Helicobacter pylori infection predicts favorable outcome in patients with gastric cancer

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Conclusions
Our study demonstrates that positive H. pylori status is a beneficial prognostic indicator in patients with gastric cancer and might suggest possible therapeutic approaches for gastric cancer. Further research is required to better understand inflammation mechanisms and cancer progression.

KEY WORDS
Helicobacter pylori, gastric cancer, survival, relapse

1. INTRODUCTION
Cancer development is characterized by stepwise accumulation of various genetic and epigenetic alterations of the genome. Approximately 10%–15% of cancers have been related to chronic infections with bacteria, viruses, or parasites. Chronic inflammation, particularly in the digestive organs, plays an important role in cancer development—for example, Helicobacter pylori–associated gastric cancer. Gastric cancer is one of the most frequently reported cancers in the world and is characterized by invasivity, metastatic potential, and poor outcomes. Despite promising developments in multimodal treatment strategies, gastric cancer maintains a profile of low survival rates and lack successful therapies, which may be a result of high rates of tumour invasion and lymph node metastasis. The addition of trastuzumab to combination chemotherapy can achieve remarkable survival advantages in patients with advanced gastric cancer positive for the human epidermal growth factor receptor 2. The AVAGAST trial evaluating bevacizumab in combination with chemotherapy demonstrated a higher response rate and a longer progression-free survival time in patients with gastric cancer.

Gastric cancer is a heterogeneous disease that results from complex interactions involving host genetic, environmental, bacterial, and molecular factors.
mechanisms\textsuperscript{7}. Currently, there is an urgent need to identify novel molecular predictive and prognostic biomarkers that will contribute to the effective use of targeted therapy in patients with gastric cancer, facilitate an understanding of gastric carcinogenesis, and reveal new molecular targets for therapeutic intervention\textsuperscript{7,8}.

First discovered by Warren and Marshall in 1982\textsuperscript{9}, \textit{H. pylori} is known to colonize the gastric epithelium in a noninvasive way\textsuperscript{10}. \textit{H. pylori} infection is a major cause of chronic gastritis, peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, and gastric cancer. In 1994, \textit{H. pylori} was classified as a definite group 1 carcinogen by the International Agency for Research on Cancer\textsuperscript{11}. Since then, infection with \textit{H. pylori} has been well established to be a crucial event associated with the risk of gastric cancer. \textit{H. pylori} infection is so common that more than 50\% of the global population harbours the bacterium\textsuperscript{12}. However, only a limited proportion of \textit{H. pylori}–infected subjects have gastric cancer, which can be explained mainly by host factors, bacterial factors, and environmental factors\textsuperscript{13,14}.

Based on an association between \textit{H. pylori} infection and the risk of gastric cancer, some investigations have focused on the relationship between \textit{H. pylori} status and gastric cancer prognosis\textsuperscript{15–19}. Currently, the available data suggest a controversial role of \textit{H. pylori} infection in the prognosis of patients with gastric cancer, and because of regional differences in \textit{H. pylori} prevalence and gastric cancer incidence, the effect of \textit{H. pylori} status on prognosis should be determined in various areas. To the best of our knowledge, only one retrospective study, by Qiu et al.\textsuperscript{18}, has been reported in China, which is well-known as a \textit{H. pylori} endemic area. In that study, overall survival (OS) in 157 gastric cancer patients had no correlation with \textit{H. pylori} status. Further studies with additional clinically relevant data from prospective cohorts are required to evaluate the prognostic significance of \textit{H. pylori} infection in patients with gastric cancer. The aim of the present study was to investigate, in a Chinese prospective cohort, the potential impact of \textit{H. pylori} status on the prognosis of patients undergoing curative resection for gastric cancer.

2. METHODS

2.1 Patients

The cohort consisted of 261 consecutive patients who underwent curative resection for gastric cancer between November 5, 2007, and November 30, 2009, at the First Affiliated Hospital of Anhui Medical University, Hefei, PR China. After surgery, all patients enrolled in the study had a histologically confirmed diagnosis of primary gastric adenocarcinoma. Patients were excluded if they had undergone non-resective surgery and if they had received neoadjuvant treatment before surgery. Cases with Siewert type 1 cardia adenocarcinoma or distant metastasis were also excluded from the study.

In all patients, the surgeries performed were partial or total gastrectomy. In accordance with the General Rules for the Gastric Cancer Study in Surgery and Pathology of the Japanese Research Society for Gastric Cancer\textsuperscript{20}, a standardized technique was used for surgical resection and lymphadenectomy. Pathology staging followed the International Union Against Cancer (UICC) criteria, 6th edition\textsuperscript{21}. Histology of the gastric cancer was classified according to Lauren’s criteria\textsuperscript{22}. Adjuvant treatments—including chemotherapy and radiotherapy—were chosen by the oncologist based on the pathology findings and patient comorbidities, without knowledge of the patient’s \textit{H. pylori} status. A database was used to record age, sex, tumour location, UICC stage, tumour grade, Lauren classification, and adjuvant treatments for each patient.

The study protocol was approved by the Institutional Ethics Committee of Anhui Medical University and was conducted according to the principles of the Declaration of Helsinki. Collection of tissue samples and clinical information was undertaken after written informed consent had been obtained from all participants.

2.2 Follow-Up Evaluation

After discharge from our hospital, all patients entered a follow-up program that was conducted according to a standard protocol\textsuperscript{23}. The patients were reviewed every 3 months for 2 years, every 6 months for 2 years, and once again after another 12 months.

The follow-up program consisted of clinical examination, hematologic analyses, measurement of tumour markers, abdominal ultrasonography and chest radiography (every 6 months), and endoscopy of the upper digestive tract (once annually). To complete staging, abdominal computed tomography imaging was performed in cases of suspected recurrence and after diagnosis of recurrence. Recurrences were documented by clinical or radiologic assessment (or both) and were categorized as local, systemic, or combined.

All patients also received monthly telephone follow-up. In cases with presentation of symptoms or suspected indicators of recurrence, further check-ups were conducted immediately. Follow-up was closed on October 16, 2012.

2.3 Endpoints

Analyses for cancer-specific survival and disease-free survival (DFS) were performed. Cancer-specific survival was measured from time of diagnosis to death from gastric cancer–related complications or to the last follow-up. The DFS was calculated from time of diagnosis to disease recurrence or last follow-up.
2.4 Histopathologic Examinations

All resected stomach specimens were fixed in neutral-buffered 10% formalin, embedded in paraffin, and cut into 4-μm sections. The sections were stained with hematoxylin–eosin for histology.

Tumour and non-neoplastic tissues were collected from the resected specimens of stomach for all patients. Non-neoplastic tissue was sampled from the antrum and corpus mucosa at least 5 cm from the tumour. When tumour involved the entire antrum, non-neoplastic mucosa was removed from the middle or upper third of the stomach. Harvested samples were immediately placed in formalin and embedded in paraffin.

Evaluation of *H. pylori* status was performed using immunohistochemical (IHC) staining. The density of *H. pylori* was classified as negative (normal) or positive (mild, moderate, marked), using the visual analog scale of the Updated Sydney System. Patients were regarded as negative for *H. pylori* if they were negative on tests of both tumour and non-neoplastic samples. Otherwise, they were regarded positive for *H. pylori*.

2.5 Immunohistochemistry

Results from the IHC staining were independently evaluated by 2 pathologists who had no prior knowledge of the clinical features and outcomes of the patients. Discrepancies between the pathologists were resolved by consensus after discussion.

Tissue sections were deparaffinized, rehydrated, and pretreated by autoclave for antigen retrieval. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide for 10 minutes at room temperature. Incubation with a polyclonal rabbit antibody *H. pylori* primary antibody (B0471: Dako Corporation, Glostrup, Denmark) at a dilution of 1:50 was conducted at room temperature for 1 hour. After samples had been washed 3 times with phosphate-buffered saline, the Dako EnVision Dual Link System–HRP (K4065: Dako Corporation) was applied for 30 minutes. Finally, sections were incubated in diaminobenzidine for 10 minutes, followed by hematoxylin counterstaining and mounting. *H. pylori*–infected gastric mucosa from chronic gastritis patients served as positive controls. Negative controls were obtained by replacing the primary antibody with phosphate-buffered saline. *H. pylori* infection in the tissue sections was confirmed when short, curved, or spiral bacilli resting on the epithelial surface, in the mucus layer, or deep in the gastric pits were observed by light microscopy with IHC staining.

2.5 Statistical Analysis

The association of *H. pylori* infection with clinical and demographic characteristics was determined using the chi-square test. The prognostic influence of *H. pylori* infection was assessed by univariate and multivariate Cox proportional hazards regression models. Hazard ratios (HRs) together with 95% confidence intervals (CIs) are presented for cancer-specific survival and DFS. All statistical analyses were performed using the SPSS statistical software package (version 13.0: SPSS, Chicago, IL, U.S.A.). Values of *p* less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Patient Characteristics

Table 1 shows the main characteristics of the cohort, which included 201 men (77.0%) and 60 women (23.0%), with a median age of 61 years (range: 26–87 years). The UICC staging yielded 79 stage I cases, 77 stage II cases, 100 stage III cases, and 5 stage IV cases. After surgery, 182 patients received adjuvant treatment postoperatively: 3 received radiotherapy, and 182 received chemotherapy. At the end of the follow-up, 51 patients had experienced tumour recurrence or distant metastasis, 48 patients had died of gastric cancer–related complications, and 2 patients had been lost to follow-up.

Quantitative detection of *H. pylori* was performed in tumour and non-neoplastic tissues in all patients. In evaluating the results of *H. pylori* IHC staining, 188 patients (72.0%) were found to be *H. pylori*–positive, and 73 (28.0%) were found to be *H. pylori*–negative (Figure 1). Of the positive patients, 42 (22.3%) showed *H. pylori* in tumour tissue; 123 (65.4%), in non-neoplastic tissue; and 23 (12.2%), in both types of tissue.

Positivity for *H. pylori* was significantly associated with younger age (*p* = 0.034). Patients who were negative for *H. pylori* had a significantly higher frequency of disease relapse (*p* = 0.042) and death (*p* = 0.043). With respect to sex, tumour location, UICC stage, grade, Lauren classification, and adjuvant treatment, we observed no statistically significant differences between the groups by chi-square test.

3.2 Association of *H. pylori* Status with Survival

In the univariate Cox regression analysis, positive *H. pylori* status was associated with better cancer-specific survival (HR: 0.486; 95% CI: 0.271 to 0.870; *p* = 0.015) and DFS (HR: 0.540; 95% CI: 0.307 to 0.950; *p* = 0.033; Table II). Mean cancer-specific survival and mean DFS were 55.2 months (95% CI: 53.4 to 56.9 months) and 53.9 months (95% CI: 51.8 to 56.0) respectively in *H. pylori*–positive patients; they were 45.1 months (95% CI: 42.2 to 47.9 months) and 43.7 months (95% CI: 40.4 to 47.0 months) in those who were *H. pylori*–negative. Figure 2 shows Kaplan–Meier survival curves for cancer-specific survival and DFS.
Multivariate analysis was used to further evaluate the potential for *H. pylori* infection to be as an independent prognostic biomarker. A multivariate Cox proportional hazards model demonstrated that *H. pylori* positivity was associated with improved survival (HR: 0.485; 95% CI: 0.265 to 0.889; \( p = 0.019 \)) independent of other clinical covariates, which included age, sex, tumour location, UICC stage, grade, Lauren classification, and adjuvant treatment (Table 1). Compared with *H. pylori*–negative patients, *H. pylori*–positive patients showed a trend toward improved DFS (HR: 0.557; 95% CI: 0.310 to 1.044; \( p = 0.051 \); Table 1), but the difference was not statistically significant.

### 4. DISCUSSION

In the present study, we identified *H. pylori* infection as an independent beneficial prognostic factor for patients with curatively resected gastric cancer. Patients negative for *H. pylori* had a poorer outlook. Multivariate analysis confirmed the prognostic value of *H. pylori* infection, even after adjustments for other well-known prognostic factors such as UICC stage, Lauren classification, and adjuvant treatment. To the best of our knowledge, this prospective study is the first in China designed to assess the potential role of *H. pylori* infection in the prognosis of patients with gastric cancer.

*H. pylori* infection may be localized in or around the tumour (classified as diffuse, localized, or none).
As a result, a histologic diagnosis may be more successful than other methods in detecting the relationship between *H. pylori* infection and prognosis in gastric cancer patients. The reported rates of *H. pylori* positivity vary from 17.5% to 86.2%. An overall positivity rate of 72.0% was observed in our patients. That value is higher than the rates reported in other Asian studies, which might be explained by epidemiologic factors and the sensitivity of the various methods used to detect *H. pylori* infection. Moreover, it is well known that a high prevalence of *H. pylori* is always accompanied by a high incidence of gastric cancer.

In agreement with most studies in the literature, we found that the rate of *H. pylori* positivity was higher in non-neoplastic tissue than in tumour tissue. The possible biologic basis might be elucidated as follows: Once cancer developed, sustained injury to the gastric mucosa persisted. The microenvironment in gastric mucosa was then no longer suitable for *H. pylori* survival. In addition, we identified a higher frequency of *H. pylori* infection in younger patients (age < 61 years: 78.2% vs. 66.4%). A possible explanation might be that inclusion of patients with gastric cancer only after curative resection led to selection bias.

Several clinicopathologic factors have been proposed to predict the prognosis of patients with gastric cancer. Among those factors, pathologic stage appears to be the most reliable. In the univariate analysis in our study, patients positive for *H. pylori* experienced better survival. Further findings in the multivariate analysis confirmed the role of *H. pylori* as an independent beneficial prognostic factor.
for patients with curatively resected gastric cancer. Stratified analysis according to T classification indicated that positive Helicobacter pylori status might be a beneficial prognostic factor mainly for patients with advanced-stage gastric cancer. Univariate Cox regression analysis showed that infection with *H. pylori* was associated with a high DFS in patients with gastric cancer. Meanwhile, multivariate analysis also identified a role for infection with *H. pylori* as a predictive factor for improved DFS (with marginal significance).

To the best of our knowledge, two prospective studies have investigated the relationship between *H. pylori* status and prognosis in gastric cancer patients. Meimarakis and colleagues identified *H. pylori* as an independent beneficial prognostic factor for OS and relapse-free survival in both univariate and multivariate analysis. The effect was pronounced in patients with early-stage cancer. Patients positive for *H. pylori* showed improved OS and relapse-free survival. A prospective study by Marrelli *et al.* indicated that negative *H. pylori* status was an indicator of poor prognosis in patients with gastric cancer.

We believe that the current prospective study is the first to confirm *H. pylori* status as a favourable prognostic factor in a large number of patients with gastric cancer in China, thus validating the effect of *H. pylori* infection status on survival in a Chinese prospective cohort. The main findings of the present study are consistent with those in previous reports. However, in our study, the association between *H. pylori* infection and survival was observed mainly in patients with advanced gastric cancer, a finding that requires further validation. Moreover, our results contrast with those from a retrospective Chinese study by Qiu *et al.*, who used real-time polymerase chain reaction to detect *H. pylori* in 157 gastric cancer patients and found no significant association between *H. pylori* infection and OS or relapse-free survival in patients who underwent curative surgery. The difference might be attributable to some combination of the different approaches used in the two studies, different *H. pylori* detection methods, or different sample sizes.

To date, the mechanisms of the reported differences in prognosis between patients with gastric cancer who are positive for *H. pylori* and those who are negative are still unclear. Several explanations for the role of *H. pylori* infection in improving the prognosis of patients with gastric cancer are plausible. The most recognized virulence markers for *H. pylori* are CagA (cytotoxic-associated gene A), VacA (vacuolating toxin A), and BabA (blood-group antigen-binding adhesion)30. Bacterial virulence factors, host genetic factors, and environmental factors constitute a complex regulatory network for persistent immune and inflammatory responses. Infection with *H. pylori* might promote the processes of tissue damage and lead to gastric malignancy. Once a multi-step process leading to
The lack of significance for the association between H. pylori status and DFS might perhaps be partly attributable to those difficulties. Second, in current study, we used only HIC to detect H. pylori. Other methods, such as the urea breath test, real-time polymerase chain reaction, serologic tests, and bacterial culture are also used for H. pylori detection. The combination of two or more methods might increase the sensitivity and specificity of a diagnosis of H. pylori infection. However, in clinical practice, performing all of those tests is impractical, given the costs and time required. Furthermore, no method of H. pylori detection is perfect. For example, Marrelli et al.17 reported that 14% of polymerase chain reaction–positive gastric cancer patients demonstrated negative serology for H. pylori. The role of serology tests therefore remains questionable for other than epidemiologic purposes. In the present study, histologic examination provided insights about the status of the gastric mucosa. Compared with other staining methods, HIC for the detection of H. pylori therefore makes the screening time much shorter and increases specificity, helping to eliminate other similarly shaped organisms43,44.

5. CONCLUSIONS

The present study demonstrates that H. pylori positivity is a beneficial prognostic indicator in patients with gastric cancer, independent of other clinicopathologic variables. In clinical practice, patients with curatively resected gastric cancer who are negative for H. pylori may need more careful follow-up and more aggressive antitumour treatment to prolong life expectancy. Further research is required to elucidate the exact mechanisms of inflammation and tumour suppression, which might provide new opportunities for personalized treatment options.

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7. CONFLICT OF INTEREST DISCLOSURES

The authors have no financial conflicts of interest to declare.

8. REFERENCES


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