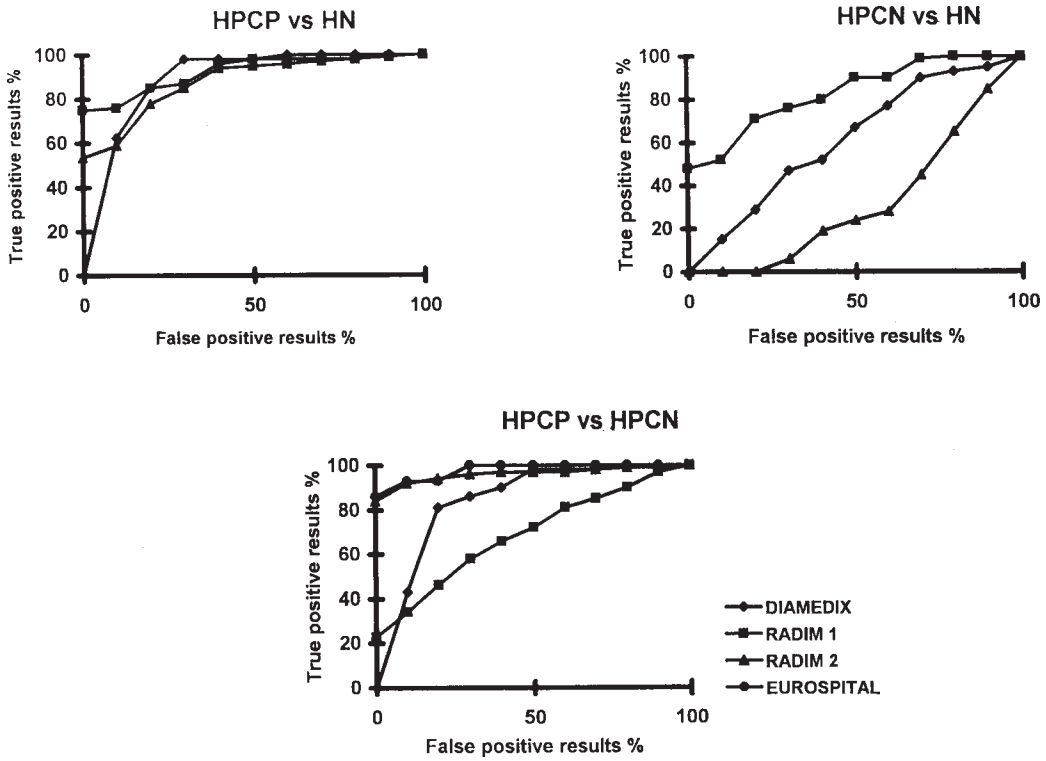

Fig. 2. Receiver operating characteristic curves (ROC) of the four methods studied to distinguish between *H. pylori*-positive patients, *cagA*-positive or -negative, from *H. pylori*-negative patients. HPCP, patients *H. pylori*-positive/*cagA*-positive; HPCN, patients *H. pylori*-positive/*cagA*-negative; HN, patients *H. pylori*-negative.

could be used for screening. However, in view of its low specificity towards *H. pylori*-negative patients already treated for the infection, we cannot recommend its use in unselected patients, unless accurate studies on the clearance of antibodies identified by this method are performed.

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