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Comparison of Four Serological Tests To Determine the CagA or VacA Status of *Helicobacter pylori* Strains

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We compared four tests for antibodies to CagA or VacA, HelicoBlot 2.0, RIDA Blot Helicobacter, CHIRON RIBA *H. pylori* SIA, and an enzyme-linked immunosorbent assay using recombinant CagA. Immunoblot assays were accurate for determining *Helicobacter pylori* status but poor for determining CagA or VacA status (accuracy, 66 to 80% for CagA status and 34 to 67% for VacA status). None can be recommended for determining CagA or VacA status.

Recently, there has been interest in detecting whether *Helicobacter pylori* infection is caused by strains that express putative virulence factors CagA and VacA. A number of reports have been published regarding the association of anti-CagA or anti-VacA antibodies with different diseases (1–6, 9, 11, 12, 15). The interpretation of these studies is critically dependent on the accuracy of the tests used. We evaluated the performance of three immunoblot assays and one enzyme-linked immunosorbent assay (ELISA) for the detection of serum anti-CagA and anti-VacA antibodies against *H. pylori*.

All patients were seen at the Kyoto Prefectural University of Medicine, Kyoto, Japan. *H. pylori* status was determined by culture, by histology (Giemsa stain), and by testing for anti-*H. pylori* antibodies in serum (second-generation kit; HM-CAP). Control sera were obtained from asymptomatic volunteers who were determined to be negative for *H. pylori* infection by a combination of negative culture, histology, and serology. Patients were excluded if they had received recent blood transfusions, had had previous gastric surgery, had severe liver disease, or had received nonsteroidal anti-inflammatory drugs, proton pump inhibitors, or antibiotics within the previous 3 months.

Positive controls for determining CagA and VacA status were as follows: for CagA status, immunoblotting and PCR for *H. pylori*, as described previously (16–20); for VacA status, a vacuolating cytotoxin assay, as described previously (20).

Serum samples were stored at −80°C until serological testing was performed. CagA and VacA status was determined by three commercial immunoblot assays (HelicoBlot 2.0 [Genelabs Diagnostics, Singapore, Republic of Singapore; HB2.0], RIDA Blot Helicobacter [R-Biopharm GmbH, Darmstadt, Germany; RIDA-BH], and CHIRON RIBA *H. pylori* SIA (Chiron Corporation, Emeryville, Calif.; Chiron-RIBA]). All tests were run according to the manufacturer's directions.

For the Chiron-RIBA test, the identities of the antigen bands were scored in relation to the intensities of the two internal immunoglobulin G controls (level I, low control; level II, high control). A cutoff for seropositivity was defined from 30 *H. pylori*-negative controls, and we regarded uncertainty range data as positive for the anti-CagA antibody and negative for *H. pylori* status and the anti-VacA antibody. CagA status was also determined by an ELISA using a recombinant CagA antigen (OraVax Inc., Cambridge, Mass.) (OraVax ELISA) (1, 19).

We tested 80 CagA protein-, *cagA* gene-positive *H. pylori*-infected patients (42 men and 38 women; mean age, 54.3 years), including 20 patients with gastric ulcer, 20 patients with duodenal ulcer, 20 patients with gastric cancer, and 20 patients with chronic gastritis. The control group consisted of 15 CagA protein-, *cagA* gene-negative *H. pylori*-infected patients (9 men and 6 women; mean age, 53.6 years), including 3 patients with gastric ulcer, 1 patient with duodenal ulcer, 1 patient with gastric cancer, and 10 patients with chronic gastritis. The *H. pylori*-negative control group consisted of 30 asymptomatic volunteers (17 men and 13 women; mean age, 54.4 years).

Most patients with *H. pylori*-associated disease had at least five or more bands appearing on the blots produced with HB2.0 and RIDA-BH kits. The sensitivities, specificities, accuracies, and positive and negative predictive values for detection of *H. pylori* infection were 98, 80, 93, 94, and 92% for RIDA-BH; 97, 90, 92, 97, and 90% for HB2.0; and 95, 93, 90, 98, and 85% for Chiron-RIBA, respectively. The sensitivities, specificities, accuracies, and positive and negative predictive values for CagA status were, respectively, 100, 67, 80, 94, and 100% for RIDA-BH; 88, 80, 70, 96, and 55% for HB2.0; 83, 93, 66, 99, and 55% for Chiron-RIBA; and 85, 80, 68, 96, and 50% for OraVax ELISA. Each of the tests was beset by a high proportion of either false-positive or false-negative results (e.g., the false-positive rate with the best test, the RIDA-BH test, was 33%) (Table 1).

The sensitivities, specificities, accuracies, and positive and negative predictive values for VacA status were, respectively, 100, 32, 67, 78, and 100% for RIDA-BH; 51, 100, 34, 100, and 46% for HB2.0; and 88, 89, 58, 95, and 76% for Chiron-RIBA. Each of the tests was beset by a high proportion of either false-positive or false-negative results (e.g., the false positive rate with the best test, the Chiron-RIBA, was 17%) (Table 1).

While each of the three immunoblot kits was useful for assessing *H. pylori* status, none could be recommended for the evaluation of the CagA or VacA status of the infecting *H. pylori*.

Cover et al. used an ELISA to detect CagA-expressing *H. pylori* and correlated the serologic results with detection of the *cagA* gene (4). They found considerable discordance between ELISA and the molecular detection of *cagA*, especially for patients infected with *cagA*-negative *H. pylori* strains, and sug-

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TABLE 1. Overall results with the four serologic tests for *H. pylori*-infected and uninfected individuals

Test and status[a]	No. of patients	% of patients that were:	
		Positive	Negative
RIDA-BH			
Hp^+ $CagA^+$	80	100	0
Hp^+ $CagA^-$	15	33	67
Hp^-	30	17	83
Hp^+ VT^+	67	100	0
Hp^+ VT^-	28	68	32
Hp^-	30	20	80
HB2.0			
Hp^+ $CagA^+$	80	88	12
Hp^+ $CagA^-$	15	20	80
Hp^-	30	7	93
Hp^+ VT^+	67	51	49
Hp^+ VT^-	28	0	100
Hp^-	30	0	100
Chiron-RIBA			
Hp^+ $CagA^+$	80	83	17
Hp^+ $CagA^-$	15	7	93
Hp^-	30	3	97
Hp^+ VT^+	67	88	12
Hp^+ VT^-	28	11	89
Hp^-	30	10	90
OraVax ELISA			
Hp^+ $CagA^+$	80	85	15
Hp^+ $CagA^-$	15	20	80
Hp^-	30	3	97

[a] Hp^+, *H. pylori* positive; Hp^-, *H. pylori* negative; $CagA^+$, CagA protein, *cagA* gene positive; $CagA^-$, CagA protein, *cagA* gene negative; VT^+, vacuolating cytotoxin positive; VT^-, vacuolating cytotoxin negative.

gested that this was due to undetected mixed infection with CagA-positive and CagA-negative strains. The results of this study suggest that false-positive results with the ELISA are at least as likely.

Shimoyama et al. reported that the positivity rates for the *cagA* gene were 100 and 92.3% in patients with gastric cancer and asymptomatic controls, respectively, in Japan (13); these rates are consistent with our observations and those of others (8, 10, 16–18). In a separate study, they reported an unexpectedly low frequency of anti-CagA antibody in patients with gastric cancer and controls, as determined by using the recombinant CagA antigen (14); this is in accordance with our result suggesting that the serological detection of CagA status may provide misleading results.

Serological profiles of *H. pylori*-positive patients tend to be quite diverse, possibly reflecting the genetic diversity of *H. pylori* worldwide. Höök-Nikanne et al. reported that although antigenic preparations from individual U.S. and Chinese strains were not optimally sensitive for the serologic detection of infection in China and the United States, respectively (7), the serologic response to CagA was retained in all groups of patients they tested.

The results of these experiments have bearing on the interpretation of seroepidemiologic studies of the association of CagA or VacA with different *H. pylori*-related diseases. Publication of any seroepidemiology study should be accompanied by confirmation that the serologic test has been validated for the population being studied. Prior associations made by using these tests without such validation should be accepted with caution.

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