Relationship of prognostic factors in stomach cancer with *helicobacter pylori*: a retrospective study

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Abstract

Background and study aims: **The prognostic value of H. pylori, which infects more than half of the human population living in the world and plays a role in gastric cancer pathogenesis, is controversial. Our aim is to investigate the relationship between H. pylori and prognostic factors in gastric cancer.**

Patients and methods: **The data of 110 patients (38 females and 72 males) that underwent surgeries due to gastric cancer between 2014 and 2017 were retrospectively analyzed. The relationships between survival (disease-free and overall) and factors such as p53, HER2/neu, Ki-67, neutrophil and platelet lymphocyte ratio (NLR / PLR), histopathological and demographic characteristics were examined. In addition, the results of H. pylori positive and negative groups were compared.**

Results: **Sixty-one (55%) patients were H. pylori negative and 49 (45%) were positive. In multivariate analysis, TNM stage, lymph node capsule invasion and NLR were determined as independent prognostic factors in both disease-free and overall survival. Age>62 and PLR>14.3 were determined as independent predictive factors of poor prognosis in overall survival. In univariate analysis, tumor diameter of >4.3 cm, lymphovascular and perineural invasion, and diffuse p53 expression were determined as predictive factors of poor prognosis in disease-free and overall survival. The effectiveness of these markers in prognosis was not different between H. pylori negative and positive groups.**

Conclusion: **While age, tumor diameter, TNM stage, lymph node capsule invasion, perineural and lymphovascular invasion, diffuse p53, PLR, and NLR were determined as prognostic factors in gastric cancer, these factors were not affected by the presence of *H. pylori*.** (Acta gastroenterol. belg., **2021,** 84**, 607-617**).

Key words: H. pylori; gastric cancer; p53; HER2; neutrophil lymphocyte ratio; platelet lymphocyte ratio.

Introduction

More than 50% of the human population living in the world is infected with Helicobacter Pylori *(H. pylori*). *H. pylori* is usually transmitted in childhood (1,2). It was accepted as a Class 1 risk factor for gastric cancer by the World Health Organization in 1994 (3). An inflammatory response develops in cells in the gastric mucosa infected with *H. pylori*. While in some patients a progressive process of inflammation-related atrophic gastritis, metaplasia, dysplasia and carcinoma can start due to gland damage in the stomach, it was observed that this process did not take place in others and the inflammation steps progressed only marginally. However, it is not known how each individual infected patient will progress (4). This uncertainty is also seen in the prognostic effect of *H. pylori*. For example, *H. pylori* positivity was found to be a good independent prognostic factor in prospective and retrospective studies conducted in Germany, Italy and Taiwan (5-7), whereas in a study in China its prognostic effect was poor (8).

Gastric cancer develops in very few patients infected with *H. pylori* (9). Cancer development is triggered when mutations occur in oncogenes, tumor suppressor genes, and genes involved in cellular regulatory mechanisms as a result of combined effects of genetics, environment and *H. pylori* (10). There is no clear knowledge about the level of mutation in gastric cancer, the correlation of the mutations with *H. pylori*, and its prognostic effects.

Our aim in this study was to investigate the relationship between survival (disease-free and overall) and prognostic factors such as p53, HER2/neu, Ki-67, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and histopathological and demographic characteristics in patients operated for gastric cancer. In addition, these results were compared between *H. pylori* positive and negative groups

Patients and methods

Ethical Consideration

The study was carried out with the approval of the ethics committee of Health Sciences University Training and Research Hospital (Date: 13.07.2017; Decision number: 18).

Patients and clinicopathological information collection

Medical records of 38 female and 72 male patients that underwent total / subtotal gastrectomy and D2 lymph node dissection due to gastric cancer at the Health Sciences University Training and Research Hospital between 2014 and 2017 were obtained from the hospital database and evaluated retrospectively. Those who had previous gastric and peptic ulcus surgery, were operated for Siewert type I cardia tumor, received neoadjuvant therapy and had distant metastases were not included in the study. The

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patients were divided into two groups according to the *H. pylori* negative and positive status in the pathology specimen. Age, gender, macroscopic tumor location, tumor size, Borman subtype, tumor differentiation, TNM stage (early stage I-II and advanced stage III-IV), lymph node capsule invasion, lymphovascular and perineural invasion status of patients in both groups were examined and recorded from pathology reports. Post-operative disease-free and overall survival times were obtained by phone calls (from the surviving patients themselves, and from close relatives of the deceased).

Immunohistochemical (IHC) staining and evaluation

Previously prepared paraffin blocks for IHC studies were re-evaluated by two independent pathologists. One paraffin block, which best reflected the tumor tissue and was the most suitable for IHC evaluation, was chosen. Multiple blocks were prepared using the mapping and addressing technique from cylindrical paraffin tissue samples with a diameter of 4 mm, which were marked first on the slide and then on the block. One of the sections were stained with H&E, while others were manually stained with primary antibodies: cErbB2 (polyclonal, Leica; 1/40 dilution, NCL-L-CB11), Ki-67 (polyclonal, Leica; 1/200 dilution, NCL-L-Ki-67-MM1), and p53 (polyclonal, Leica; 1/800 dilution, NCL-L-p53-DO-7). For IHC staining, the sections placed on lysine slides were deparaffinized at 60°C for 1 hour, and then incubated in a pH 6.0 citrate solution at 65°C for 20 minutes in the DAKO PT LINK device. Slides were allowed to cool in buffer solution for 5 minutes. The slides were then incubated at room temperature with primary antibodies for one hour and were stained by avidin-biotin method. Sections stained with cErbB2, Ki-67 and p53 primary antibodies were examined under a light microscope. Paraffin blocks, which included areas in the gastric mucosa that did not show non-neoplastic atrophy and intestinal metaplasia, were stained with Warthin Starry stain for *H. pylori* (Figure 1 (A, B, C, D)). Intestinal metaplasia status was evaluated with PAS-AB pH 2.5 stain from non-tumor preparations. Mucosal atrophy was evaluated histomorphologically.

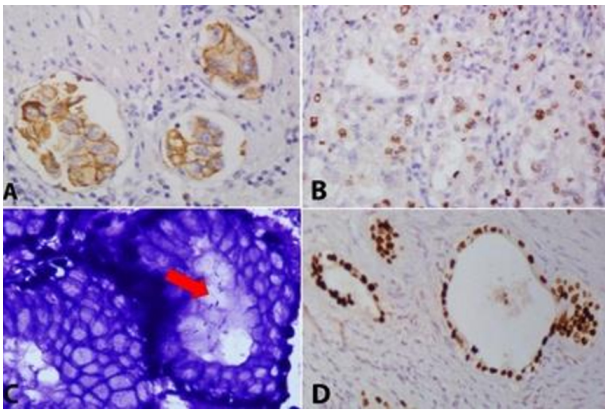

Figure 1. — A) Strong membranous HER2/ neu expression is remarkable (DAB x 400). B) A gastric cancer exhibiting high proliferative Ki67 index (DAB x 200). C) Note the small group of H pylori-like bacteria (red arrow) in mucus (Toluidine blue x 1000). D) Presence of high nuclear p53 positivity (DAB x 200)..

HER2 / neu was evaluated IHC according to the ACSO / CAP 2013 protocol (11). Samples were considered HER2 negative if HER2 / neu were 0 and 1 in IHC and negative in fluorescence in situ hybridization (FISH). Samples were considered HER2 positive, if HER2 / neu were 3 + in IHC and positive in FISH (12).

The p53 protein expression level in the tumor was evaluated according to nuclear staining. The cell cytoplasmic staining was defined as negative, 10-50% staining of the tumor cell nucleus was evaluated as focal involvement and more than 50% staining was defined as diffuse type (13).

The Ki-67 proliferation index in the tumor was evaluated as low if it was ≤10%, medium if 10-40% and high if ≥40% (14).

Biochemical analyses

NLR, PLR, CEA, CA19-9 values were determined from the blood samples preoperatively. Platelet, neutrophil, and lymphocyte levels were analyzed using a CBC analyzer with Coulter Principle impedance and multi-angle laser scatter (DxH 800, Beckman Coulter Inc., USA). Serum levels of CEA and CA19-9 were measured by chemiluminescent methodology using a DxI analyzer (Beckman Coulter Inc., USA).

Follow-up

None of the patients had a history of preoperative radiotherapy or chemotherapy. In the postoperative period, patients were referred to the medical oncology clinic and adjuvant chemotherapy was initiated based on the histological stage of the tumor. Patients were followed with tumor markers (CEA, CA19-9) and thoraco-abdominal computed tomography evaluation every three months for the first two years, and every six months for the next 3 years, and annually for the following years.

Statistical methods

SPSS 25.0 (IBM Corporation, Armonk, New York, United States) program was used in the analysis of variables. The suitability of the data for homogeneous distribution was evaluated by Shapiro-Wilk test and variance homogeneity by Levene method. Independent-Samples T test was used together with Bootstrap results, while Mann-Whitney U test was used with Monte Carlo simulation technique to compare quantitative variables in two independent groups. Pearson Chi-Square, Fisher Exact and Fisher-Freeman-Halton tests and Monte Carlo Simulation technique were used for comparing categorical variables with each other. The sensitivity

Table 1. — **The distribution of demographic and histopathological features, immuno-histochemical and biochemical markers in *H. pylori* negative and positive groups**

	***H. pylori* negative** (n=61)	***H. pylori* positive** (n=49)	**Total** (N=110)	**p**
Gender [3]				
Female	23 (37.7)	15 (30.6)	38 (34.5)	0.546 [a]
Male	38 (62.3)	34 (69.4)	72 (65.5)	
Age (years) [1]	61.00±12.43 - 37 / 88	62.69±11.19 - 34 / 88	61.75±11.87 - 34 / 88	0.441 [x]
Tumor diameter (cm)[2]	5.5 (1.8 / 16)	5 (1 / 14)	5.5 (1 / 16)	0.671 [y]
CEA [2]	2.91 (0.63 / 124.7)	3.95 (0.48 / 547.2)	3.67 (0.48 / 547.2)	0.186 [y]
CA 19-9 [2]	12.56 (0.06 / 769.8)	24.44 (0.6 / 1135.6)	18.77 (0.06 / 1135.6)	0.040 [y]
NLR [2]	2.54 (0.20 / 16.16)	2.86 (0.23 / 38.54)	2.61 (0.20 / 38.54)	0.398 [y]
PLR [2]	14.05 (0.97 / 35.59)	14.78 (1.54 / 38.85)	14.48 (0.97 / 38.85)	0.934 [y]
P53				
Negative	22 (36.1)	16 (32.7)	38 (34.5)	0.173 [a]
Focal	24 (39.3)	13 (26.5)	37 (33.6)	
Diffuse	15 (24.6)	20 (40.8)	35 (31.8)	
Ki67				
Low	18 (29.5)	9 (18.4)	27 (24.5)	0.160 [a]
Moderate	28 (45.9)	20 (40.8)	48 (43.6)	
High	15 (24.6)	20 (40.8)	35 (31.8)	
HER2 [3]				
Negative	56 (91.8)	41(83.7)	97 (88.2)	0.240 [c]
Positive	5 (8.2)	8(16.3)	13 (11.8)	
Localization [3]				
Proximal	17 (27.9)	19 (38.8)	36 (32.7)	0.307 [a]
Distal	44 (72.1)	30 (61.2)	74 (67.3)	
Resection[3]				
Total	37 (60.7)	34 (69.4)	71 (64.5)	0.423 [c]
Subtotal	24 (39.3)	15 (30.6)	39 (35.5)	
Atrophic gastritis [3]				
Absent	39 (75.0)	35 (79.5)	74 (77.1)	0.634 [a]
Present	13 (25.0)	9 (20.5)	22 (22.9)	
Intestinal metaplasia [3]				
Absent	15 (28.8)	11 (25.0)	26 (27.1)	0.818 [a]
Present	37 (71.2)	33 (75.0)	70 (72.9)	
Differentiation [3]				
Undifferentiated	33 (54.1)	24 (49.0)	57 (51.8)	0.701 [a]
Differentiated	28 (45.9)	25 (51.0)	53 (48.2)	
Borman Classification [2]	3 (1 / 3)	3 (1 / 4)	3 (1 / 4)	0.368 [y]
Perineural Invasion [3]				
Absent	15 (25.0)	13 (26.5)	28 (25.7)	0.999 [c]
Present	45 (75.0)	36 (73.5)	81 (74.3)	
Lymphovascular Invasion [3]				
Absent	16 (26.2)	8 (16.3)	24 (21.8)	0.251 [c]
Present	45 (73.8)	41 (83.7)	86 (78.2)	
Stage [a]				
Early (I + II)	20 (32.8)	13 (26.5)	33 (30.0)	0.534 [a]
Advanced (III + IV)	41 (67.2)	36 (73.5)	77 (70.0)	
Lymph node capsule invasion				
Absent	29 (47.5)	19 (38.8)	48 (43.6)	0.440 [a]
Present	32 (52.5)	30 (61.2)	62 (56.4)	
Disease-free survival [2]	27 (1 / 81)	20 (1 / 77)	21.5 (1 / 81)	0.362 [y]
Overall survival [2]	31 (1 / 81)	24 (1 / 77)	30 (1 / 81)	0.633 [y]

[x] Independent Samples t test(Bootstrap), [y] Mann Whitney U Test(Monte Carlo), [a] Pearson Chi square Test (Monte Carlo), [b] Fisher Freeman Halton Test (Monte Carlo), [c] Fisher Exact Test (Monte Carlo). [1] Data are shown as Mean ± Standard deviation-Min / Max, [2] data are shown as Median (Min / Max), [3] data are shown as n (%). $p < 0.05$ was considered as statistically significant.

and specificity rates for the relationship between classification of cut-off value calculated according to mortality and the actual classification were examined and expressed by Receiver Operating Curve (ROC)

Table 2. — **Roc Curve Analysis of prognostic markers in gastric cancer**

Dependent variable: Mortality	Optimal Cut-off	Sensitivity	Specificity	AUC (SE.)	p
Age (year)	>62	59.2	71.8	0.634 (0.054)	**0.020**
Tumor diameter (cm)	>4.3	77.46	51.28	0.707 (0.051)	**<0.001**
CEA	>3.43	6.6	61.5	0.595 (0.055)	0.100
CA 19-9	>34.72	35.1	82.1	0.567 (0.056)	0.246
NLR	>2.86	54.9	84.6	0.673 (0.053)	**0.003**
PLR	>14.53	62	74.4	0.679 (0.051)	**0.002**

Roc Curve Analysis (Youden index J - Honley & Mc Nell), AUC: Area under the ROC curve, SE: Standard Error, NLR: Neutrophil/Lymphocyte ratio, PLR: Platelet/Lymphocyte ratio, p< 0.05: Statistically significant.

curve analysis. The variables were reconstructed with the estimation values obtained here. Kaplan-Meier (product limit method) analysis was used for categorized variables and other variables created by ROC analysis to examine the effects of factors on mortality and lifespan. The Cox Regression analysis Backward Stepwise method was used for variables that were found to be significant in Kaplan Meier analysis, which was applied to measure the effects of prognostic variables on survival according to the main factor. Quantitative variables were shown in tables as mean ± SD (Standard Deviation), Minimum / Maximum and median (Minimum / Maximum), and categorical variables as n (%). Variables were analyzed at a 95% confidence level, and a p value of less than 0.05 was considered significant.

Results

Thirty-eight (34.5%) patients were female and 72 (65.5%) were male. Sixty-one (55%) of 110 patients operated on for gastric cancer were *H. pylori* negative and 49 (45%) were positive (table 1).

Prognostic factors affecting disease-free and overall survival

Age

According to univariate analysis, age was effective in disease-free and overall survival. In the ROC analysis, the cut-off value for age was 62 years with the sensitivity of 59.2%, specificity of 71.8%, and AUC 0.634 (p = 0.002) (table 2). In patients >62 years of age, 3/5 year disease-free and overall survival was 30.2/19.3 and 34/19.1 months, respectively, while in patients with age ≤62 these values were longer (51.9/47.7 and 54.4/48.4, respectively) (p = 0.002, p = 0.001) (table 3). The multivariate analysis showed that advanced age was an independent risk factor for overall survival (Hazard Ratio (HR) 2.75 [95% Confidence interval (CI) CI 1.65-4.57, p <0.001], p <0.001) (table 5).

The mean age was 61.00 ± 12.43 years in the *H. pylori* negative group and 62.69 ± 11.19 years in the *H. pylori* positive group and this difference was not significant. When *H. pylori* positive and negative groups were compared, no difference was observed between disease-free and overall survival in terms of age (>62, ≤62) (p> 0.05) (table 3).

Tumor localization and diameter

The tumor location in 36 (32.7%) cases was proximal (cardia, fundus, corpus) and distal (antrum, prepyloric, pylori) in 74 (63.7%) cases. There was no significant difference in gastric location of the tumor between *H. pylori* negative and positive groups (p> 0.05) (table 1). In the univariate analysis, although the location of the tumor in the stomach did not have an effect on disease-free and overall survival, tumor diameter did (table 3). Tumor diameter cut-off value was found as 4.3 cm in ROC analysis. The sensitivity of the cut-off value was 77.46, specificity was 51.28, and AUC was 0.707 (p <0.001) (table 2). When the tumor diameter was greater than the cut-off value, 3/5 year disease-free and overall survival was 33.8/23.8, 31.1/24.3 months, respectively, when it was smaller than the cutoff these values were higher (63.9/55.1 and 63/54, respectively). This difference was significant (p = 0.004, p = 0.004), (table 3). However, no difference was found between *H. pylori* negative and positive groups in terms of tumor diameter (p> 0.05) (table 2).

Histopathological TNM Stage

Thirty-three (30%) cases were early stage (I, II), while 77 (70%) were advanced stage (III, IV) (table 1). Univariate analysis indicated that TNM stage had an effect on disease-free and overall survival. While the 3/5 years disease-free and overall survival rates in the early stage were 72.7 / 61.9 and 72.7 / 61.9 months, they were shorter in the advanced stage (27.7 / 21.6 and 32.5 / 22 months, respectively) (p = 0.001, p = 0.001), (table 3). Multivariate analysis revealed that TNM stage was an independent prognostic factor in disease-free and overall survival (HR: 1.79 /2.75 [CI 95% 1.24-15.89 / 1.16-4.96, (p = 0.095, p <0.001)), (table 5). The disease-free and overall survival effect of the TNM stage was not significantly different between the *H. pylori* negative and positive groups (p> 0.05), (table 3).

Perineural invasion

While perineural invasion was not observed in 28 (25.7%) cases, it was seen in 81 (74.3%) cases (table 1).

Table 3. — **The relationship between prognostic factors with disease-free and overall survival in *H. pylori* negative and positive groups**

		Progression-free Survival		P	Overall Survival		P
		Mean ± SE.	EPS at the 3/5 Year		Mean ± SE.	EPS at the 3/5 Year	
H. pylori							
	Negative	40.1±4.51	45.9/40.1	0.242	42.4±4.29	47.5/40.1	0.366
	Positive	31.4±4.37	35.5/26.2		36.1±4.11	46.9/27.7	
Overall		36.7±3.26	41.3/33.8		40.0±3.07	44.5/34.2	
Age (years)							
≤62		46.3±4.63	51.9/47.7	**0.002**	49.8±4.21	54.4/48.4	**0.001**
>62		26.4±4.09	30.2/19.3		29.2±3.95	34/19.1	
≤62	HP (-)	51.5±5.67	61.1/54.4	0.126	53.9±5.21	61.1/54.4	0.208
	HP (+)	35.7±7.12	35.4/35.1		41.4±6.48	42.9/38.1	
>62	HP (-)	23.4±5.94	24/19.2	0.420	25.6±5.80	28.1/19.2	0.308
	HP (+)	28.6±5.40	35.7/20.8		32.1±5.14	39.3/20.8	
Tumor diameter (cm)							
≤4.3		50.9±5.83	63/54	**0.004**	52.5±5.55	63.9/55.1	**0.004**
>4.3		30.0±3.68	31.1/24.3		33.8±3.46	33.8/23.8	
≤4.3	HP (-)	56.4±7.67	68.4/62.7	0.255	56.9±7.52	68.4/62.7	0.361
	HP (+)	39.6±7.38	56.7/43.2		42.5±6.82	58.8/46.3	
>4.3	HP (-)	32.7±5.18	35.7/30	0.465	35.9±4.90	38.1/30	0.593
	HP (+)	26.0±4.84	25/18.2		31.1±4.59	31.3/18.2	
TNM Stage							
Early (I,II)		58.2±5.48	72.7/61.9	**<0.001**	58.4±5.45	72.7/61.9	**<0.001**
Advanced (II,III)		27.4±3.52	27.7/21.6		32.2±3.34	32.5/22.6	
Early	HP (-)	58.2±7.13	70/63	0.894	58.5±7.06	70/63	0.894
	HP (+)	56.0±8.06	76.9/60.6		56.0±8.06	76.9/60.6	
Advanced	HP (-)	31.3±5.22	34.1/28.9	0.291	34.6±4.93	36.6/28.9	0.487
	HP (+)	22.1±4.17	20.1/13.8		28.7±4.08	27.8/16.2	
Lymphovascular invasion							
Absent		55.5±6.89	66.7/62.2	**0.004**	56.6±6.66	70.8/62.2	**0.008**
Present		31.4±3.49	34.2/25.9		35.4±3.29	37.2/26.6	
Absent	HP (-)	53.7±8.87	62.5/62.5	0.837	55.3±8.49	68.8/62.5	0.893
	HP (+)	52.6±8.68	75/62.5		52.6±8.68	75/62.5	
Present	HP (-)	35.3±5.05	40/32.1	0.264	37.8±4.78	40/32.1	0.457
	HP (+)	26.4±4.46	27.6/19.4		32.1±4.25	34.1/21.3	
Perineural invasion							
Absent		49.6±6.27	62.1/50.6	**0.028**	51.2±5.93	62.1/50.6	**0.044**
Present		32.1±3.68	36.3/27.9		36.1±3.49	38.3/28.7	
Absent	HP (-)	56.9±8.12	75/60.6	0.231	58.1±7.64	75/60.6	0.229
	HP (+)	39.2±8.70	53.8/38.5		41.2±8.25	46.2/38.5	
Present	HP (-)	34.1±5.11	35.6/32.8	0.498	36.8±4.88	37.8/32.8	0.765
	HP (+)	28.3±4.85	31.6/22.2		33.9±4.59	38.9/24.3	
Lymph node capsule invasion							
Absent		51.8±4.64	64.6/50.1	**<0.001**	52.6±4.50	64.6/50.1	**<0.001**
Present		24.8±3.91	23/20.9		30.3±3.75	29/22	
Absent	HP (-)	53.3±6.34	65.5/58	0.512	54.2±6.16	65.5/58	0.489
	HP (+)	47.5±5.97	63.2/39		48.2±5.74	63.2/39	
Present	HP (-)	28.0±5.61	25.1/23.4	0.371	31.7±5.31	31.3/23.4	0.675
	HP (+)	20.3±5.03	17.4/17.4		27.8±5.00	26.7/20	

Kaplan Meier Test-Log Rank (Mantel-Cox), SE: Standard Error, EPS: Estimate Proportion Surviving, IHC: Immunohistochemical, HP (-): Helicobacter pylori negative, H P(+): Helicobacter pylori positive ,p< 0.05: Statistically significant.

It was found to be a predictive factor for poor prognosis in univariate analysis. While 3/5 year disease-free and overall survival rates were 62.1/50.6 and 62.1/50.6 months in patients without perineural invasion, they were significantly shorter in patients with invasion (36.3/27.9 and 38.3/28.7 months) (p = 0.028, p = 0.044) (table 3). The effect of perineural invasion on disease-free and overall survival in *H. pylori* positive and negative groups was not significantly different (p> 0.05) (table 3).

Lymphovascular invasion

While lymphovascular invasion was not observed in 24 (21.8%) cases, it was seen in 86 (78.2%) cases (table 1). Lymphovascular invasion was determined

Table 4. — **The relationship of prognostic factors (p53 protein expression, neutrophil and platelet lymphocyte ratio) with disease-free and overall survival in *H. pylori* negative and positive groups**

		Progression-free Survival		P	Overall Survival		P
		Mean ± SE.	EPS at the 3/5 year		Mean ± SE.	EPS at the 3/5 year	
P53							
Negative	**I**	34.8±5.16	39.5/28.1	p (I-II)=0.155	39.4±4.78	55.3/28.1	p (I-II)=0.164
Focal	**II**	45.9±6.00	53/50.2	p (I-III)=0.520	48.5±5.65	54.1/51.4	p (I-III)=0.351
Diffuse	**III**	29.6±5.42	31.4/23.9	**p (II-III)=0.045**	32.0±5.16	31/4	**p (II-IIII)=0.034**
Negative	HP (-)	37.0±6.94	45.5/30.3	0.626	39.3±6.61	23/9	0.871
	HP (+)	30.7±7.23	31.3/25		38.5±6.41	50/30.3	
Focal	HP (-)	45.6±7.34	50/50	0.995	48.5±6.82	43.8/25	0.958
	HP (+)	40.8±8.91	58.7/50.3		42.8±8.54	50/50	
Diffuse	HP (-)	36.4±9.50	40/40	0.357	37.8±9.20	61.5/53.8	0.453
	HP (+)	24.3±5.79	25/13.3		27.4±5.40	40/40	
	HP (+)	21.6±6.45	17.6/17.6		24.2±6.15	53.1/33.1	
NLR							
≤2.86		47.9±4.31	58.8/47.7	**<0.001**	50.6±3.98	60.9/48.3	**<0.001**
>2.86		19.7±3.46	17.4/14.9		23.7±3.39	21.7/14.9	
≤2.86	HP (-)	51.5±5.36	62.5/53.6	0.270	53.0±5.06	62.5/53.6	0.415
	HP (+)	40.3±6.67	52.5/37.5		45.2±6.01	58/40.1	
>2.86	HP (-)	16.6±4.84	14.3/14.3	0.396	20.3±4.81	19/14.3	0.459
	HP (+)	22.1±4.72	20/15		26.2±4.58	24/15	
PLR							
≤14.53		48.0±4.68	55.7/49.7	**<0.001**	50.0±4.40	56.4/50.4	**0.001**
>14.53		25.2±3.93	27.3/16.9		29.5±3.75	32.7/16.5	
≤14.53	HP (-)	50.3±5.98	61.3/50.4	0.663	52.1±5.56	61.3/50.4	0.703
	HP (+)	42.6±7.02	48/48		45.1±6.65	50/50	
>14.53	HP (-)	29.4±6.15	30/30	0.363	32.3±5.96	33.3/30	0.511
	HP (+)	20.5±4.15	24/5.3		26.8±3.97	32/5.3	

Kaplan Meier Test-Log Rank (Mantel-Cox), SE: Standard Error, EPS: Estimate Proportion Surviving, IHC: Immunohistochemical, HP(-): Helicobacter pylori negative, HP(+): Helicobacter pylori positive, NLR: Neutrophil/Lymphocyte ratio, PLR: Platelet/Lymphocyte ratio, p< 0.05: Statistically significant.

Table 5. — **Multivariate-Cox Regression analysis results**

	P	HR. Lower	95% CI for HR.	Hazard probability at the 1 / 3 / 5 Year		
			Upper	Helicobacter pylori		
				Absent	Present	
Overall survival						
Age (>62)	**<0.001**	2.75	1.65	4.57	25.9 / 57.4 / 69.9	15.4 / 47.8 / 68.4
Stage (advanced)	**0.018**	2.38	1.16	4.90		
LNCI	**0.010**	2.12	1.20	3.75		
NLR (>2.86)	**0.005**	2.22	1.27	3.88		
PLR (>14.53)	**0.028**	1.90	1.07	3.37		
Disease-free survival						
Stage (advanced)	**0.022**	4.44	1.24	15.89	21.4 / 33.9 / 40.3	23.3 / 38.4 / 38.4
LNCI	**0.044**	2.30	1.02	5.17		
NLR (>2.86)	**0.095**	1.79	0.90	3.57		

Cox Regression-Backward Stepwise (Wald) Method, CI: Confidence interval, HR. Hazard Ratio, NLR: Neutrophil/Lymphocyte ratio, PLR: Platelet/Lymphocyte ratio, LNCI: Lymph node capsule invasion, p< 0.05: Statistically Significant.

as a predictive factor for poor prognosis of disease-free and overall survival. The 3/5 year disease-free and overall survival rates of patients without lymphovascular involvement were determined as 66.7/62.2 and 70.8/62.2 months, however these rates were significantly lower in patients with lymphovascular involvement (34.2/25.9, 37.2/26.6 months) (p = 0.004, p = 0.008), (table 3). The effect of lymphovascular invasion on disease-free and

overall survival was not significantly different between *H. pylori* positive and negative groups (p> 0.05), (table 3).

Lymph Node Capsule Invasion

While there was no capsule invasion in 48 (43.6%) of the cases, it was seen in 62 (56.4%) patients (table 1). Both the univariant and multivariant analysis indicated that lymph node capsule invasion was an indicator of poor prognosis. In patients without lymph node capsule invasion the 3/5 year disease-free and overall survival times were 64.6/50.1 and 64.6/50.1 months, but in patients with capsule invasion these survival rates were significantly lower (23 / 20.9 and 29/22 months) (p = 0.001, p = 0.001), (table 3). Disease-free and overall survival in multivariate analysis were HR: (2.30 / 2.12) [95% CI: 1.02-5.17 / 1.20-3.75, (p = 0.044, p = 0.010), respectively (table 5). There was no significant difference between *H. pylori* positive and negative groups in terms of lymph node capsule invasion's effect on disease-free and overall survival (p> 0.05), (table 3).

HER2/neu

The HER2/neu was negative in 97 (88.2%) and positive in 13 (11.8%) patients (table 1). Positive or negative HER2 / neu amplification had no effect on disease-free and overall survival (p> 0.05). There was no significant difference between *H. pylori* positive and negative groups in terms of the effect of HER2 / neu gene amplification on disease-free and overall survival (p> 0.05)

P53

Thirty-eight (34.5%) cases were negative for p53 protein expression. While focal involvement was detected in 37 (33.6%) cases, 35 (31.8%) cases had diffuse involvement (table 1). Diffuse involvement was found to be a predictive factor for poor prognosis in univariate analysis. In focal involvement, 3/5 year disease-free and overall survival was 53/50.2 and 54.1/51.4 months, respectively, while these times were shorter in diffuse involvement (31/4, 31.4 / 23.9 months) (p = 0.045, p = 0.034) (table 4). There was no significant difference between *H. pylori* positive and negative groups in terms of the effect of diffuse p53 protein expression on disease-free and overall survival (p> 0.05) (table 4).

Ki-67

Patients were divided into 3 groups based on the Ki-67 proliferation index as low, medium and high. Twenty-seven (24.5%) cases had low proliferative index, 48 (43.6%) had moderate, and 35 (31.8%) had high proliferative index (table 1). It was determined that Ki-67 had no effect on survival (p> 0.05). There was no significant difference between *H. pylori* positive and negative groups in terms of the effect of Ki-67 proliferation index on disease-free and overall survival (p> 0.05), (table 4).

NLR

The univariate analysis indicated that NLR can be used as a prognostic factor for disease-free and overall survival. The cut-off value for NLR was 2.86 with sensitivity of 54.9%, specificity of 84.6% and AUC value of 0.673 (p = 0.003) (table 2). When the NLR ratio was below the cut-off value, the 3/5 year disease-free survival was 58.8/47.7 and 60.9/48.3 months, but when it was higher than the cut-off value, these times were significantly shorter (17.4/14.9 and 21.7/14.9 months) (p <0.001, p <0.001), (table 4).

In multivariant analysis, NLR was determined as an independent risk factor for disease-free and overall survival (HR: 1.79 / 2.2 [95% CI: 0.90-3.57 / 1.27-3.88, p = 0.095 / p = 0.005]) (table 5). There was no significant difference between *H. pylori* positive and negative groups in terms of the effect of NLR level on disease-free and overall survival (p> 0.05), (table 4).

PLR

PLR was found to be a prognostic factor for disease-free and overall survival in univariate analysis. The cut-off value for PLR was found to be 14.53. The specificity of the cut-off value was 62%, sensitivity was 74.4%, and AUC was 0.679 (P = 0.002) (table 2). The cut-off value was significant in determining the prognosis (p = 0.002). When PLR is below the cut-off value, 3/5 year disease-free and overall survival are 55.7/49.7 and 56.4/50.4 months, respectively, but when it was higher than the cut-off value, these values were significantly lower (27.3/16.9, 32.7/16.5 months) (table 4).

The multivariate analysis revealed that PLR was an independent prognostic factor for overall survival (HR: 1.9 [95% CI: 1.07-3.37, p = 0.028]) (table 5). There was no significant difference between *H. pylori* positive and negative groups in terms of the effect of PLR level on disease-free and overall survival (p> 0.05), (table 4).

Effect of H. pylori status on disease-free and overall survival

In the *H. pylori* positive group, disease-free and overall survival at 1, 3 and 5 years were 23.3 / 38.4 / 38.4 and 15.4 / 47.8 / 68.4, respectively, while in the *H. pylori* negative group these values were 21.4 /33.9 / 40.3 and 25.9 / 57.4 / 69.9 (p = 0.265) (table 5) (figure 2, 3).

Discussion

Both the biological and genetic diversity of gastric cancer (15) and the large difference (up to 15-20 times) in

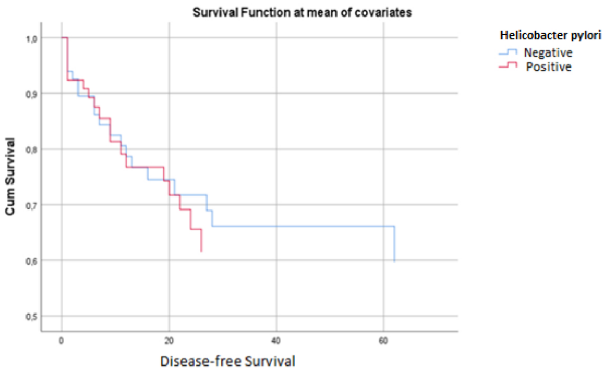

Figure 2. — Disease-free survival rates of patients in *H. pylori* negative and positive groups.

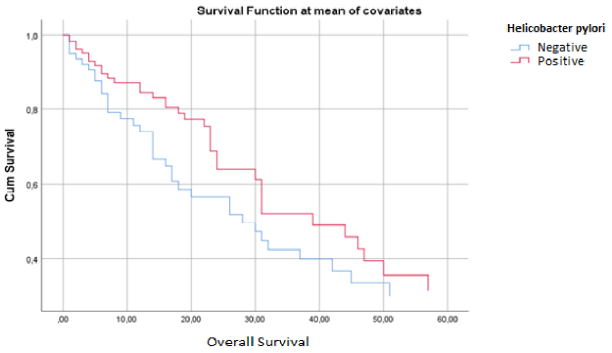

Figure 3. — Overall survival rates of patients in *H. pylori* negative and positive groups.

cancer incidence between high-risk and low-risk regions (16) create confusion in determining prognostic factors. Although *H. pylori*, which is considered to be among the risks of stomach cancer, has variable prevalence in different regions in the world, it infects more than half of the human population. Therefore, determining its efficacy as a prognostic focator may contribute to the follow-up and treatment of stomach cancer. In our study, it was observed that clinical and histopathological features, immunophenotypic markers, and biochemical markers affect disease-free and overall survival in gastric cancer, but the presence of *H. pylori* did not affect these prognostic factors.

Our multivariate and univariate analyses revealed that advanced age, large tumor size, advanced TNM stage, lymphovascular and perineural invasion, and lymphovascular capsule invasion were indicators of poor prognosis in patients that underwent R0 resection for gastric cancer. However, when the relationship of these factors with survival were compared between the *H. pylori* negative and positive groups, there was no significant difference. In their prospective study of 166 cases in Germany, which is not in the high risk area for gastric cancer, Meimarakis et al. reported that age, tumor stage, tumor depth, and lymph node involvement were prognostic factors according to the results of univariate and multivariate analyses (5). However, these prognostic factors were not significantly different between the *H. pylori* positive and negative groups. These findings are in line with the results of our study. Unlike our study, in Meimarakis et al.'s study, *H. pylori* positivity was detected by three different tests (histological, serological, bacterial culture) and *H. pylori* negativity was found to be an independent factor indicating poor prognosis in the multivariate analysis (5). In a retrospective study conducted by Marelli et al. in Italy, which is a region with low incidence of gastric cancer, *H. pylori* positive status was detected by serological and polymerase chain reaction (PCR) tests. They reported that tumor invasion depth, serosa involvement, and lymph node involvement were higher in the *H. pylori* negative group compared to the *H. pylori* positive group (6). In both aforementioned studies, the 5-year disease-free and overall survival was shorter in *H. pylori* negative group than in the positive group. They commented that the reason for the good prognosis in the *H. pylori* positive group may be due to the antitumoral effect of the antibody developed against *H. pylori*.

In a retrospective study conducted in Taiwan, which is the low-risk area for gastric cancer, *H. pylori* negativity was reported as a predictive factor for poor prognosis (7). They found that in *H. pylori* negative cases, the tumor was more aggressive than in positive cases and overall survival was shorter (7). Age, tumor depth and lymph node involvement were other prognostic factors (7).

In our study conducted in Turkey, which is not located in the geographical risk zone for gastric cancer, although the presence of *H. pylori* did not show significant prognostic capability, minimal survival advantage was observed in positive cases. In addition, the fact that the histopathological features that adversely affect the prognosis in our study were not affected by the presence of *H. pylori* suggests that *H. pylori* positivity may not be an indicator of poor prognosis.

Qiu et al. conducted a study of 156 cases in China, a high-risk area for gastric cancer, and interestingly, disease-free survival was found to be longer in the group with a high number of *H. pylori* DNA copies (by PCR method) (17). This result supports the thesis that the presence of *H. pylori* is not an indicator of poor prognosis in cases with developed cancers.

In a study conducted in Japan, a high-risk area for gastric cancer, 96 *H. pylori* positive cases that underwent endoscopic submucosal dissection due to early gastric cancer were compared with 96 cases with previously eradicated *H. pylori* (18). The histopathological evaluation showed that the submucosal and lymphatic invasion was higher in the *H. pylori* eradicated group compared to the *H. pylori* positive group. The results of these studies suggest that the presence of H pylori in gastric cancers may slow down the aggressive behavior of the tumor. It even suggests that *H. pylori* eradication may weaken this positive effect.

In a retrospective study of 162 cases conducted by Li et al. in China, it was reported that *H. pylori* positivity was an independent predictive factor for poor prognosis

and that the survival time was significantly shorter in *H. pylori* positive patients compared negatives (8). The fact that in Li et al's study the presence of *H. pylori* showed an opposite effect compared to other studies, suggest that different factors may play a role in the prognostic effect of *H. pylori*. In their study, as in other studies, tumor size, advanced age, and advanced TNM stage were found to be other predictive factors of poor prognosis.

Genetic mutations have led to increased expression of HER-2 / neu, which is involved in normal cell proliferation, and this increase leads to acceleration of aggressiveness and spread of the tumor and resistance to conventional chemotherapy treatment (8,9). However, there is no consensus yet on the therapeutic and prognostic value of HER2 / neu (13).

In our study, the positivity rate in HER2 / neu amplification was 11.8%. There was no correlation between HER2 / neu positivity rate and survival. We think this may be because of our study's low HER2 / neu positivity rate.

In a study of 78 cases examining the relationship between HER2 / neu and *H. pylori*, HER2 / neu positivity rate among patients infected with *H. pylori* was very low (19). Shim et al. reported higher HER2 / neu expression in *H. pylori* positive group than in negative group. However, its relationship with tumor invasion depth and lymph node metastasis was not observed (20). In our study, there was no significant difference between *H. pylori* negative and positive groups in terms of HER2 / neu amplification.

When the p53 tumor suppressor gene is mutated, it loses the ability to repair DNA damage and eliminate oncogenic mutations in normal cells, which is the main step in the aggressive behavior of the tumor (21). Although studies have reported that p53 protein expression increases in precancerous (intestinal metaplasia, gastric dysplasia) and cancerous lesions of the stomach, its predictive prognostic value is not yet clear (21,22).

In the recent study by Grosser et al., aberrant p53 (more than 60% staining / no staining) was detected in 50.2% of 562 gastric cancer patients that did not receive neoadjuvant chemotherapy, and aberrant p53 was reported to be an independent predictive factor for poor prognosis and chemoresistance (22). In our study, 31.8% of patients had more than 50% p53 protein expression (diffuse p53) immunophenotypically. Patients with diffuse p53 protein expression had significantly shorter disease-free and overall survival than those with focal expression. However, there was no significant difference in diffuse p53 expression between *H. pylori* negative and positive groups. In other studies where p53 protein expression was not differentiated between focal and diffuse, a strong correlation was found between *H. pylori* and p53 (23).

Ki-67 is a nuclear protein that shows the rate of cell proliferation. It is present in all stages of the cell cycle except the resting phase (24). Ki-67 has a role in tumor development by affecting the p53 signaling pathway. Although its prognostic value in gastric cancer is controversial, recent reviews and meta-analyzes have reported that Ki-67 is a predictive factor for poor prognosis (25). In our study, the relationship between Ki-67 proliferation index and prognosis was not determined. Moreover, its prognostic effect was not different between *H. pylori* positive and negative groups.

The fact that the prognostic effects of HER2 / neu gene amplification, p53 protein expression (due to genetic mutations), and Ki 67 proliferation index were similar in *H. pylori* negative and positive groups in our study suggests that *H. pylori* may not have an effective role on these genetic changes.

Some interleukins (eg IL-6) released from neutrophils, which are one of the main components of inflammation, increase the capacity of tumor progression and metastasis. Lymphocytes have an antitumor effect by suppressing cytokines. Although the mechanism of thrombocytes' involvement is not yet known, they have been found to increase tumor proliferation and metastasis rates. Numerous studies have reported that NLR and PLR ratios have prognostic value in gastric tumors (26,27).

In their study including 3012 cases Hu et al. reported that increased NLR rate in early and advanced gastric cancer is an independent predictive factor for poor prognosis (28). Kosuga et al. evaluated 429 cases and stated that the NLR rate was superior to tomography in determining the extent of lymph node involvement in advanced gastric cancer with tumor resection (29). In the same study, 5-year survival was found to be significantly lower in those with NLR $\geq$1.6 compared to those with NLR <1.6.

In a study in which 730 gastrectomies were performed due to gastric cancer, NLR was specified as an independent prognostic factor and tumor invasion, lymph node metastasis number, and peritoneal metastasis rate increased, while 5-year overall survival decreased in patients with NLR $\geq$ 4 compared to those with NLR of <4 (30) . In our study, NLR values above the cut-off of 2.86 predicted poor prognosis and decreased disease-free and overall survival. Multivariate analyses also found NLR as an independent factor indicating poor prognosis.

Studies have reported that increased PLR was associated with increased rate of tumor proliferation and metastasis and shortened disease-free and overall survival time (31). Wang et al. evaluated 466 gastric cancer cases and reported that high NLR and PLR were predictive factors for poor prognosis in the advanced stage (28). A meta-analysis showed a relationship between the PLR rate and lymph node involvement, serosal invasion, and advanced TNM stage. It was observed that the disease-free and overall survival time was shorter in those with high PLR compared to those with low (31). In our study, it was observed that the overall survival time in cases with PLR values above the cut-off value (14.53) was shorter than those with PLR below the cut-off value.

H. pylori infection begins with acute inflammation involving neutrophils and T lymphocytes and continues

with a chronic inflammatory process (32). In our study, *H. pylori* positivity did not correlate with NLR and PLR ratio. These findings suggest that *H. pylori*, which plays a role in acute and chronic inflammatory processes in the gastric mucosa, is not related to markers that are effective in tumor prognosis.

The retrospective design, small number of patients, and the use of a single test in the detection of *H. pylori* can be considered as limitations of our study.

Conclusion

Age, advanced stage, tumor diameter, lymphovascular and perineural invasion, lymph node capsule invasion, diffuse p53 protein expression, high NLR and PLR were found to be effective in gastric cancer prognosis. However, there was no relationship between these prognostic factors and *H. pylori*.

Data availability

The data sets in the current study are available from the corresponding author on reasonable request.

Conflict of interest

All authors declare that they have no conflict of interest related to study.

Financial support

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Informed consent

All patients provided written, informed consent for the iranonymized data to be used for study purposes.

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