Helicobacter pylori Infection and Risk of Gastric Cancer in Shanghai, China: Updated Results Based upon a Locally Developed and Validated Assay and Further Follow-Up of the Cohort[1]

Jian-Min Yuan,[2] Mimi C. Yu, Wei-Wen Xu, Myles Cockburn, Yu-Tang Gao, and Ronald K. Ross

Department of Preventive Medicine, University of Southern California/Norris Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles, California 90033-0800 [J.-M. Y., M. C. Y., M. C., R. K. R.]; Shanghai Institute of Digestive Diseases, Shanghai 200001, People's Republic of China [W.-W. X.]; and Shanghai Cancer Institute, Shanghai 200032, People's Republic of China [Y.-T. G.]

Abstract

Infection with *Helicobacter pylori* has been associated with an increased risk of gastric cancer in low-risk populations. However, our previous results (P. M. Webb *et al.*, Int. J. Cancer, *67:* 603–607, 1996) from an ongoing prospective study in Shanghai, China, a relatively high-risk population, failed to show an association between *H. pylori* infection and the subsequent risk of gastric cancer. That previous study had a relatively short time period of follow-up and the enzyme-linked immunosorbent assay (ELISA) used was based on strains found in Southern England and without validation among the Chinese. Either one of these two factors could have had an impact on the validity of those earlier observations. An ELISA developed and validated among Shanghai residents was used in the present study to reexamine specific antibodies to *H. pylori* in 188 gastric cancer patients and 548 control subjects. All of the cases of gastric cancer were identified during the first 12 years of follow-up of a cohort of 18,244 men, ages 45–64 years in Shanghai, from whom blood samples were collected at enrollment during 1986–1989. For each cancer case, three cancer-free control subjects were randomly selected from the cohort and matched to the index cases by age (within 2 years), month and year of sample collection, and neighborhood of residence. The Shanghai-based ELISA detected a higher prevalence of serum antibodies to *H. pylori* than the English-based assay in both gastric cancer cases (86 *versus* 53%) and control subjects (85 *versus* 56%). Virtually all of the subjects (98%) who were *H. pylori*-seropositive by the English-based assay tested positive by the Shanghai-based assay. On the other hand, 73% of gastric cancer cases and 68% of control subjects who were seronegative according to the English-based assay tested positive by the Shanghai-based assay. Using this alternative assay, combined with increased follow-up, our latest data contradict our earlier findings and show a statistically significant association between *H. pylori* seropositivity and gastric cancer risk (odds ratio, 1.84; 95% confidence interval, 1.08–3.11). We noted an increasing rate of seropositivity among cases as the time interval between cohort enrollment and cancer diagnosis increased. Among subjects followed for 5 or more years after enrollment, the odds ratio for gastric cancer related to *H. pylori* seropositivity was 3.74 (95% confidence interval, 1.51–9.30).

Introduction

It has been suggested that infection with the bacterium *Helicobacter pylori* is a risk factor for gastric cancer (1). The best evidence in support of this association has come from a number of case-control studies nested within prospective cohorts, which have shown a significantly increased risk of gastric cancer with *H. pylori* infection as assessed by the presence of specific antibodies (2–6). Previously, data from an ongoing prospective cohort study in men in Shanghai, China, a relatively high-risk population for this malignancy (51.7 per 100,000 person-years; Ref. 7), did not support a positive association between *H. pylori* infection and risk of gastric cancer (8). In that publication, we raised two possible concerns regarding the validity of those results:

(*a*) the specific antibodies to *H. pylori* were assessed using an enzyme-linked immunosorbent assay (ELISA) developed in Southern England, using *H. pylori* strains obtained from local patients (9). That English-based ELISA has been validated in several western populations with varying rates of sensitivity and specificity. Rates of sensitivity and specificity among tested subjects in Northern Ireland were 88 and 72%, respectively (10). The corresponding rates among the populations of Rochester, Minnesota; Copenhagen, Denmark; and Victoria, Australia were 93 and 96%, 74 and 71%, and 86 and 65%, respectively (11–13). There is no information on the validity of this English-based assay in a Chinese population. Given that strains of *H. pylori* may vary in different populations, it is possible that the English-based assay is suboptimal (or even inappropriate) for detecting infection in a Chinese population; and

(*b*) the cohort had been followed for a relatively short period of time (an average of 2.4 years between blood collection and the diagnosis of gastric cancer; Ref. 8). There is evidence that with the development of advanced gastric disease, the *H. pylori* bacterium can be cleared from the stomach, resulting in seroconversion (from positive to negative for *H.*

Received 11/25/98; revised 4/12/99; accepted 5/24/99.
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] This study was supported by NIH Grants R01 CA43092, R35 CA53890, and P30 ES07048.
[2] To whom requests for reprints should be addressed, at Department of Preventive Medicine, USC/Norris Comprehensive Cancer Center, M/S # 44, University of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033-0800. Phone: (323) 865-0825; Fax: (323) 865-0136; E-mail: jyuan@hsc.usc.edu.

pylori antibodies; Ref. 14). Therefore, gastric cancer patients (but not control subjects) may have been misclassified as seronegative, even though they were infected in the past. In the presence of such differential misclassification, estimates of the association between *H. pylori* seropositivity and gastric cancer would have been biased downwards.

In the present study, we used a recently developed and validated assay that was based on patient materials collected locally to assess *H. pylori* seropositivity. Follow-up of the cohort is now extended to an average of 5.2 years postenrollment. This report presents our latest findings on the *H. pylori*-gastric cancer association within the Shanghai Male Cohort.

Subjects and Methods

Subjects. The study design has been described in detail previously (8, 15). Between January 1986 and September 1989, all men, ages 45–64 years, living in four small, geographically defined areas of the city of Shanghai were invited to participate in a prospective study of diet and cancer. A total of 18,244 men were recruited, and each subject was interviewed in person to obtain information on current diet, medical history, and other life-style variables such as tobacco smoking and alcohol consumption. Each subject also provided blood and spot urine samples.

By March 1998, 195 incident cases of gastric cancer were identified among the cohort members. For each of the cases, three cancer-free controls matched to the index cases by age (within 2 years), month and year of sample collection, and neighborhood of residence at recruitment were selected from the cohort (8). The first 85 of these cases and 255 of their matched controls were tested for serum *H. pylori* antibodies using the English-based assay (8). Serum samples on 7 gastric cancer cases and an additional 16 control subjects not matched to these 7 cancer cases were depleted after the English-based serological testing. Therefore, 188 cases and 548 controls were included in the present study. Seventy-eight of these patients and 218 of their matched controls were included in the comparison of *H. pylori* positivity rates derived from the English-based *versus* the Shanghai-based ELISA.

Laboratory Tests. Blood samples from all of the cohort subjects were processed shortly after collection and stored at −20°C and −70°C until analysis. Serum samples in the present study were taken from the −20°C aliquots and had been thawed, once when they were used to test for the presence of *H. pylori* antibodies using the English-based assay (8). Serum samples (which were blinded with respect to case/control status and other characteristics of the individual subjects) were sent on dry ice to the Shanghai Institute of Digestive Diseases for *H. pylori* antibody testing using an enhanced version of ELISA developed by Pan *et al.* (16, 17). All of the serum samples from a given case-control matched set were tested in the same batch, which routinely contained two positive and two negative control sera for quality assurance. Duplicate measurements were made on 332 (45%) of the 736 tested samples; the remaining 404 samples were measured only once to conserve serum samples for other biomarker measurements. Only seven (2%) samples yielded inconsistent duplicate results. A third measurement was made on each of these seven specimens and the majority finding was recorded (two were antibody-positive, and five were antibody-negative). We also submitted two independent serum samples on 10% of study subjects ($n = 74$) to further assure high laboratory quality. Results were consistent for 72 of these subjects. The two subjects with inconsistent results were tested a third time, and the majority finding was recorded for each (one was antibody-negative, and the other was antibody-positive).

Table 1 *H. pylori* antibody status in gastric cancer patients and matched control subjects by the Shanghai-based and the English-based ELISAs

Shanghai-based ELISA	English-based ELISA		Total subjects (%)
	Antibody status		
	Negative (%)	Positive (%)	
Antibody status among cases			
Negative	10 (27)	1 (2)	11 (14)
Positive	27 (73)	40 (98)	67 (86)
Total	37 (100)	41 (100)	78 (100)
Antibody status among controls			
Negative	30 (32)	2 (2)	32 (15)
Positive	65 (68)	121 (98)	186 (85)
Total	95 (100)	123 (100)	218 (100)

The ELISA method of Pan *et al.* (17) is briefly described as follows. A bacterial suspension from each of 15 *H. pylori* strains obtained locally was sonicated, pooled, and used as a group antigen. The optimal antigen concentration was 10 μg/ml as determined by checkerboard titrations. Testing serum samples were diluted to 1:200. The absorbance was read at 490 nm on a spectrophotometer (Denatach Minireader II) after stopping the reaction with 50 ml of 1 M sulfuric acid. A serum sample was considered positive when the ratio of its absorbance to that of the standard negative sera was greater than 1.7.

The Pan *et al.* ELISA method (17) was validated among Shanghai residents. Two hundred twenty-five patients with various gastrointestinal symptoms were recruited. Gastric biopsies and serum samples were collected from all of the study subjects. Culture and smear of biopsied materials showed that 171 subjects were infected with *H. pylori.* One hundred sixty-one of the 171 infected patients were seropositive (sensitivity rate, 94%), and 47 of the 54 uninfected subjects were seronegative (specificity rate, 87%; Ref. 16).

The assay used in the present study was an enhanced version of the ELISA described above (17). An additional 15 strains of *H. pylori* obtained from local patients were added to the antigen pool (thus, 30 strains in total). To validate this enhanced version of the ELISA, a separate group of 152 patients with gastrointestinal symptoms were recruited. One hundred nineteen (78%) patients were men; their mean age was 44 years (range, 22–62 years). Gastric biopsies and serum samples were collected from all of the study subjects. Culture and smear of biopsied materials showed that 117 subjects were infected with *H. pylori.* One hundred sixteen of the 117 infected patients were seropositive (sensitivity rate, 99%), and 33 of the 35 uninfected subjects were seronegative (specificity rate, 94%).

Statistical Analysis. Data were analyzed by standard matched-pair methods (18). The association between *H. pylori* infection and gastric cancer was measured by OR[3] with 95% CI. We also examined this association by subsite of gastric cancer and by time interval between blood sample collection and diagnosis of gastric cancer. Conditional logistic regression models were used to examine *H. pylori*-gastric cancer association with or without adjustment for other potential confounders. The current set of possible confounding variables was identical to the one used in the analysis of our first study (8) and included the level of education (primary school or less, middle school, college or

[3] The abbreviations used are: OR, odds ratio; CI, confidence interval.

Table 2 *H. pylori* seropositivity in relation to risk of gastric cancer by time interval between cohort enrollment and cancer diagnosis and subsite of cancer

	Cases		Controls		OR[a] (95% CI)
	Total	*H. pylori*-positive (%)	Total	*H. pylori*-positive (%)	
All cases	188	168 (89)	548	451 (82)	1.84 (1.08–3.11)
By time interval between cohort enrollment and cancer diagnosis (year)					
<5, by cancer subsite[b]	97	83 (86)	275	231 (84)	1.10 (0.57–2.13)
Cardia	24	21 (88)	67	60 (90)	0.75 (0.17–3.25)
Noncardia	61	51 (84)	172	141 (82)	1.10 (0.50–2.39)
≥5, by cancer subsite[b]	91	85 (93)	273	220 (81)	3.74 (1.51–9.30)
Cardia	19	18 (95)	57	48 (84)	4.32 (0.45–41.80)
Noncardia	53	49 (92)	159	131 (82)	2.67 (0.88–8.11)

[a] OR for gastric cancer in *H. pylori*-positive *versus H. pylori*-negative individuals.
[b] Cases who had an unspecified subsite of gastric cancer were excluded from this analysis.

higher), cigarette smoking (ever *versus* never), alcohol consumption (weekly *versus* less frequently), history of peptic ulcer (yes *versus* no), blood group (O, A, B, or AB type), and consumption of dark leafy vegetables (tertiles), cured meats (tertiles) and pickled vegetables (ever *versus* never). Results from "unadjusted" and "adjusted" analyses were remarkably similar. The unadjusted results are presented in this report.

Results

The mean age (±SD) of gastric cancer patients at the time of diagnosis was 63.4 years (±5.6). The average time interval between blood collection and the diagnosis of gastric cancer was 5.2 years (range, 0.1–11.8 years).

There were 78 patients with gastric cancer and 218 control subjects who had *H. pylori* serological results from both the English-based and the Shanghai-based assays. Table 1 compares these results. Forty (98%) of the 41 gastric cancer patients who were antibody-positive according to the English assay tested positive by the Shanghai assay. On the other hand, 27 (73%) of the 37 gastric cancer patients who were antibody-negative according to the English assay tested positive by the Shanghai assay. Similar findings were seen in control subjects. Almost all (98%) of the positive control subjects and a substantial proportion (68%) of the negative control subjects by the English assay were seropositive in the present study.

Table 2 shows the association between *H. pylori* seropositivity and risk of gastric cancer. One hundred sixty-eight (89%) of the 188 gastric cancer patients and 451 (82%) of the 548 control subjects were *H. pylori*-seropositive, yielding a statistically significant OR for gastric cancer (OR, 1.84; 95% CI, 1.08–3.11).

We examined the effect of length of follow-up (*i.e.,* the time interval between blood collection and diagnosis of gastric cancer) on the association between *H. pylori* seropositivity and the risk of gastric cancer. There was no increase in gastric cancer risk among those with less than 5 years of follow-up. On the other hand, a statistically significant, 3.7-fold increase in risk of gastric cancer associated with *H. pylori* positivity was observed among subjects with 5 or more years of time interval between blood collection and the diagnosis of cancer (Table 2). The positive association remained unchanged after an adjustment for potential confounding factors (data not shown). When cases were stratified by subsite of cancer, the risk for cardia cancer was comparable to that for noncardia cancer, regardless of length of follow-up (Table 2).

Discussion

With increased follow-up, we now find *H. pylori*-infected individuals in Shanghai to be at substantially increased risk for gastric cancer. Consistent with the report of Forman *et al.* (19), we noted an increasing rate of seropositivity among cases as the time interval between cohort enrollment and cancer diagnosis increased. Among cases diagnosed 5 or more years after enrollment, 93% were seropositive at recruitment; the comparable figure for those with less than 5 years of follow-up was 86%.

Using data from three nested case-control studies to examine the *H. pylori*-gastric cancer association according to time interval between blood collection and cancer diagnosis, Forman *et al.* reported that the OR for gastric cancer was 2.1 among subjects with less than 5 years of follow-up, 2.3 in those with 5–9 years of follow-up, 4.4 in those with 10–14 years of follow-up, and 8.7 in those with 15 or more years of follow-up (19). As previously reported (8), we find no clear increase in risk (OR, 1.1) among subjects with less than 5 years of follow-up. The observed OR of 3.7 among those with 5 or more years (ranging from 5 to 12 years) of follow-up is compatible with the Forman *et al.*'s results (19).

The present study clearly indicates that the English-based assay is suboptimal in identifying *H. pylori*-infected individuals among Shanghai Chinese. Although it had few false-positive readings relative to the Shanghai-based assay, the English-based assay exhibited a substantial rate of false negatives. These differences are likely due to strain-specific differences in *H. pylori* bacteria inhabiting the British *versus* the Chinese population. A study in Thailand has shown that a locally derived ELISA is more sensitive and specific in identifying *H. pylori*-infected individuals than a United States-based assay, although the discrepancy is not as dramatic as that observed in the present study between the English- and the Shanghai-based assay. Whereas the Thai-based assay had sensitivity and specificity rates of 98% and 76%, respectively, the corresponding figures for the United States-based assay were 86% and 66%, respectively (20).

There is sparse information on *H. pylori* seropositivity rates in Chinese populations based on locally derived and validated ELISAs. In a rural county in Shandong, China, where gastric cancer mortality in men is about 70 per 100,000 person-years (21), Zhang *et al.* (22) reported an *H. pylori* seropositivity prevalence rate of 68% in healthy men, ages 35–64 years, using an assay derived from local strains; no information was given regarding the sensitivity and specificity of the assay. It is unclear whether the difference in rates of *H. pylori* infection

between Shanghai and Shangdong men is real or due to possibly lower sensitivity of the Shangdong assay.

Several epidemiological studies have found a statistically significant association of *H. pylori* seropositivity with noncardia cancer but not with cardia cancer (3–5). In the present study, there is no evidence of a differential risk between cardia and noncardia cancer in relation to *H. pylori* seropositivity.

In summary, the present data demonstrate a significant, positive association between *H. pylori* seropositivity and gastric cancer in Shanghai, China, a relatively high-risk population, that was observed only among subjects with at least 5 years of follow-up. The positive association remains unchanged after adjustment for potential confounding factors.

Acknowledgments

We thank Y.-B. Xiang, X.-L. Wang, Y.-L. Zhang, and J.-R. Cheng of the Shanghai Cancer Institute for their assistance in data collection and management; the staff of the Shanghai Cancer Registry for their assistance in verifying cancer diagnosis; and Kazuko Arakawa of the University of Southern California for her assistance in data management and analysis.

References

1. Correa, P. Is gastric carcinoma an infectious disease? N. Engl. J. Med., *325:* 1170–1171, 1991.

2. Forman, D., Newell, D. G., Fullerton, F., Yarnell, J. W. G., Stacey, A. R., Wald, N., and Sitas, F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. Br. Med. J., *302:* 1302–1305, 1991.

3. Nomura, A., Stemmermann, G. N., Chyou, P-H., Kato, I., Perez-Perez, G. I., and Blaser, M. J. *Helicobacter pylori* infection and gastric carcinoma among Japanese-Americans in Hawaii. N. Engl. J. Med., *325:* 1132–1136, 1991.

4. Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N., and Sibley, R. K. *Helicobacter pylori* infection and the risk of gastric carcinoma. N. Engl. J. Med., *325:* 1127–1131, 1991.

5. Siman, J. H., Forsgren, A., Berglund, G., and Floren, C-H. Association between *Helicobacter pylori* and gastric carcinoma in the city of Malmö, Sweden: a prospective study. Scand. J. Gastroenterol., *32:* 1215–1221, 1997.

6. Watanabe, Y., Kurata, J. H., Mizuno, S., Mukai, M., Inokuchi, H., Miki, K., Ozasa, K., and Kawai, K. *Helicobacter pylori* infection and gastric cancer. A nested case-control study in a rural area of Japan. Dig. Dis. Sci., *42:* 1383–1387, 1997.

7. Parkin, D. M., Muir, C. S., Whelan, S. L., Gao, Y-T., Ferlay, J., and Powell, J. Cancer Incidence in Five Continents, Vol. 6, IARC Scientific Publications No. 120. Lyon, France: IARC, 1992.

8. Webb, P. M., Yu, M. C., Forman, D., Henderson, B. E., Newell, D. G., Yuan, J.-M., Gao, Y.-T., and Ross, R. K. An apparent lack of association between *Helicobacter pylori* infection and risk of gastric cancer in China. Int. J. Cancer, *67:* 603–607, 1996.

9. Steer, H. W., Hawtin, P. R., and Newell, D. G. An ELISA technique for the serodiagnosis of *Campylobacter pyloridis* infection in patients with gastritis and benign duodenal ulceration. Serodiagn. Immunother., *1:* 253–259, 1987.

10. Tham, T. C. K., McLaughlin, N., Hughes, D. F., Ferguson, M., Crosbie, J. J., Madden, M., Namnyak, S., and O'Connor, A. F. Possible role of *Helicobacter pylori* serology in reducing endoscopy workload. Postgrad. Med. J., *70:* 809–812, 1994.

11. Talley, N. J., Newell, D. G., Ormand, J. E., Carpenter, H. A., Wilson, W. R., Zinsmeister, A. R., Perez-Perez, G. I., and Blaser, M. J. Serodiagnosis of *Helicobacter pylori:* comparison of enzyme-linked immunosorbent assays. J. Clin. Microbiol., *29:* 1635–1639, 1991.

12. Jensen, A. K. V., Andersen, L. P., and Wachmann, C. H. Evaluation of eight commercial kits for *Helicobacter pylori* IgG antibody detection. Acta Pathol. Microbiol. Immunol. Scand., *101:* 795–801, 1993.

13. Schembri, M. A., Lin, S. K., and Lambert, J. R. Comparison of commercial diagnostic tests for *Helicobacter pylori* antibodies. J. Clin. Microbiol., *31:* 2621–2624, 1993.

14. Karnes, W. E., Jr., Samloff, I. M., Siurala, M., Kekki, M., Sipponen, P., Kim, S. W. R., and Walsh, J. H. Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. Gastroenterology, *101:* 167–174, 1991.

15. Ross, R. K., Yuan, J.-M., Yu, M. C., Wogan, G. N., Qian, G.-S., Tu, J.-T., Groopman, J. D., Gao, Y.-T., and Henderson B. E. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet, *339:* 943–946, 1992.

16. Xiao, S.-D., Pan, Z.-J., Zhang, Z.-H., and Jiang, S.-J. Enzyme-linked immunosorbent assay for detection of immunoglobulin G antibody against *Helicobacter pylori.* Chin. Med. J. (Engl. Ed.), *104:* 904–908, 1991.

17. Pan, Z.-J., Xiao, S.-D., Zhang, Z.-H., Xu, W.-W., and Jiang, S.-J. Development of an ELISA kit for diagnosis of the infection with *Helicobacter pylori* in human (in Chinese). Shanghai J. Immunol., *11:* 157–159, 1991.

18. Breslow, N. E., and Day, N. E. Statistical Methods in Cancer Research, Vol I. The Analysis of Case-Control Studies. IARC Scientific Publications No. *32:* Lyon, France: IARC, 1980.

19. Forman, D., Webb, P., and Parsonnet J. *H. pylori* and gastric cancer (Letter). Lancet, *343:* 243–244, 1994.

20. Bodhidatta, L., Hoge, C. W., Churnratanakul, S., Nirdnoy, W., Sampathanukul, 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.

21. You, W.-C., Blot, W. J., Li, J.-Y., Chang, Y.-S., Jin, M.-L., Kneller, R., Zhang, L., Han, Z.-X., Zeng, 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.

22. 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., Jr. *Helicobacter pylori* antibodies in relation to precancerous gastric lesions in a high-risk Chinese population. Cancer Epidemiol. Biomark. Prev., *5:* 627–630, 1996.