

# World Journal of *Gastroenterology*

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### FRONTIER

- 6161** Significance of gut microbiota in alcoholic and non-alcoholic fatty liver diseases  
*Sharma SP, Suk KT, Kim DJ*

### OPINION REVIEW

- 6180** Surveillance for hepatocellular carcinoma at the community level: Easier said than done  
*Del Poggio P, Mazzoleni M, Lazzaroni S, D'Alessio A*

### REVIEW

- 6191** Challenges and opportunities in the application of artificial intelligence in gastroenterology and hepatology  
*Christou CD, Tsoulfas G*

### MINIREVIEWS

- 6224** Impact of *Helicobacter pylori* infection on gut microbiota  
*Iino C, Shimoyama T*
- 6231** Therapeutic drug monitoring in inflammatory bowel disease: The dawn of reactive monitoring  
*Albader F, Golovics PA, Gonczi L, Bessissow T, Afif W, Lakatos PL*

### ORIGINAL ARTICLE

#### Basic Study

- 6248** Increased systemic RNA oxidative damage and diagnostic value of RNA oxidative metabolites during *Shigella flexneri*-induced intestinal infection  
*Nie JJ, Pian YY, Hu JH, Fan GQ, Zeng LT, Ouyang QG, Gao ZX, Liu Z, Wang CC, Liu Q, Cai JP*

#### Retrospective Cohort Study

- 6262** Hepatitis B virus persistent infection-related single nucleotide polymorphisms in HLA regions are associated with viral load in hepatoma families  
*Hsieh AR, Fann CSJ, Lin HC, Tai J, Hsieh SY, Tai DI*

#### Retrospective Study

- 6277** Recently acquired hepatitis C virus infection among people living with human immunodeficiency virus at a university hospital in Taiwan  
*Huang MH, Sun HY, Ho SY, Chang SY, Hsieh SM, Sheng WH, Chuang YC, Huang YS, Su LH, Liu WC, Su YC, Hung CC*

**Observational Study**

- 6290** *Helicobacter pylori* in gastric cancer: Features of infection and their correlations with long-term results of treatment  
*Senchukova MA, Tomchuk O, Shurygina EI*

**SYSTEMATIC REVIEWS**

- 6306** Determination of gluten immunogenic peptides for the management of the treatment adherence of celiac disease: A systematic review  
*Coto L, Mendia I, Sousa C, Bai JC, Cebolla A*

**CASE REPORT**

- 6322** Pancreatic paraganglioma diagnosed by endoscopic ultrasound-guided fine needle aspiration: A case report and review of literature  
*Lanke G, Stewart JM, Lee JH*
- 6332** Abdominal cocoon in children: A case report and review of literature  
*Keese D, Schmedding A, Saalabian K, Lakshin G, Fiegel H, Rolle U*

**LETTER TO THE EDITOR**

- 6345** Gastrointestinal symptoms in patients with COVID-19: Is there a relationship with mortality and new variations of SARS-CoV-2?  
*Ribeiro IB, de Moura DTH, de Moura EGH*

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## Observational Study

# *Helicobacter pylori* in gastric cancer: Features of infection and their correlations with long-term results of treatment

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## Abstract

### BACKGROUND

*Helicobacter pylori* (*H. pylori*) is a spiral-shaped bacterium responsible for the development of chronic gastritis, gastric ulcer, gastric cancer (GC), and MALT-lymphoma of the stomach. *H. pylori* can be present in the gastric mucosa (GM) in both spiral and coccoid forms. However, it is not known whether the severity of GM contamination by various vegetative forms of *H. pylori* is associated with clinical and morphological characteristics and long-term results of GC treatment.

### AIM

To establish the features of *H. pylori* infection in patients with GC and their correlations with clinical and morphological characteristics of diseases and long-term results of treatment.

### METHODS

Of 109 patients with GC were included in a prospective cohort study. *H. pylori* in the GM and tumor was determined by rapid urease test and by immunohistochemically using the antibody to *H. pylori*. The results obtained were compared with the clinical and morphological characteristics and prognosis of GC. Statistical analysis was performed using the Statistica 10.0 software.

### RESULTS

*H. pylori* was detected in the adjacent to the tumor GM in 84.5% of cases, of which a high degree of contamination was noted in 50.4% of the samples. Coccoid forms of *H. pylori* were detected in 93.4% of infected patients, and only coccoid-in 68.9%.

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It was found that a high degree of GM contamination by the coccoid forms of *H. pylori* was observed significantly more often in diffuse type of GC ( $P = 0.024$ ), in poorly differentiated GC ( $P = 0.011$ ), in stage T3-4 ( $P = 0.04$ ) and in N1 ( $P = 0.011$ ). In cases of moderate and marked concentrations of *H. pylori* in GM, a decrease in 10-year relapse free and overall survival from 55.6% to 26.3% was observed ( $P = 0.02$  and  $P = 0.07$ , respectively). The relationship between the severity of the GM contamination by the spiral-shaped forms of *H. pylori* and the clinical and morphological characteristics and prognosis of GC was not revealed.

## CONCLUSION

The data obtained indicates that *H. pylori* may be associated not only with induction but also with the progression of GC.

**Key Words:** Gastric cancer; *Helicobacter pylori*; Coccoid and spiral forms of bacteria; Rapid urease test; Relapse free survival; Overall survival

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**Core Tip:** The role of *Helicobacter pylori* (*H. pylori*) in the progression of gastric cancer (GC) is not well understood. Our results indicate that in GC, a high degree of gastric mucosa contamination by coccoid forms of *H. pylori* is associated with advanced stages of the disease and deterioration of long-term results of treatment.

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## INTRODUCTION

Gastric cancer (GC) continues to be one of the most common malignant diseases in the world[1,2]. Despite a decreasing trend in the incidence of GC in most countries of the world, the treatment results of this pathology cannot be considered satisfactory. In the structure of mortality from malignant neoplasms, this pathology firmly occupies 2<sup>nd</sup> place in most developed countries of the world, and the 5-year survival rate of radically operated patients does not exceed 15%-30%[3,4].

It is important to note that it is impossible to improve the long-term results of malignant neoplasms treatment without knowledge of the mechanisms associated with their progression[3]. Clinical studies in recent years indicate that inflammatory infiltration of the tumor stroma and surrounding tissues can have an important prognostic value and affect the long-term results of malignant neoplasm treatment[5-9]. A number of studies have shown that inflammatory infiltration of the tumor stroma is associated with the body's adequate immune response to the tumor, and may be a favorable prognosis factor in various malignant neoplasms[10,11], including GC[12,13]. At the same time, the data obtained by other researchers indicates that pronounced inflammatory infiltration of the tumor stroma, especially T-reg lymphocytes and macrophages, may be a factor contributing to the progression of malignant neoplasms[14,15]. There was a decrease in the overall survival (OS) and relapse-free survival (RFS) of GC patients with a high content of Foxp3 + T-reg in the tumor stroma and regional metastases[16-18] and macrophages[16,19]. It is believed that inflammatory infiltration of the tumor stroma can contribute to tumor progression by activating the mechanisms of angiogenesis, expression of E- and L-selectins, formation of the products of lipid peroxidation and free radicals, destruction of connective tissue matrix and basement membranes of epithelia by proteolytic enzymes, and activation of epithelial-mesenchymal transformation[20-23].

When studying the role of inflammation in the progression of GC, it is impossible to ignore the problem of *Helicobacter pylori* (*H. pylori*) infection. *H. pylori* is a gram-negative, spiral-shaped bacterium, the habitat of which is the gastric mucosa (GM) and

duodenum. *H. pylori* differs from other bacteria in a set of properties that make it possible to colonize the GM and persist for a long time under conditions that are unfavorable for other microorganisms[24,25]. These include: (1) The ability to produce a special enzyme-urease; (2) Synthesis of lytic enzymes that cause the depolymerization and dissolution of gastric mucus, consisting mainly of mucin; (3) The mobility of the bacterium, which is ensured by the presence of 5-6 flagella; (4) The high adhesiveness of bacteria to GM epithelial cells of the GM and elements of connective tissue due to the interaction of bacterial ligands with the corresponding cells receptors; (5) Production of various exotoxins (VacA, CagA, and others); (6) Instability of the *H. pylori* genome; (7) The presence of vegetative and coccoid forms of bacteria; and (8) possibility of intracellular persistence and translocation outside the GM [26-29].

It should be noted that despite the huge number of studies devoted to *H. pylori*, it is still not clear whether *H. pylori* is involved only in the initiation of the tumor process in the stomach, or whether it can affect the mechanisms of tumor progression. The relationship between the severity of *H. pylori* infection and the clinical and morphological characteristics of GC and long-term results of this pathology treatment remains poorly studied, and in this connection, the question of the expediency of anti-Helicobacter therapy in patients with invasive GC remains open.

### Objective

To assess the features of *H. pylori* infection in patients with Stage I-IIIb GC and their correlation with the clinical and morphological characteristics of the disease, the presence of antibiotic therapy (AT) before surgery, and long-term treatment results.

## MATERIALS AND METHODS

### The patients

One hundred and nine patients with GC who had undergone radical surgery (R0) between May 2007 and March 2010 at the Orenburg Regional Clinical Oncology Center, were included in this prospective cohort pilot study. Study inclusion criteria were: Histologically proven invasive GC; no evidence of distant metastases; radical surgery (R0); no prior gastric surgery; no previous chemotherapy or radiotherapy. The study did not include patients with decompensation of cardiovascular and renal diseases, exacerbation of chronic inflammatory processes, severe allergic processes, or who received glucocorticoids, antihistamines, and non-steroidal anti-inflammatory drugs. The study was performed in accordance with the Helsinki Declaration and internationally recognized guidelines, and the privacy of patients was protected by decoding the data according to the privacy regulations of the Orenburg Regional Clinical Oncologic Center (Russia, Orenburg). All patients provided written informed consent. The protocol was approved by the Institutional Review Board of the Orenburg State Medical University (Russia, Orenburg).

Clinical and pathological data including age, tumor localization, stage, type of surgery, histology, the presence of AT before surgery, postoperative therapy, and long-term results of treatment were retrieved from the routine reports for analyses. The distribution of patients according to the clinical and pathological characteristics of GC is presented in Table 1.

When interviewing patients, it was found that 45 patients received AT before admission to the clinic due to a preliminary wrong diagnosis of gastric ulcer or chronic gastritis. The following combinations of antibacterial drugs were most commonly ordered: Amoxicillin + clarithromycin (34 patients), amoxicillin + clarithromycin + metronidazole (6 patients), amoxicillin + metronidazole (3 patients), other drugs (2 patients). Information about the antibacterial drugs, the timing and duration of their intake was entered into the primary patient documentation and then taken into account in the analysis. We considered only those patients who underwent AT in the period from 1 to 1.5 mo before the operation, lasting at least seven days, and using two or more antibacterial drugs.

The long-term results of treatment were assessed for the period from May 12, 2007 to April 12, 2021. The median follow-up period was 86.2 mo. As of April 12, 2021, 26 (24.5%) patients were alive, 54 (50.9%) had died from the progression of GC, 20 (18.9%) had died from causes other than GC, and six (5.6%) left the region at different follow-up periods. Malignant tumors of other localizations were diagnosed in 8 patients at different times after the operation: Non-Hodgkin's lymphomas-in three, prostate cancer-in one, lung cancer-in two, laryngeal cancer-in one, and breast cancer-in one patient. With the exception of one patient with non-Hodgkin's lymphoma, the other

**Table 1** The distribution of patients according to the clinical and pathological characteristics of gastric cancer

Characteristics of gastric cancer	<i>n</i>	Percent
<b>Age</b>	61.7 ± 1.03 years (from 24 to 81 years, the median—was 61 years)	
<b>Sex</b>		
Men	72	66.1
Women	37	33.9
<b>Tumor localization</b>		
Upper third	18	16.5
Middle third	32	29.4
Lower third	57	52.3
Total gastric cancer	2	1.8
<b>T status</b>		
T1	12	11
T2	30	27.5
T3	62	56.9
T4	5	4.6
<b>N status</b>		
N0	57	52.3
N1	20	18.3
N2	32	29.3
<b>TNM</b>		
T1-2N0M0	40	36.7
T3N0M0	17	15.6
T3-4N1M0	20	18.3
T3-4N2M0	32	29.3
<b>Types of GC</b>		
Intestinal type	50	45.9
Diffuse-types	59	54.1
<b>Grade (histology)</b>		
G1	31	28.4
G2	19	17.4
G3	33	30.3
Signet ring cell carcinoma	26	23.9
<b>Type surgery</b>		
Subtotal distal resection	85	78
Subtotal proximal resection	18	16.5
Gastrectomy	7	6.4

GC: Gastric cancer.

patients died from the progression of these diseases. The causes of death were not associated with malignant tumors for the other patients. During the period 2020-2021, seven patients contracted coronavirus disease-19, one of whom died from the disease, but the rest are alive.

### Detection *H. pylori* infection

*H. pylori* in the GM and tumor was determined by rapid urease test (RUT) and by immunohistochemically (IHC) using the antibody to *H. pylori*.

### RUT

After removal of the stomach (within 30 min) a greater curvature of the organ was opened and biopsy samples were taken from the tumor and the adjacent macroscopically non-tumorous GM at a distance of 3-5 cm from the tumor margin. The samples were placed on test strips (HELPII-test, "AMA", Russia) for three minutes. The presence and the severity of the infection were evaluated by the color change of the indicator from yellow to blue.

According to the intensity and the time of the appearance of a blue color, we distinguished three degrees of infection: 3+: Marked (+++)-bright staining in the first minute of the study; 2+: Moderate (++)-for an average intensity of staining for 2 min and 1+ mild (+)-weak staining for three minutes. If the color of the indicator did not change, or became dirty gray, and if after repeated research the same result was received, the test was evaluated as negative. The same samples were later used for histological analysis and IGH.

### Immunohistochemistry

The presence and features of *H. pylori* infection were studied in samples of the GM adjacent to the tumor, in the tumor tissue, in the omentum, and regional lymph nodes of 46 patients, by immunohistochemistry.

The sections for IGH were dewaxed and rehydrated by sequential immersion in xylene and graded ethanol and water. For antigen retrieval, the sections boiling for 10 min in citrate buffer (pH 6) and endogenous peroxidase activity was blocked with 30 mL/L hydrogen peroxide solution. Slides were incubated at room temperature with the anti-*H. pylori* (RB-9070, Thermo Fisher Scientific, the immunogen is purified *H. pylori*) rabbit polyclonal antibodies in diluted at 1:1000 for 30 min.

The visualization system included DAB (UltraVision LP Detection System HRP Polymer & DAB Plus Chromogen) and hematoxylin counterstaining. For negative control sections, the primary antibody was replaced with phosphate-buffered saline and processed in the same manner.

The concentration of *H. pylori* in the GM detected by IGH was graded as 1+ for mild, 2+ for moderate, and 3+ for marked according to the Sydney system[30]. The presence of point inclusions giving a positive reaction with antibodies to *H. pylori* in the cytoplasm of epithelial cells of deep gastric glands and of the lymphoid cells of the lamina propria of GM as well as in omentum and lymph node, were taken into account.

All sections were carefully and completely scanned by two of the authors (MS and OT) without knowledge of the clinical and pathological data.

The data obtained was compared with clinical features of GC: Stage, localization, histology, the presence of AT before surgery, and 10-year OS and RFS.

### Statistics

Statistical analysis was performed using the Statistica 10.0 software. The correlations between different data were evaluated using the nonparametric Spearman's rank correlation or gamma correlation. Chi-square tests were carried out to analyze the difference of distribution among the categorized data. Mann-Whitney U nonparametric test was used to compare the value of the quantitative data. The survival was analyzed using the Kaplan-Meier method. The log-rank test was used to compare survival curves between subgroups of patients. A value of  $P < 0.05$  was considered statistically significant.

## RESULTS

### The features of *H. pylori* infection in GC

Of 93 patients (84.5%) demonstrated positive RUT. According to RUT the concentration of *H. pylori* in GM was mild (1+) in 38 (34.8%) patients, moderate (2+)-in 37 (33.9%) and marked (3+)-in 8 (16.5%). RUT was negative in 16 patients (14.7%).

It was found that urease activity 2+ and 3+ were significantly more frequent in GM than in tumors (in 55.5% and 13.3% of cases, respectively,  $P = 0.01$ ). In more than half the cases (53.3%), the urease activity in the tumor was lower than in the adjacent GM.



Forty-six samples of GM were stained by IHC. The coccoid forms of *H. pylori* were found to prevail in GM adjacent to the tumor (Figure 1A). Coccoid forms of *H. pylori* only were identified in 31 (63.1%) patients, and coccoid and spiral in 12 (30.4%) patients (Figure 1B). No signs of infection were found in 3 (6.5%) patients. The concentration of coccoid and spiral forms of *H. pylori* in GM according to IGH was 1+ in 24 (52.2%) and 1 (19.6%) patients, 2+-in 11 (23.9%) and 4 (8.7%), and 3+-in 8 (17.4%) and 1 (2.2%) patients. A positive correlation between the concentration of coccoid and spiral forms of *H. pylori* ( $\gamma = 0.642$ ,  $P < 0.0001$ ) was noted.

The localization of *H. pylori* in GM was observed not only in the surface mucous gel layer of the stomach (Figure 1C) but also within the cytoplasm of the gastric superficial-foveolar epithelium (Figure 1D). The point inclusions giving a positive reaction with antibodies to *H. pylori* were also revealed in the cytoplasm of the epithelial cells of deep gastric glands (in 41.3% samples) and of the immune cells (Figure 1E) of the lamina propria of GM (in 43.5% samples), as well as of the intra-epithelial lymphocytes (Figure 1F). The close relationship between the presence of point inclusions in immune cells and epithelial cells ( $\gamma = 0.642$ ,  $P < 0.0001$ ) was noted. The individual cocci or their small clusters, and sometimes the short rod bacterium, were also often detected in the lamina propria of the GM. We also found bacteria in the omentum and lymph nodes. In the omentum the bacteria were presented predominantly by cocci between 0.5 and 1  $\mu\text{m}$  in diameter (Figure 2A). Cocci were arranged most commonly by the small compact groups up to 10-15 cells in the immediate vicinity of the LN capsule. The bacteria were located mainly between the adipocytes. However, it was not clear whether bacteria were outside of the cells or in a narrow rim of cytoplasm of the fat cells.

In the tissue of lymph nodes we usually observed the small accumulations of cocci between 10 and 15 cells (Figure 2B). Bacteria were located between lymphocytes of the paracortical area, but not so compact, as in the omentum. Quite often the concentration of bacteria was observed around the nuclei of cells that can testify to their intracellular localization.

#### **Correlations of clinical and pathological characteristics of GC with the severity of *H. pylori* infection according to RUT data**

The gamma correlation coefficient test ( $\gamma$ ) showed that the severity of *H. pylori* in GM according to RUT positively correlated with the T status ( $\gamma = 0.537$ ,  $P < 0.00001$ ), N status ( $\gamma = 0.371$ ,  $P = 0.0007$ ) and stage ( $\gamma = 0.520$ ,  $P < 0.00001$ ), and negatively correlated with the presence of AT in anamnesis ( $\gamma = -0.418$ ,  $P = 0.003$ ). The marked (+++) and moderate (++) degrees of *H. pylori* infection were more often observed in Grade 2 and Grade 3, in T3-4 status, in N1 status, in the T3-4N1-2 stage, and in the absence of AT in anamnesis (Table 2). Correlations of *H. pylori* concentration in GM according to RUT with 10-year OS and RFS of GC patients were not determined.

It is important to note that the presence in AT 1-1.5 mo before surgery was associated with a significant improvement in RFS and OS (Figure 3), however, this applied only to patients with local GC (T1-3N0). In advanced GC (T3-4N1 and T3-4N2) there were no significant differences in patient survival (Figure 4).

#### **Correlations of clinical and pathological characteristics of GC with the concentration of spiral and coccoid forms of *H. pylori* in the GM**

It is important to note that correlations between the clinical and pathological characteristics of GC and the concentration of *H. pylori* spiral forms in GM were not found. However, the concentration of *H. pylori* coccoid forms correlated with age ( $\rho = -0.502$ ,  $P = 0.0006$ ), histology ( $\gamma = 0.550$ ,  $P = 0.0004$ ), T status ( $\gamma = 0.709$ ,  $P = 0.0001$ ), N status ( $\gamma = 0.509$ ,  $P = 0.002$ ) stage ( $\gamma = 0.636$ ,  $P = 0.0002$ ), and 10-year RFS ( $\gamma = -0.521$ ,  $P = 0.008$ ) and OS ( $\gamma = -0.500$ ,  $P = 0.044$ ). In cases with a moderate and marked concentration (2+ or 3+) of *H. pylori* coccoid forms in GM compared to cases with a low concentration (1+ or without infection) the patients were younger ( $57.9 \pm 2.5$  years vs  $66.2 \pm 1.4$  years, respectively,  $P = 0.004$ ) and the diffuse type of GC, poorly differentiated tumors (G3), T3-4 stage and N1 stage of GC were more often observed (Table 3). In cases of moderate and marked concentrations of *H. pylori* in GM, a decrease in 10-year progression-free survival (PFS) and OS survival from 55.6% to 26.3% was observed ( $P = 0.02$  and  $P = 0.07$ , respectively). PFS and OS curves, depending on the concentration of coccoid forms of *H. pylori* in GM, are presented in Figure 5.

**Table 2 Clinical and pathological characteristics of gastric cancer depending on the data rapid urease test**

Characteristics of gastric cancer	Degrees of infection according to RUT test				P value
	No or mild, n (%)		Moderate or marked, n (%)		
Age	63.8 ± 10.1		58.8 ± 11.2		0.008
T status					
T1	9	75	3	25	0.01
T2	20	66.7	10	33.3	
T3	24	38.7	38	61.3	
T4	1	20	4	80	
N status					
N0	37	64.9	20	35.1	0.0001
N1	2	10	18	90	
N2	15	46.9	17	53.1	
TNM					
T1-2N0M0	28	70	12	30	0.002
T3N0M0	9	52.9	8	47.1	
T3-4N1-2	17	32.7	35	67.3	
Types of GC					
Intestinal type	27	54	23	46	0.39
Diffuse-types	27	45.8	32	54.2	
Grade (histology)					
G1	22	70	9	30	0.005
G2	5	26.3	14	73.7	
G3	12	36.4	21	63.6	
Signet ring cell carcinoma	15	57.6	11	42.3	
Antibiotic therapy before surgery					
No	25	41.2	37	68.5	0.025
Presence	28	52.8	17	31.5	

RUT: Rapid urease test; GC: Gastric cancer.

There were no correlations between the presence of point inclusions in the cytoplasm of epithelial cells of deep gastric glands, in the stroma immune cells, and in the intraepithelial lymphocytes with the clinical and pathological characteristics of GC.

## DISCUSSION

A large amount of clinical and experimental data testifies to the important role of *H. pylori* in the occurrence of GC[31-34], but, there is less research into the features of *H. pylori* infection in patients with GC and its role in tumor progression, and the results are quite contradictory. These contradictions relate to many aspects, such as: (1) The frequency of infection in patients with GC. This data varies widely and ranges from 36% to 100%[35-40]; (2) The relation of infection with GC prognosis. Some researchers have noted an improvement in the long-term results of GC treatment in infected patients[41,42], while others, on the contrary, found that the presence of *H. pylori* infection was associated with a decrease in patient survival[43,44]; and (3) The connection between the infection and a histologic type of GC. In some studies, it was noted that patients with an intestinal type of GC are more often infected with *H. pylori* than patients with the diffuse type of GC[45,46]. Other researchers did not find a

**Table 3 Clinical and pathological characteristics of gastric cancer depending on the concentration of *Helicobacter pylori* coccoid forms in the gastric mucosa**

Characteristics of gastric cancer	Degrees of infection according to RUT test				P value
	No or mild, n (%)		Moderate or marked, n (%)		
Age	66.2 ± 1.4		57.9 ± 2.5		0.004
T status					
T1	6	85.7	1	14.3	0.04
T2	9	81.8	2	18.2	
T3	12	44.4	15	55.6	
T4	0	0	1	100	
N status					
N0	18	78.3	5	21.7	0.01
N1	1	16.7	5	83.8	
N2	8	47.1	9	52.9	
TNM					
T1-2N0M0	14	82.3	3	17.6	0.02
T3N0M0	4	66.7	2	33.3	
T3-4N1-2	9	39.1	14	60.9	
Types of GC					
WIntestinal type	18	75	6	25	0.02
Diffuse-types	9	40.9	13	59.1	
Grade (histology)					
G1	14	93.3	1	6.7	0.008
G2	4	44.4	5	55.6	
G3	2	28.3	5	71.4	
Signet ring cell carcinoma	7	46.7	8	53.3	
Antibiotic therapy before surgery					
No	16	64.5	10	38.5	0.655
Presence	11	55	9	45	

RUT: Rapid urease test; GC: Gastric cancer.

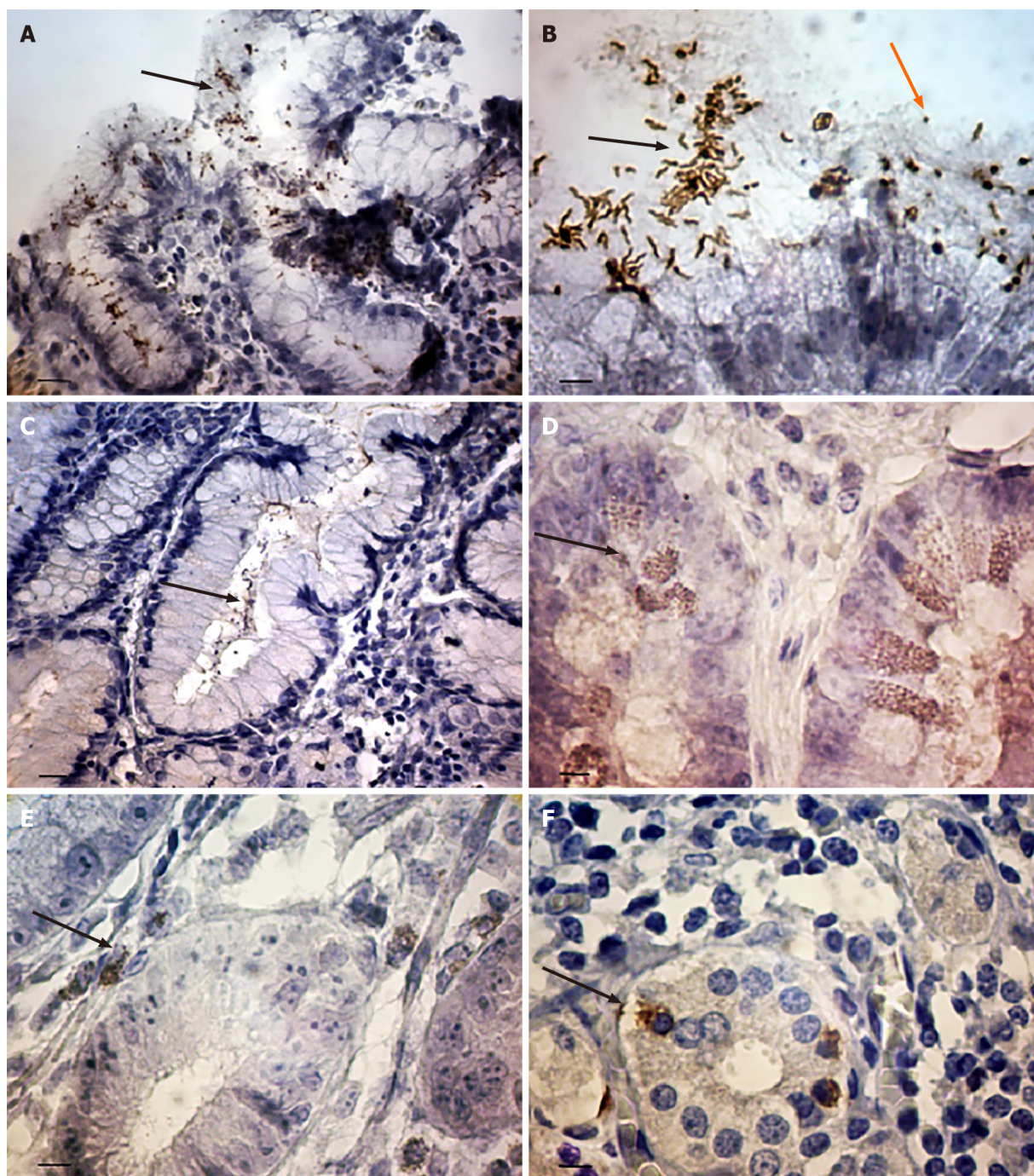
difference in *H. pylori* infection in patients with different histological types of tumors [47].

It is believed that the differences noted are associated with the fact that to reveal the infection the authors used the methods that were significantly different in their sensitivity, and primarily, to coccoid forms of bacteria. Most of the studies were carried out without considering coccoid forms of *H. pylori* and the concentration of bacteria in GM. The use of the biochemical method for the detection of urease activity and immunohistochemistry for visualization of bacteria in our study allowed us not only to assess the presence of infection in patients with GC, but also to mark some of its features associated with the localization of bacteria in the stomach, with a ratio of cocci and spiral forms, and the degree of bacterial contamination of GM.

Our data for the Orenburg region recorded a high rate of *H. pylori* infection in patients with GC (84.5%). The coccoid forms of *H. pylori*, preserving a high degree of urease activity, dominated in GM in patients with GC. They were found in 93.4% of infected patients, with only coccoid forms of *H. pylori*-in 68.9%.

It is known that the coccoid forms of *H. pylori* can arise in response to unfavorable environmental factors, such as AT [47,48]. These forms are resistant to AT[49,50] and are able to form biofilms[51] and avoid the immune system[50]. They express a higher rate of *cagE* mRNA than their spiral counterparts[52], and by increasing the synthesis



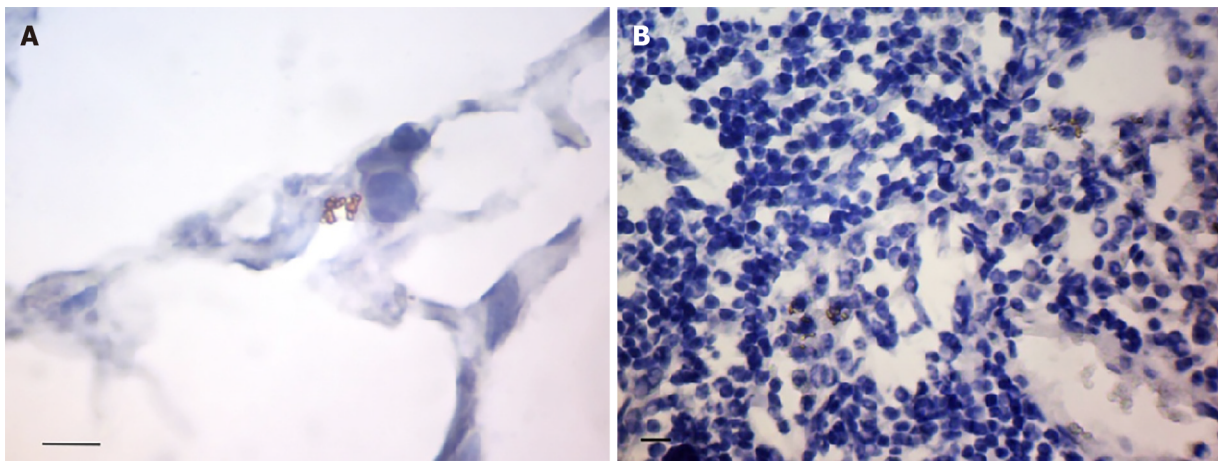


**Figure 1** The features of *Helicobacter pylori* localization in gastric mucosa in patients with gastric cancer. A: Coccoid forms of *Helicobacter pylori* (*H. pylori*) in the gastric pit. The some bacteria within the cytoplasm of epithelium cells (arrows); B: The spiral (black arrows) and coccoid (orange arrows) forms of *H. pylori* on the surface of superficial-foveolar gastric epithelium; C: The bacteria in the surface mucous gel layer of stomach (arrows); D: The point inclusions giving a positive reaction with antibodies to *H. pylori* within the cytoplasm of epithelial cells of deep gastric glands (arrows); E: The point inclusions giving a positive reaction with antibodies to *H. pylori* within the cytoplasm of the immune cells of the lamina propria of gastric mucosa (arrows); F: The point inclusions giving a positive reaction with antibodies to *H. pylori* within the cytoplasm of intraepithelial lymphocytes (arrows). Immunoperoxidase staining with anti-*H. pylori* antibody, immersion. Bars: A: 20  $\mu$ m; B: 10  $\mu$ m; C: 20  $\mu$ m; D-F: 10  $\mu$ m.

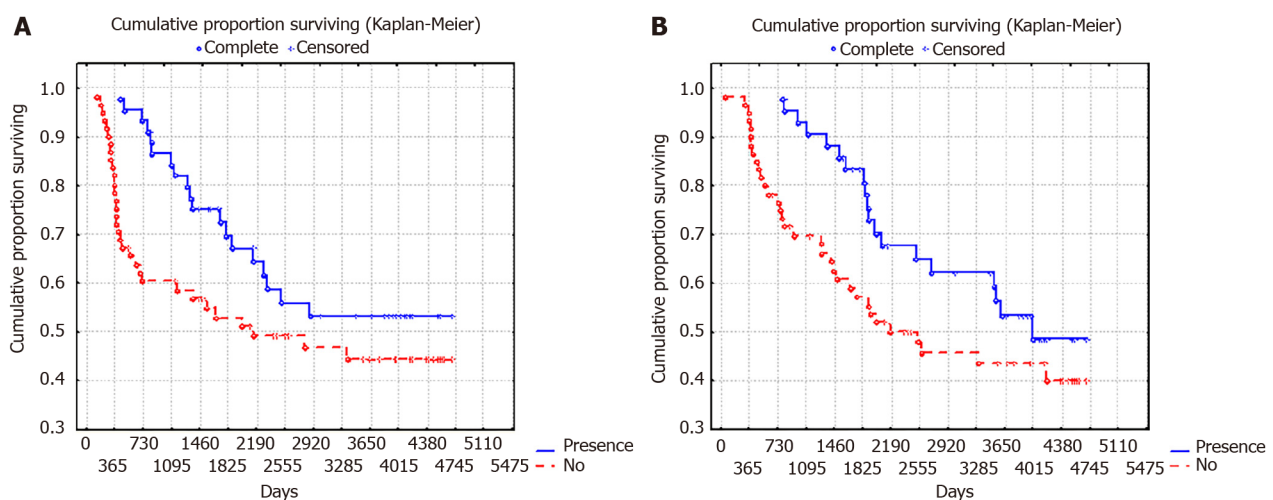
of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-inducing protein (Hps), which is introduced into the cytosol and cell nuclei, they can activate nuclear factor-kappaB (NF- $\kappa$ B) and the expression of TNF- $\alpha$  and other cytokines involved in carcinogenesis[53,54]. The effect on the proliferation of gastric epithelial cells in the *H. pylori* coccoid forms is also stronger than in the helical forms[55], and they can induce the expression of VEGF-A and transforming growth factor- $\beta$ [56]. The ability to transform into coccoid forms was also found to be characteristic of the most virulent strains of *H. pylori*[50,54].

It should be noted that the higher infection by coccoid forms of *H. pylori* in patients with GC, compared to patients with gastritis or gastric ulcer, had been mentioned by





**Figure 2** The features of *Helicobacter pylori* localization in omentum and lymph node in patients with gastric cancer. A: The small group of cocci located in the central part of the omentum adipocyte; B: The congestions of bacteria around the nucleus of lymphocytes in the paracortical area of lymph node. Immunoperoxidase staining with anti-*Helicobacter pylori* antibody, immersion, Bars: 10  $\mu$ m.

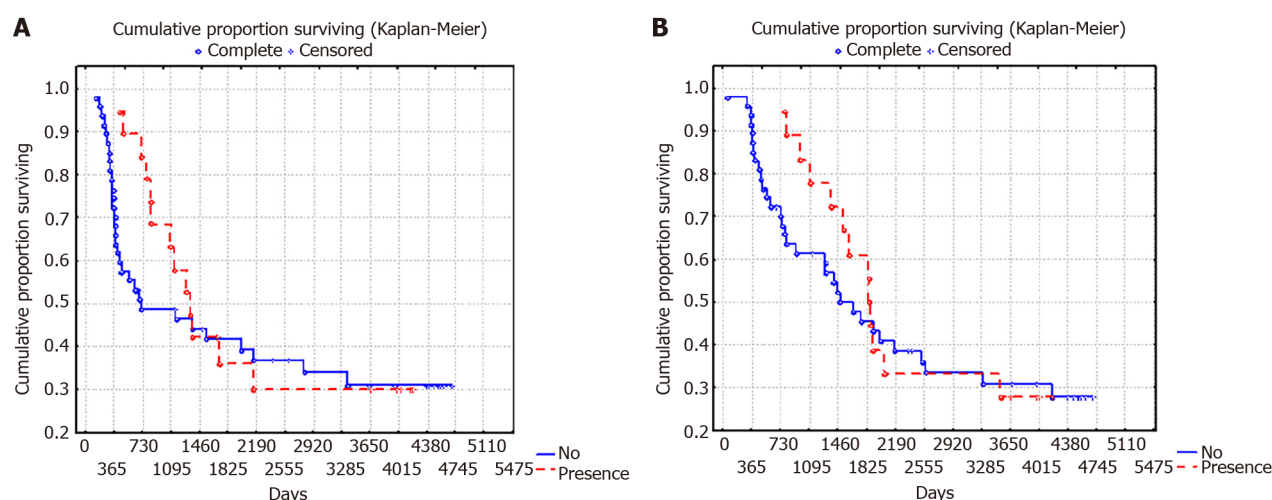


**Figure 3** 10-year overall surviving and relapse-free surviving of patients with gastric cancer depending on the presence of antibiotic therapy 1-1.5 mo before surgery ( $P = 0.02$ ). A: 10-year overall surviving; B: Relapse-free surviving.

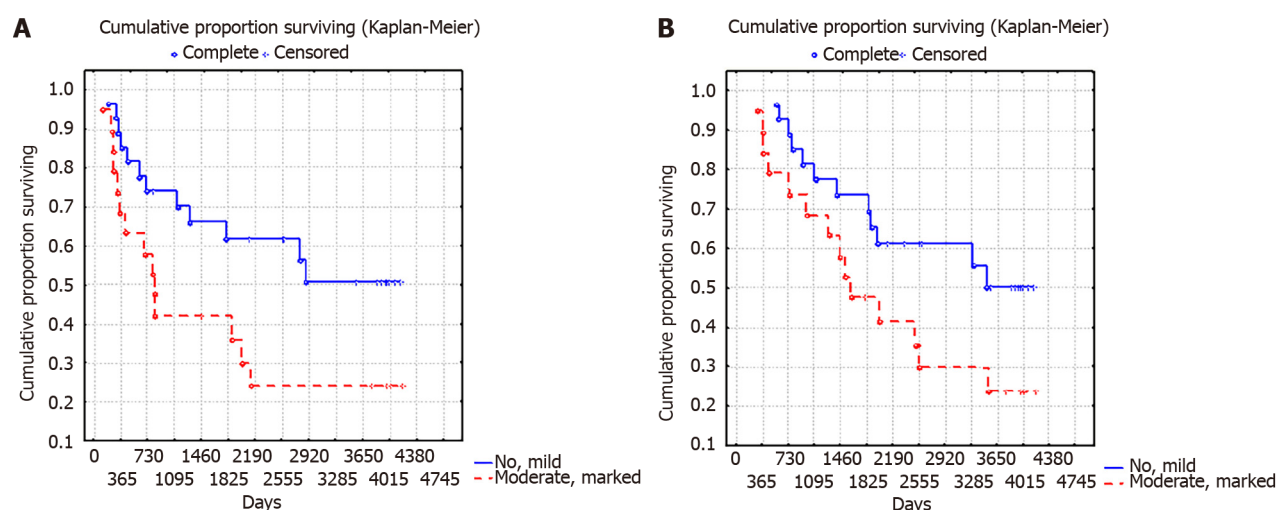
other researchers[57]. A number of studies have shown that coccoid forms of *H. pylori* retained urease activity[58] and the expression of such antigens as CagA, UreA, porin, components of the Cag type IV secretion system (TFSS), antigen-binding adhesin of the blood group BabA and others[59,60].

The use of immunohistochemistry in this study made it possible to detect the bacteria not only in the gastric mucus and on the surface of epithelial cells, but also within the cytoplasm of epithelial and immune cells of GM. Such intracellular expression was characterized by point inclusions giving a specific reaction with antibodies to *H. pylori*.

The intracellular persistence of *H. pylori* has been demonstrated by many investigators. They found *H. pylori* in the cytoplasm of epithelial cells, intercellular spaces, in the lamina propria of GM, and in the lumen of small vessels[61-63]. We assume that the point inclusions in the cytoplasm of epithelial and immune cells giving a positive reaction with antibodies to *H. pylori* is similar to those particle-rich cytoplasmic structure (PaCS) described earlier in the human superficial-foveolar epithelium and its metaplastic or dysplastic foci[64]. The authors found that the PaCS are a colocalization of VacA, CagA, urease, and outer membrane proteins with NOD1 receptor, ubiquitin-activating enzyme E1, polyubiquitinated proteins, proteasome components, and potentially oncogenic proteins like SHP2 and ERKs[64]. They believe that PaCS is a novel, proteasome-enriched structure arising in ribosome-rich cytoplasm at sites of *H. pylori* product accumulation.



**Figure 4** 10-year overall surviving and relapse-free surviving of patients with T3-4N1-2M0 stages of gastric cancer depending on the presence of antibiotic therapy 1-1.5 mo before surgery ( $P = 0.78$ ). A: 10-year overall surviving; B: Relapse-free surviving.



**Figure 5** 10-year overall surviving and relapse-free surviving of patients with gastric cancer depending on the concentration of *Helicobacter pylori* coccoid forms in the gastric mucosa ( $P = 0.02$ ). A: 10-year overall surviving; B: Relapse-free surviving.

We believe that the immune cells with point inclusions in the lamina propria of GM are likely to be macrophages. The data obtained by several researchers suggests this conclusion[65,66]. It is noted that even the absorbed bacteria retains their viability in macrophages, which may be associated with the violation of the phagosome maturation[66-68]. The use of confocal microscopy enabled the localization of the bacteria within the cells to be associated with the endosomal and lysosomal markers, and found that *H. pylori* could use the vesicles of autophagosomes (autophagic vesicles) for its own replication[63,69].

The study found that the concentration of *H. pylori* coccoid forms in GM was the most significant clinical factor. This factor was associated with the tumor histology, T status, N status, stage, 10-year PFS, and OS. The moderate and marked concentrations of coccoid forms of *H. pylori* were more often found in the diffuse type of GC ( $P = 0.024$ ) and T3-4 ( $P = 0.04$ ) stage. Interestingly, the high concentration of *H. pylori* is more frequent in Stage N1 than in N2 (at 90.0% and 53.1%, respectively,  $P = 0.024$ ).

The moderate and marked concentrations of coccoid forms of *H. pylori* represented a prognostic factor associated with the decrease of 10-year RFS and OS from 55.6% to 26.3% ( $P = 0.02$  and  $P = 0.07$ , respectively).

It should be noted that the results of this study do not allow us to unambiguously judge the effect of *H. pylori* on GC progression. A decrease in OS and DFS in patients with moderate and marked concentrations of *H. pylori* coccoid forms in the GM may be due to the fact that these patients had more advanced stages and more aggressive

forms of GC. Meanwhile, there are more and more studies showing that *H. pylori* infection can promote GC progression by activating the NF- $\kappa$ B signaling pathway and induction of interleukin-8 secretion[70], the activation of epithelial-mesenchymal transformation[71-74] and angiogenesis[75,76], as well as increasing the invasive properties of tumor cells[77]. It can be assumed that the administration of AT before surgery contributes to the reduction of the inflammatory process activity and normalization of the adhesive properties of tumour cells, which in turn decreases metastasis risk and improves the long-term results of the treatment of GC. The data literature on the improvement of the long-term results of malignant tumours treatment when using antibacterial drugs testify in favour of this hypothesis[78-80].

## CONCLUSION

The data obtained indicates that *H. pylori* may be associated not only with induction but also with the progression of GC. It can be assumed that the prevalence of coccoid forms of bacteria and their intracellular persistence can affect the mechanisms of tumor progression. Further appropriate studies regarding the role of *H. pylori* in the progression of GC are obviously advisable.

## ARTICLE HIGHLIGHTS

### Research background

Gastric cancer (GC) continues to be one of the most common malignant diseases in the world. It is known that *Helicobacter pylori* (*H. pylori*) infection, initiating the development of a chronic inflammatory process in the gastric mucosa (GM), is the leading risk factor for GC. At the same time, clinical studies indicate that inflammatory infiltration of the tumor stroma and surrounding tissues can have an important prognostic value and affect the long-term results of malignant neoplasm treatment.

### Research motivation

It is still not clear whether *H. pylori* is involved only in the initiation of the tumor process in the stomach, or whether it can affect the mechanisms of tumor progression.

### Research objectives

The aim of this study was to establish the features of *H. pylori* infection in patients with GC and their correlations with clinical and morphological characteristics of diseases and long-term results of treatment.

### Research methods

In this prospective observational study, we included all patients with GC who had undergone radical surgery (R0) between May 2007 and March 2010 at the Orenburg Regional Clinical Oncology Center. Features of the *H. pylori* infection and its severity was determined by rapid urease test and by immunohistochemically using the antibody to *H. pylori*. The data obtained we compared with clinical features of GC: Stage, localization, histology, the presence of antibiotic therapy (AT) before surgery, and 10-year overall and disease-free survival.

### Research results

We found *H. pylori* infection in the adjacent to the tumor GM in 84.5% of cases. We have established that the coccoid forms of *H. pylori* predominate in the GM of patients with GC. A high rate of infection by coccoid forms of *H. pylori* has been associated with more aggressive type of GC, advanced stage, and decline of a 10-year overall and disease-free survival. The presence of AT 1-1.5 mo before the operation was associated with an improvement in the 10-year survival rate of patients with local (T1-3N0M0), but not advanced (T3-4N1-2M0) stages of GC.

### Research conclusions

These results indicate that *H. pylori* may be associated not only with induction but also with the progression of GC.

# Research perspectives

The results obtained do not allow one to draw unambiguous conclusions about the role of *H. pylori* in the progression of GC. Further appropriate prospective studies regarding the role of *H. pylori* in the progression of GC are obviously advisable.

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