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Accuracy of a Commercial Enzyme-Linked Immunosorbent Assay for CagA in Patients from Brazil with and without Gastric Carcinoma

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We validated a commercial enzyme-linked immunosorbent assay for the detection of anti-CagA antibodies in Brazilian patients with *Helicobacter pylori* infection. The test presented high sensitivity (97.4%) and specificity (88.9%) when employed in patients without gastric carcinoma. However, in gastric carcinoma patients, the test was neither sensitive nor specific enough to detect *cagA*-positive *H. pylori* infection.

Among the *Helicobacter pylori* putative virulence factors, the *cag* pathogenicity island, which includes *cagA*, has been associated with peptic ulcer and distal gastric carcinoma in human beings (3, 6, 14). *cagA* status may be determined by detection of the *cagA* gene in the bacterium strain or gastric mucosa by molecular methods or by evaluation of serum anti-CagA antibodies (7, 8). Although anti-CagA antibodies have been evaluated by enzyme-linked immunosorbent assay (ELISA) in several studies, in most of them (4, 10, 13), the test had not been previously validated for the population. Besides this factor, all of those studies were conducted in developed countries, where the low prevalence of the infection may have a negative effect on the positive predictive value of the test. In addition, becausethe serum response to CagA may depend on factors linked to the patient, such as *H. pylori*-associated disease (15), or the genetic diversity of the bacterium worldwide (18), validation of the tests employed to detect anti-CagA antibodies in different geographic regions should be done (8, 19).

Therefore, from 1995 to 1997, we studied 123 patients (64 male and 59 female; mean age, 48.2 ± 15.8 years; range, 19 to 95 years) who underwent endoscopy for the evaluation of symptoms involving the upper gastrointestinal tract ($n = 86$) or had been treated for gastric carcinoma ($n = 37$) at the Surgical Clinic of the University Hospital/Universidade Federal de Minas Gerais (UFMG), at Luxemburgo Hospital, and at Mário Penna Hospital of Oncology. Part of the sera tested in the present study were from patients with gastric carcinoma included in a previous study (15). This project was approved by the Ethics Committee of Hospital das Clínicas, UFMG, Brazil, and informed consent was obtained from all patients.

At endoscopy, biopsy specimens were obtained from the antral and oxyntic gastric mucosa for culture, preformed urease test, and carbolfuchsin-stained smears. In patients with gastric carcinoma, gastric mucosa fragments from the antrum and body were obtained from stomachs removed by gastrectomy. Patients were considered to be *H. pylori* positive if at least two of the three test results were positive or if the culture alone was positive and negative if the three test results were negative.

As a "gold standard," the presence of the *cagA* gene was evaluated by PCR. Genomic DNAs from the bacterial strains ($n = 92$) or the gastric mucosa ($n = 5$ each from *H. pylori*-positive or -negative patients) were extracted with QIAamp (Qiagen, Germany) and PCR amplified with two sets of oligonucleotide primers as previously described (11, 12). The PCR products were resolved in 1% agarose gels containing Tris-borate-EDTA by using 100 bp as a molecular size marker. The *ureA* extracted from the tissue or bacterial growth was amplified as a control for the presence of *H. pylori* DNA (5).

Venous blood samples were drawn from each patient at the time of endoscopy or before surgery. Sera were assayed for anti-CagA antibodies by using a commercial ELISA, *Helicobacter pylori* p120 CagA (Viva Diagnostika, Hürth, Germany). The antigen employed in the kit is a highly purified protein of 120 kDa, and the assay was performed according to the recommendations of the manufacturer.

Two of the 123 patients evaluated were excluded because they had only one positive test result for *H. pylori*. One patient had a positive urease test result, and the other had only a positive stained smear. The data from the remaining 121 patients (63 male and 58 female; mean age, 48.3 ± 15.9 years; range, 19 to 95 years) were used for final analysis. Among them, 97 were *H. pylori* positive: 28 had duodenal ulcer, 32 had gastritis only, and 37 had gastric carcinoma. Twenty-four patients were *H. pylori*-negative. The analysis of the accuracy of the ELISA for the gastric carcinoma group was done separately.

First we evaluated the performance of the test in the group of patients without gastric carcinoma. In this group, 39 strains were *cagA* positive: 23 of 28 (82.1%) were isolated from the duodenal ulcer patients, and 16 of 32 (50%) were isolated from *H. pylori*-positive patients without the disease ($P = 0.009$). Anti-CagA antibodies were detected in 38 (97.4%) of these patients and in 2 of 21 (9.5%) patients, both with duodenal ulcer, infected by *cagA*-negative strains. Anti-CagA antibodies were also detected in 3 of 24 (12.5%) noninfected patients. The sensitivity, specificity, and positive and negative predictive

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values of the ELISA were 97.4 (95% confidence interval [CI], 84.9 to 99.9%), 88.9 (95% CI, 75.2 to 95.8%), 88.4 (95% CI, 74.1 to 95.6%), and 97.6% (95% CI, 85.6 to 99.9%), respectively.

With regard to the gastric carcinoma patients, 32 of 37 (86.5%) were colonized by *cagA*-positive strains. Among them, 24 of 32 (75.0%) developed anti-CagA antibodies. Three patients (60.0%) colonized by *cagA*-negative strains also developed anti-CagA antibodies. The sensitivity of the test was 75.0% (95% CI, 56.2 to 87.8%). When *H. pylori*-positive gastric carcinoma patients were compared with patients with gastritis only plus duodenal ulcer, significant differences ($P = 0.008$) in the sensitivity of the test were observed.

As we previously observed for children (16), this commercial ELISA showed high sensitivity for the detection of anti-CagA antibodies in adults from Brazil. Employing commercial ELISAs, similar results were observed by other authors in a developed country (2, 9).

With regard to the specificity of the test, approximately 10% of patients without *H. pylori* infection and 10% of patients infected by *cagA*-negative strains have serological reactivity against CagA. Similarly, Fusconi et al. (9) in Italy observed that 10% of *H. pylori*-negative patients developed anti-CagA antibodies, and Figueiredo et al. (8) in The Netherlands reported that 13% of false-positive results were due to patients infected by *cagA*-negative strains. This finding seems to be related to either a previous *H. pylori* infection, a mixed infection by *cagA*-positive and -negative strains, or the detection of cross-reacting antibodies. In fact, it has been demonstrated that anti-CagA antibodies can persist up to 32 months after eradication of the bacterium with antimicrobial drugs (17) and that mixed infections occur in approximately 8% of patients from Brazil (1). However, because we determined the *cagA* status in a pool of colonies, infection with multiple *H. pylori* strains seems not to be a factor in the false-positive results observed in our study.

Although the ELISA tested in this study showed high accuracy for the determination of *cag*A status in adults from Brazil, caution is required in the interpretation of this serologic test in gastric carcinoma patients. In this group of subjects, the test was neither sensitive nor specific enough to confirm or rule out the diagnosis. This result may explain the low accuracy of a commercial anti-CagA ELISA reported by Yamaoka et al. (19) in Japan. Among the patients evaluated by Yamaoka et al., 25% had gastric carcinoma, which could contribute to the 85% sensitivity and 80% specificity observed for the test.

In conclusion, this ELISA can be useful for the diagnosis of infection by *cagA*-positive *H. pylori* strains, except in gastric carcinoma patients. Because these subjects represent less than 1% of the infected patients, the test can also be useful in epidemiologic studies.

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