STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		Helicobacter pylori in gastric cancer. Features of infection and their correlations with long-term results of treatment: an observational cohort study	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
		In observational cohort study, the features of H. pylori infection were studied in 109 patients who underwent radical surgery for gastric cancer. The results obtained were compared with the clinical and pathological characteristics of gastric cancer and long-term results of treatment. The presence of H. pylori in the gastric mucosa and tumor was determined using an express test for urease and immunohistochemically using antibodies to H. pylori. It was found that patients with gastric cancer have a high rate of H. pylori infection (84,5%), and coccoid forms of bacteria predominate in the gastric mucosa, which were detected in 93.4% of infected patients. A high rate of infection by coccoid forms of H. pylori has been associated with more aggressive type of gastric cancer, advanced stage, and decline of a 10-year overall and disease-free survival. The presence of antibiotic therapy 1 - 1.5 months before the operation, since the preliminary diagnosis of gastritis or gastric ulcer, 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 gastric cancer.	2-3

Introduction

	Item No	Recommendation	page
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	
		The role of H. pylori in the progression of gastric cancer remains unclear, in this connection, the question of the advisability of antibiotic therapy in patients with invasive gastric cancer remains open.	4,5
Objectives	3	State specific objectives, including any prespecified hypotheses	
		To establish the features of H. pylori infection in patients with gastric cancer and their correlations with clinical and morphological characteristics of diseases and long-term results of treatment.	5

Methods

	Item No	Recommendation	page
Study design	4	Present key elements of study design early in the paper	
		Observational cohort study	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
		All patients with gastric cancer who had undergone radical surgery (R0) between May 2007 and March 2010 at the Orenburg Regional Clinical Oncology Center	5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	
		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. Clinical and pathological data including age, tumor localization, stage, type of surgery, histology, the presence of antibiotic therapy (AT) before surgery, postoperative therapy, and long-term results of treatment were retrieved from the routine reports for analyses. The long-term results of treatment were assessed for the period from May 12, 2007 to April 12, 2021.	5,6

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
		H. pylori in the GM and tumor was determined by rapid urease test and by immunohistochemically (IHC) using the antibody to H. pylori.	
		The data obtained was compared with clinical features of GC: stage, localization, histology, the presence of antibiotic therapy before surgery, and 10-year overall and disease-free survival.	7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
		Rapid urease test (RUT) Samples of tumor and the adjacent macroscopically non-tumorous GM were placed on test strips (HELPIL–test, "AMA", Russia) for three minutes. 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 minutes 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 sections for IGH were stained with the anti-H. pylori (RB-9070, Thermo Fisher Scientific, the immunogen is purified H. pylori) rabbit polyclonal antibodies. 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 (Dixon et al, 1996). 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.	7,8
Bias	9	Describe any efforts to address potential sources of bias	
		All sections were carefully and completely scanned by two of the authors (MS and OT) without knowledge of the clinical and pathological data.	8
Study size	10	Explain how the study size was arrived at	
		All patients with gastric cancer who had undergone radical surgery (R0) between May 2007 and March 2010 at the Orenburg Regional Clinical Oncology Center were included in study	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
		The frequency of high and low degrees of contamination of the gastric mucosa by various vegetative forms of H. pylori was assessed, depending on the age of the patients, stage of the disease, histology, type of stomach cancer, and the presence of antibiotic therapy before surgery. 10-year overall and relapse-free survival was studied in the groups with high and low levels of H. pylori contamination and those who received and did not receive antibiotic therapy before surgery.	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		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 and categorical data. The survival was analyzed using the Kaplan-Meier method. The log-rank test was used to compare survival curves between subgroups of patients.	8,9
		(*b*) Describe any methods used to examine subgroups and interactions	
		The correlations between different data were evaluated using the nonparametric Spearman's rank correlation or gamma correlation.	8

		(*c*) Explain how missing data were addressed	
		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.	6,7

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and Analysed	
		The patients were divided into three groups depending on the stage (T1-2N0M0 - local cancer, T3N0M0 - advanced cancer without metastases and T3-4N1-2 advanced cancer with metastases to the lymph nodes); 4 groups - depending on the T status (T1-4), 3 groups - depending on the presence of lymph nodes metastases (N0-2); 4 groups - depending on the histology (G1-3 and signet ring cell carcinoma), 2 groups - depending on the type of cancer (intestinal and diffuse); two groups - depending on the presence of antibiotic therapy before surgery, and two groups - depending on the degree of gastric mucosa contamination by various vegetative forms of H. pylori	31
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		There were 72 men (66,1%) and 37 women (33,9%). The average age of the patients was 61.7±1,03 years (from 24 to 81 years, the median – was 61 years). The involvement of the upper third of the stomach was in 18 patients (16,5%), the middle third – in 32 patients (29,3%), the lower third - in 57 patients (52,3%), and the total gastric cancer was in 2 patients (1,8%). In accordance with the TNM classification of malignant tumours [TNM Classification of Malignant Tumours] the distribution of patients according to the stages of the disease was as follows: T1-2N0M0 was in 40 patients (36,7%), T3N0M0 - in 17 (15,6%), T3-4N1M0 - in 20 (18,4%) and T3-4N2M0 - in 32 (29,4%). Intestinal and diffuse-types of GC were in 50 cases (45,9%) and 59 cases (54,1%) respectively. Tumours were good-differentiated (G1) in 31 patients (28,4%), moderate (G2) - in 19 (17,4%), and poor (G3) - in 33 (30,3%), and 26 patients (23,8%) had signet ring cell carcinoma. Subtotal distal resection was performed in 85 cases (78%), subtotal proximal resection - in 10 cases (11%), and gastrectomy - in 7 cases (11%).	31
		(b) Indicate number of participants with missing data for each variable of interest	
		Six patients left the region at different follow-up periods	6
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
		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 months.	6
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		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 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 COVID-19, one of whom died from the disease, but the rest are alive.	6,7

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included

H. pylori were found in 84.5% of cases and coccoid forms of H. pylori predominated in the gastric mucosa of patients with gastric cancer.

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. Correlations of H. pylori concentration in GM according to RUT with 10-year OS and RFS of GC patients were not determined.

The presence in AT 1 - 1.5 months 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.

The concentration of H. pylori coccoid forms correlated with age (ρ=-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±2.5 years vs. 66.2±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 PFS and OS survival from 55.6% to 26.3% was observed (p=0.02 и p=0,07, respectively).

9-11, 28-31, 33,34

Discussion

Key results	18	Summarise key results with reference to study objectives

Coccoid forms of H. pylori predominate in the gastric mucosa of patients with gastric cancer. A high rate of infection by coccoid forms of H. pylori has been associated with more aggressive type of gastric cancer, advanced stage, and decline of a 10-year overall and disease-free survival. The presence of antibiotic therapy 1 - 1.5 months before the operation, since the preliminary diagnosis of gastritis or gastric ulcer, 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 gastric cancer.

12-14

Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias

The results of this study do not allow us to unambiguously judge the effect of H. pylori on GC progression. A decrease in overall and disease-free survival in patients with moderate and marked concentrations of H. pylori coccoid forms in the gastric cancer may be due to the fact that these patients had more advanced stages and more aggressive forms of gastric cancer.

14,15

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence

Some studies showing that H. pylori infection can promote GC progression by activating the NF-κB signaling pathway and induction of IL-8 secretion, the activation of epithelial-mesenchymal transformation and angiogenesis, as well as increasing the invasive properties of tumor cells. 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.

12-15

| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | |
| | | The results obtained do not allow one to draw unambiguous conclusions about the role of H. pylori in the progression of GC and the consistency of antibiotic therapy. Further appropriate prospective studies regarding the role of H. pylori in the progression of GC are obviously advisable. | 14,15 |

Other information

| | | Give the source of funding and the role of the funders for the present study and, if applicable, or the original study on which the present article is based | |
| Funding | 22 | no funding | |