Short Communication

Comparison of Two Enzyme-linked Immunosorbent Assay Tests for Diagnosis of *Helicobacter pylori* Infection in China[1]

Frank D. Groves,[2] Lian Zhang, Ji-you Li, Wei-cheng You, Yun-sheng Chang, Lei Zhao, Wei-dong Liu, Charles S. Rabkin, Guillermo I. Perez-Perez, Martin J. Blaser, and Mitchell H. Gail

Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland 20892-7368 [F. D. G., W-c. Y., C. S. R., M. H. G.]; Beijing Institute for Cancer Research and School of Oncology, Beijing Medical University, Beijing, People's Republic of China 100034 [L. Zhang, J-y. L., W-c. Y., L. Zhao]; Weifang Medical Institute, Weifang, Shandong, People's Republic of China 261041 [Y-s. C.]; Linqu Public Health Bureau, Linqu, Shandong, People's Republic of China 262600 [W-d. L.]; and Vanderbilt University Medical Center, Nashville, Tennessee 37232-2605 [G. I. P-P., M. J. B.]

Abstract

An ELISA based on a pool of United States strains of *Helicobacter pylori* was compared with a newly developed ELISA based on a pool of Chinese strains. Both assays were tested using sera from 132 Chinese study subjects with biopsy-proven *H. pylori* infection. Using cutpoints designed to yield equal specificities of 94.9% in an uninfected control population, the sensitivity of the Chinese assay was 100.0%, compared to 97.7% for the United States assay ($P = 0.25$ by McNemar test). These results suggest that a *H. pylori* assay based on pooled antigens from United States strains will perform as well in the rural Chinese population as one based on antigens from Chinese strains.

Introduction

The rural county of Linqu in Shandong Province in northeast China has among the highest age-adjusted stomach cancer mortality rates in the world (1). A population-based endoscopic screening program in Linqu in 1989 revealed a high prevalence of chronic atrophic gastritis (98.1%), intestinal metaplasia (52.8%), and dysplasia (20.4%; Ref. 2). These lesions are believed to be steps in the progression from normal gastric epithelium to gastric adenocarcinoma of the intestinal type (3). The curved bacillus *Helicobacter pylori* has been shown to cause chronic atrophic gastritis (4, 5), which may progress to intestinal metaplasia. The seroprevalence of *H. pylori* infection in Linqu is 72% overall, and the seroprevalence is highest among those with most severe chronic atrophic gastritis (6).

It was reported previously (7) that a commercial *H. pylori* assay based on United States strains performed poorly (sensitivity, 85%; specificity, 66%) in a seroprevalence survey in Thailand, and that a new assay based on indigenous Thai strains of *H. pylori* performed better (sensitivity, 98%; specificity, 76%). This result raised concerns that comparison of studies in different countries would be problematic and that it would be necessary to develop specific assays for each country. We developed a new serological test based on indigenous Chinese strains of *H. pylori* for use in a clinical trial in rural China (6). We report here the results of a validation study to compare this new assay with a widely used assay based on United States *H. pylori* strains.

Materials and Methods

Study Subjects and Specimens. Subjects resided in the rural Chinese village of Bei Duan in Linqu County in Shandong Province, a population at high risk of stomach cancer. There were 292 villagers ages 35–64, of whom 277 were recruited for the study. 263 of these completed the initial interview; two of these failed the physical examination. Of the remaining 261, 239 (91.6%) underwent endoscopy in 1989, with biopsies taken from seven standard sites in the stomach. Twenty subjects with fewer than seven biopsies were excluded, leaving 219 subjects who had a full set of seven biopsies. Biopsies were preserved in 10% neutral buffered formalin, embedded in paraffin, and sectioned at the Beijing Institute for Cancer Research. Biopsies were reviewed by a panel of three senior pathologists at the Beijing Institute for Cancer Research and interpreted according to the protocol of the Chinese Association of Gastric Cancer (8). The presence or absence of superficial gastritis, chronic atrophic gastritis, intestinal metaplasia, and dysplasia was recorded for each biopsy; diagnostic criteria were published previously (2). Each biopsy was given an overall diagnosis based on the most advanced lesion, and each subject was assigned a global diagnosis based on the most advanced lesion among any of the seven biopsies.

An additional unstained slide from each biopsy was stained with Lennert's Giemsa and read by one of us (F. D. G.). Any biopsy in which curved bacilli were seen overlying the gastric mucosa was rated as positive for *H. pylori* infection, and any case with at least one out of seven positive biopsies was rated as a positive case. All slides from negative cases and a sampling of positive slides were submitted for expert review.

Serology. Gastric biopsies were cultured from 20 of the Chinese subjects; strains from five of seven *H. pylori*-infected subjects were pooled to provide antigenic material for serology. The "Chinese" antigen, derived from the five *H. pylori* isolates, was prepared in essentially the same way as the "United States" antigen (9). The five strains were grown on blood agar plates and then resuspended in distilled water. The cell suspensions ware sonicated six times for 30 s each, and protein determination was performed by bicinchonic acid assay (Pierce Chemical Co., Rockford, IL) prior to pooling the solutions. Microtiter plates were prepared using 1 μg total protein per well. Sera

Received 11/1/96; revised 2/20/97; accepted 2/24/97.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported in part by Grant R01-CA-58834 from the National Cancer Institute.
[2] To whom requests for reprints should be addressed, at Biostatistics Branch, Room 431-C, Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6130 Executive Boulevard, MSC 7368, Bethesda, MD 20892-7368.

obtained from 176 of the 219 subjects at the time of endoscopy in 1989 were tested for IgG antibodies to *H. pylori* using the Chinese ELISA and a previously described United States ELISA (10). Serum samples were diluted 1:800, and peroxidase conjugates of goat antihuman IgG (Biosource, Camarillo, CA) were diluted 1:4000. All assays were performed on coded samples in duplicate on at least 2 separate days. The intra-assay and interassay variations were <5%, with four positive and two negative sera as controls.

Statistical Analysis. Specificity was based on the seronegative rate of each assay in a previously described (11) cohort of 39 United States children who had undergone endoscopy and biopsy and were known to be uninfected based on negative histology, negative culture, and negative direct (tissue) urease assay. These children have been used previously as the control population in other studies (12, 13) of the United States ELISA. The cutpoint for each assay was set to yield two false positives in the control population, so that the estimated specificity of each assay was 94.9% (37 of 39). We did not use a higher cutpoint because the two highest values of the United States assay were tied. The corresponding cutpoints were: relative optical density ($A_{405\ nm}$) > 0.555 for the United States assay and $A_{405\ nm}$ > 0.514 for the Chinese assay. We defined a sample as positive if the optical density of the serum exceeded these cutpoints. Sensitivity of each assay was defined as the proportion of the 132 Chinese study subjects with biopsy-confirmed *H. pylori* infection who were seropositive using that assay. Ninety-five % CIs[3] were calculated (14).

Results

Of the 132 subjects who had at least one of seven biopsies demonstrating *H. pylori* infection by light microscopy, 57 had seven positive slides, 31 had six positive slides, 9 had five positive slides, 12 had four positive slides, 5 had three positive slides, 3 had two positive slides, and 15 had only one positive slide. Two subjects had superficial gastritis as their worst lesion, 63 had chronic atrophic gastritis, 42 had intestinal metaplasia, and 25 had dysplasia. On the basis of the chosen cutpoints, the Chinese serology had no false negatives (sensitivity, 100.0%, 95% CI, 96.4–100.0%), and the United States serology had three false negatives (sensitivity, 97.7%; 95% CI, 92.9–99.4%). The difference in the sensitivities was not statistically significant; McNemar's χ^2, 1.33, with $P = 0.25$. The three subjects with false-negative results on the United States serology each had extensive chronic atrophic gastritis, but none had intestinal metaplasia or dysplasia; two had *H. pylori* on only one slide, and one had *H. pylori* on three slides.

Discussion

Histopathological identification of the organism in biopsies was used as the reference method for diagnosis of *H. pylori* infection in the present study, and the United States and Chinese ELISAs were tested against this benchmark. Both assays, one derived from indigenous Chinese strains of *H. pylori* and the other based on antigens from United States strains, had excellent sensitivity using cutpoints designed to yield high specificity. These findings suggest that the *H. pylori* assay based on antigens from United States strains will perform comparably in the rural Chinese population to an assay based on antigens from indigenous Chinese strains. Our United States antigen also has been tested against sera from Canada (10) ,New Zealand (15), and Peru.[4] Taken together, these validation studies suggest that the United States antigen will be useful in a variety of populations.

It would be interesting to know why the commercial United States assay had lower sensitivity and specificity in the Thai population when compared with an assay based on antigens from four indigenous Thai strains of *H. pylori* (7). Preliminary results from additional validation studies have shown that an ELISA based on only one Chinese strain is less sensitive than an ELISA prepared in the same manner using five Chinese strains (data not shown). If the United States commercial assay used in the Thai study was not based on a pool of *H. pylori* strains, that may account for the differences in performance.

Acknowledgments

We thank Drs. Bernardo Ruiz and Pelayo Correa (Department of Pathology, Louisiana State University Medical Center, New Orleans, LA) for expert histopathological review.

References

1. You, W. C., Blot, W. J., Chang, Y. S., Ershow, A. G., Yang, Z. T., An, Q., Henderson, B., Xu, G. W., Fraumeni, J. F., and Wang, T. G. Diet and high risk of stomach cancer in Shandong, China. Cancer Res., *48:* 3518–3523, 1988.

2. You, W. C., Blot, W. J., Li, J. Y., Chang, Y. S., Jin, M. L., Kneller, R., Zhang, L., Han, Z. X., Zung, X. R., Liu, W. D., Zhao, L., Correa, P., Fraumeni, J. F., and Xu, G. W. Precancerous gastric lesions in a population at high risk of stomach cancer. Cancer Res., *53:* 1317–1321, 1993.

3. Correa, P. A human model of gastric carcinogenesis. Cancer Res., *48:* 3554–3560, 1988.

4. Fontham, E. T., Ruiz, B., Perez, A., Hunter, F., and Correa, P. Determinants of *Helicobacter pylori* infection and chronic gastritis. Am. J. Gastroenterol., *90:* 1094–1101, 1995.

5. Ruiz, B., Correa, P., Fontham, E. T., and Ramakrishnan, T. Antral atrophy, *Helicobacter pylori* colonization, and gastric pH. Am. J. Clin. Pathol., *105:* 96–101, 1996.

6. Zhang, L., Blot, W. J., You, W. C., Chang, Y. S., Kneller, R. W., Jin, M. L., Li, J. Y., Zhao, L., Liu, W. D., Zhang, J. S., Ma, J. L., Samloff, I. M., Correa, P., Blaser, M. J., Xu, G. W., and Fraumeni, J. F. *Helicobacter pylori* antibodies in relation to precancerous gastric lesions in a high-risk Chinese population. Cancer Epidemiol., Biomarkers, & Prev., *5:* 627–630, 1996.

7. Bodhidatta, L., Hoge, C. W., Churnratanakul, S., Warawadee, N., Sampathanakul, P., Tungtaem, C., Raktham, S., Smith, C. D., and Echeverria, P. Diagnosis of *Helicobacter pylori* infection in a developing country: comparison of two ELISAs and a seroprevalence study. J. Infect. Dis., *168:* 1549–1553, 1993.

8. Chinese Association of Gastric Cancer. Classification and Diagnostic Criteria for the Histologic Gastric and Duodenal Biopsy Specimens. Liaoning, China: Liaoning Publishing House, 1981.

9. Perez-Perez, G. I., Dworkin, B. M., Chodos, J. E., and Blaser, M. J. *Campylobacter pylori* antibodies in humans. Ann. Intern. Med., *109:* 11–17, 1988.

10. Drumm, B., Perez-Perez, G. I., Blaser, M. J., and Sherman, P. M. Intrafamilial clustering of *Helicobacter pylori* infection. N. Engl. J. Med., *322:* 359–363, 1990.

11. Glassman, M. S., Dallal, S., Berezin, S. H., Bostwick, H. E., Newman, L. J., Perez-Perez, G. I., and Blaser, M. J. *Helicobacter pylori*-related gastroduodenal disease in children: diagnostic utility of enzyme-linked immunosorbent assay. Dig. Dis. Sci., *35:* 993–997, 1990.

12. Blaser, M. J., Kobayashi, K., Cover, T. L., Cao, P., Feurer, I. D., and Perez-Perez, G. I. *Helicobacter pylori* infection in Japanese patients with adenocarcinoma of the stomach. Int. J. Cancer, *55:* 799–802, 1993.

13. Parsonnet, J., Blaser, M. J., Perez-Perez, G. I., Hargrett-Bean, N., and Tauxe, R. V. Symptoms and risk factors of *Helicobacter pylori* infection in a cohort of U.S. epidemiologists. Gastroenterology, *102:* 41–46, 1992.

14. Fleiss, J. L. Statistical Methods for Rates and Proportions, Ed. 2, pp. 13–15. New York: John Wiley & Sons, Inc., 1981.

15. Perez-Perez, G. I., Bhat, N., Gaensbauer, J., Taylor, D., Kuipers, E. J., Zhang, L., You, W. C., and Blaser, M. J. Country-specific constancy by age in cagA+ proportion of *H. pylori* infections. Gut, *39* (suppl. 2)*:* A83, 1996.

[3] The abbreviation used is: CI, confidence interval.

[4] G. I. Perez-Perez, personal communication.