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Original article

The impact of clinicopathological parameters and the expression of HER-2 proteins on the survival of gastric cancer patients

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Abstract

Objective: Many studies offer controversial data related to factors that influence prognosis in gastric cancer. The aim of study was to identify the associated impact of clinicopathological parameters and expression of HER-2 protein on survival in gastric cancer.

Methods: Tissue samples of 50 patients, with Billroth II surgery resection, were selected for this pathological condition at the Department of Pathology, School of Medicine, University of Sarajevo. Clinical and morphological data, for each case, were classified and interpreted according to the evaluation protocol recommended by the American Joint Committee of Cancer (AJCC) and International Union against Cancer (IUCC). HER-2 protein expression was assessed by immunohistochemical staining.

Results: There was significant difference in survival distributions for the grade 1 vs 2, $\chi^2(1)=8.852$, $p=0.003$, grade 1 vs 3, $\chi^2(1)=7.131$, $p=0.008$, and grade 1 vs 4, $\chi^2(1)=8.284$, $p=0.004$. The survival distributions for the pT stages were significantly different, $\chi^2(3)=8.744$, $p<0.05$, especially for the pT2 vs. pT3 stage, $\chi^2(1)=6.595$, $p=0.010$. The survival distributions for pN stages were statistically significantly different, $\chi^2(3)=11.468$, $p<0.01$, especially for the pN0 vs. pN3 stage $\chi^2(1)=7.449$, $p=0.006$. There was no statistically significant difference in survival between patients with HER-2 positive and HER-2 negative tumors ($p>0.05$).

Conclusion: Results of our study confirmed the significance of standard clinicopathological parameters as prognostic factors in term of overall survival. HER-2 expression was not found as significant prognostic factor of survival.

Keywords: gastric cancer, clinicopathological parameteres, HER-2, survival

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Introduction

Gastric cancer remains one of the most common causes of cancer-related mortality worldwide (1).

Despite therapy improvement and increased detection of rate in early stages of disease, gastric cancer is dominantly found in advanced stage with low survival rate (2).

The most frequently used histological system of classification for gastric cancer is the Lauren's classification which defines two main histological subtypes- intestinal and diffuse type. Both of these types represent distinct clinical and epidemiologic characteristics and could have targetable protein expressions such has human epidermal growth factor receptor-2 (HER-2) expression (3). HER-2 protein is a member of the epidermal growth factor receptor family whose enhanced expression on the tumor cell membrane and could influence cell proliferation, differentiation, adhesion, migration and apoptosis (4).

HER-2 overexpression can be found in a variety of solid organ malignancies including breast cancer, colorectal cancer, lung cancer, ovarian cancer and gastric cancer (2).

Overexpression of HER-2 protein in gastric cancer was detected in 1986 by Sakai and coleagues (5) and since that period, many studies made an effort to elucidate the importance of its positivity in gastric cancer (6,7,8,9). HER-2 protein overexpression varies from 4-44% of primary gastric cancers, probably reflecting different methods of immunohistochemical assesment, ethnical and geographical differences, as well as sample sizes (9).

Unlike breast cancer, where the HER-2 positivity is established as a poor prognostic factor, HER-2 positivity in gastric cancer, its association with clinicopathological features and impact on survival is still controversial, although numerous studies showed evidence of unfavorable role of HER-2 in gastric cancer (4).

Recent studies have devoted special attention to investigation the role of molecular targeted agents, as ToGA study, which demonstrated the efficacy of combining trastuzumab with chemotherapy (10).

We aimed to identify association of clinicopathological factors and prognosis of gastric cancer, as well as the impact of HER-2 protein expression on survival of patients suffering from primary invasive gastric carcinoma.

Material and methods

Tissue samples of 50 patients with Billroth II surgery resection were selected for this pathological condition at the Department of Pathology, Medical Faculty University of Sarajevo. They were fixed in 10% buffered neutral formalin at room temperature and embedded in paraffin blocks, microtomically sectioned at 3-5 μm, put on the glass slides and stained by hematoxylin-eosin (standard tissue histochemical staining method). Tissue samples were treated with HER2 antibody too, following the manufacturer's protocol (HercepTestTM Kit, DakoCytomation, Glostrup, Denmark) and visualized by EnVision (DakoCytomation, Glostrup, Denmark) following the manufacturer's instructions.

For all cancer samples was determined Goseki and Borrmann grade. Goseki histologic grade in gastric cancer refers as degree of differentiation tubular structures and mucin production. Gastric adenocarcinoma with well-differentiated tubular structures and poor mucin production is defined as grade I. Grade II tumors show well-differentiated tubular structures and rich mucin production. Gastric cancers with poor-differentiated tubular structures and poor mucin production are defined as grade III, while tumors with poor-differentiated tubular structures and rich mucin production are defined as Goseki grade IV (11).

Based on Borrmann's classification, the gross appearance of advanced gastric cancers could be divided into type I or polypoid growth, type II or fungating growth, type III or ulcerating growth, and type IV or diffusely infiltrating growth which is also referred to as „linitis plastica" or „signet ring cell" cancer, where the most of gastric wall is involved by infiltrating tumor cells (12).

Survival time was calculated from the date of surgery until the time of patient's death. Clinical and morphological (macroscopic and microscopic) data for each case were classified and interpreted according to the evaluation protocol recommended by the American Joint Committee of Cancer (AJCC) (13) and International Union against Cancer (IUCC) (14).

Results are expressed as median and interquartile range (IQR) in case of non-normal distributed continue variables. The inspection of histograms and quantilediagrams and the Kolmogor–Smirn's test with a Lilliefors' significance level were used for testing normality of distribution of continuous numerical variables. In case of categorical variables, counts and percentages were reported. A log rank test was conducted to determine if there were differences in the survival distributions according to socio-demographic and histo-pathological characteristics. Pairwise log rank comparisons were conducted to determine who had different survival distributions. A Breslow's test (Generalized Wilcoxon) was conducted to determine if there were differences in the survival distributions according to Borrmann's classification of gastric cancer. A Bonferroni's correction was made for: tumor grades, pT and pN stages with statistical significance accepted at the $p< 0.0125$ level and cancer types by Borrmann's with statistical significance accepted at the $p< 0.0167$ level. A p-value <0.05 was considered significant. Statistical analysis was performed by Statistical Package for the Social Sciences (SPSS Release 19.0; SPSS Inc., Chicago, Illinois, United States of America) software.

Results

Among the 50 gastric cancer patients, there were 34 (68.0%) males and 16 (32.0%) females. The median age was 66.5 y (57.5 to 70.0). The characteristics of the gastric cancer patients according to a median survival time are reported in Table 1. and Table 2.

Patients with tumor grade 1 had a median survival time of 2.0 (95% CI, 0.0 to 4.3) years. That was longer than other tumor grades (Table 1). The survival distributions for tumor grades were statistically significantly different, $\chi^2(3) = 14.743$, $p< 0.01$. There was a statistically significant difference in survival distributions for the grade 1 vs 2, $\chi^2(1) = 8.852$, $p = 0.003$, grade 1 vs 3, $\chi^2(1) = 7.131$, $p = 0.008$, and grade 1 vs 4, $\chi^2(1) = 8.284$, $p = 0.004$. However, the survival distributions for other tumor grades were not statistically significantly different (Fig.1). Regarding the tumor size, low-pT stages were associated with longer median survival time (Table 1). The survival distributions for pT stages were statistically significantly different, $\chi^2(3) = 8.744$, $p< 0.05$. There was a statistically significant difference in survival distributions for the pT2 vs. pT3 stage, $\chi^2(1) = 6.595$, $p = 0.010$. However, the survival distributions for other pT stages were not statistically significantly different (Figure 1).

Concerning the nodal status, it was shown that node-negative tumors were associated with longer median survival time (Table 1). The survival distributions for pN stages were statistically significantly different, $\chi^2(3) = 11.468$, $p< 0.01$. There was a statistically significant difference in survival distributions for the pN0

Table 1. Clinicopathological characteristics of 50 patients with invasive gastric cancer according to a median survival time.

Variables		Frequency	Median				p – value
			Estimate	Std.Error	95% Confidence Interval		
					Lower Bound	Upper Bound	
Sex	Male	34 (68%)	1.0	0.2	0.6	1.4	0.915
	Female	16 (32%)	1.0	0.7	0.0	2.3	
Age (y)	‹65	21 (42%)	1.0	0.5	0.1	1.9	0.387
	≥65	29 (58%)	1.0	0.2	0.6	1.4	
Localization	Cardia	5 (10%)	1.0	0.4	0.1	1.9	0.622
	Fundus	4 (8%)	0.0	.	.	.	
	Corpus	9 (18%)	1.0	0.7	0.0	2.4	
	Antrum	20 (40%)	1.0	0.4	0.3	1.7	
	Pylorus	12 (24%)	1.0	0.3	0.3	1.7	
Grade	1	12 (24%)	2.0	1.2	0.0	4.3	0.002
	2	15 (30%)	1.0	0.3	0.4	1.6	
	3	14 (28%)	1.0	0.4	0.3	1.7	
	4	9 (18%)	0.0	.	.	.	
pT	1	2 (4%)	2.0	.	.	.	0.033
	2	16 (32%)	2.0	0.2	1.5	2.5	
	3	26(52%)	1.0	0.2	0.6	1.4	
	4	6 (12%)	1.0	0.2	0.6	1.4	
pN	0	9 (18%)	2.0	0.5	1.1	2.9	0.009
	1	23 (46%)	1.0	0.3	0.4	1.6	
	2	10 (20%)	1.0	0.4	0.2	1.8	
	3	8 (16%)	0.0	.	.	.	

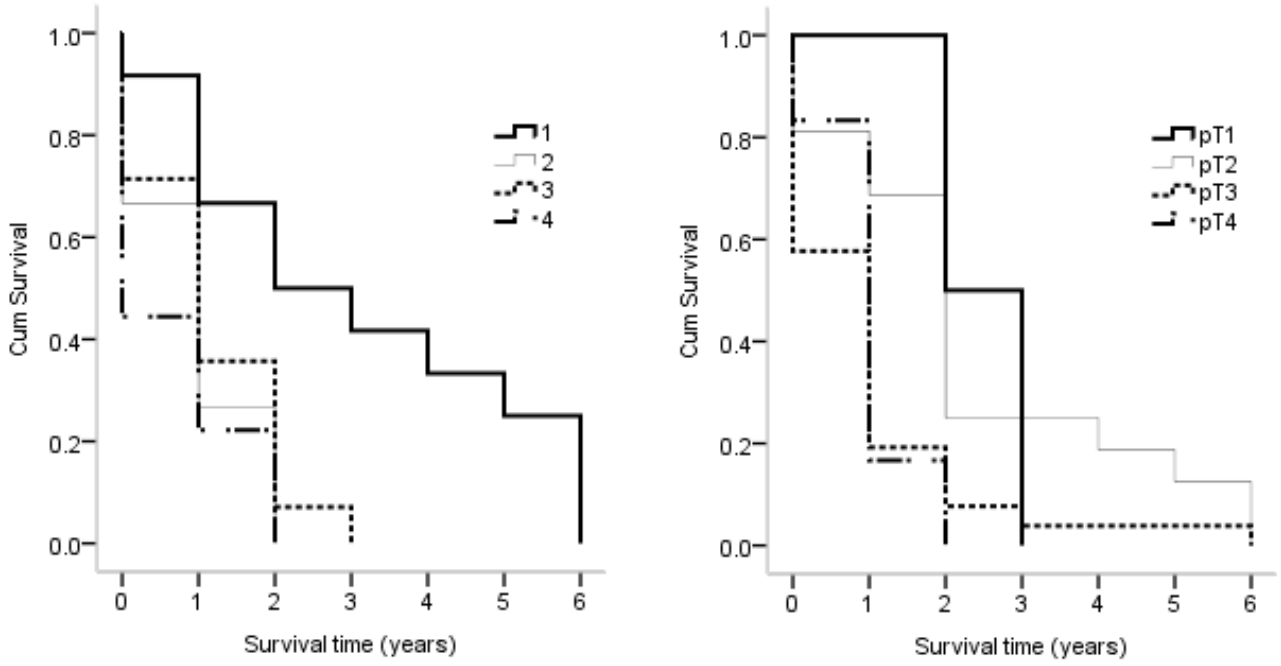

Figure 1. Survival time in years according to grade (left) and tumor size (right)

vs. pN3 stage $\chi^2(1) = 7.449$, $p = 0.006$. However, the survival distributions for the other pN stages were not statistically significantly different (Fig. 2).

Patients with Borrmann's cancer type III had a median

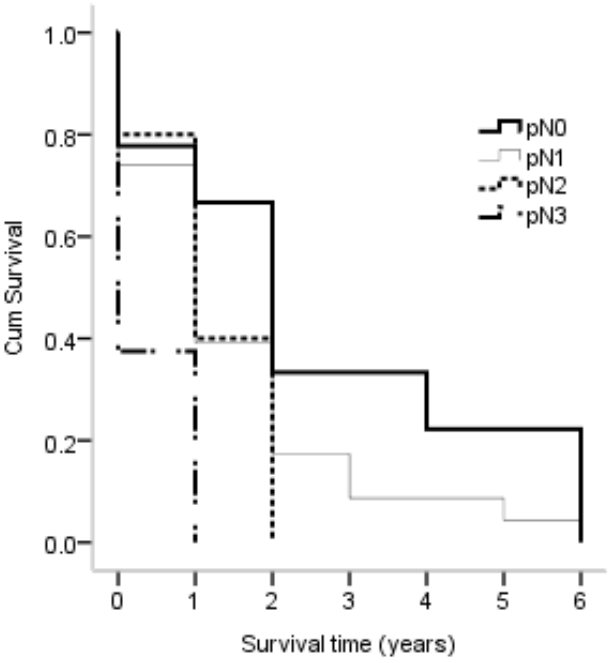

Figure 2. Survival time in years according to status of regional lymph nodes (pN).

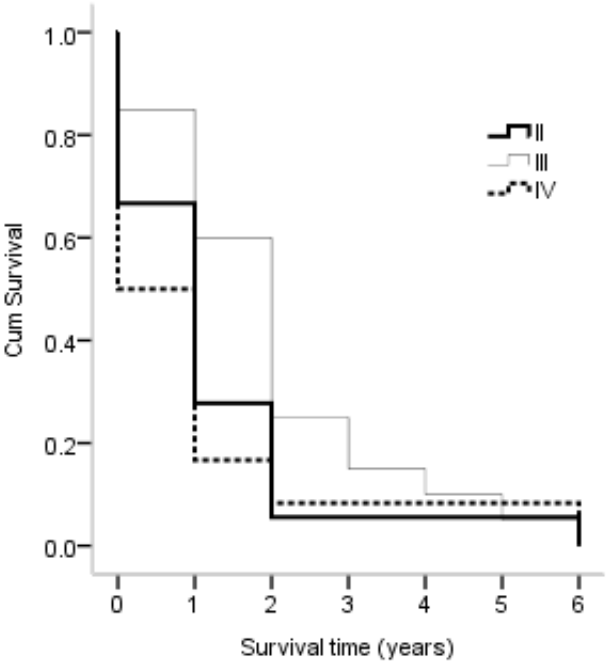

Figure 3. Survival time in years according to Borrmann gastric cancer type.

survival time of 2.0 (95% CI, 1.5 to 2.5) years. That was longer than other macroscopic types of gastric cancer by Bormann's (Table 2).

The survival distributions for the cancer types by Bormann's were statistically significantly different, $\chi^2(3) = 7.226$, $p< 0.05$. There was a statistically significant difference in survival distributions for the type III vs IV, $\chi^2(1)=5.880$, $p=0.015$. However, the survival distributions for the other types by Borrmann's were not statistically significantly different (Figure 3).

Discussion

The purpose of this study was to identify the clinicopathological factors which could be associated with prognosis and survival of patients with gastric cancer. The analysis of the potential prognostic factors included parameters related to the patient (sex, age), as well

Table 2.Median survival time (y) according to characteristics of invasive gastric cancer.

Variables		Frequency	Median				p - value
			Estimate	Std.Error	95% Confidence Interval		
					Lower Bound	Upper Bound	
Borrmann type	II	18 (36%)	1.0	0.3	0.5	1.5	0.027*
	III	20 (40%)	2.0	0.3	1.5	2.5	
	IV	12 (24%)	0.0	.	.	.	
Lauren classification	intestinal	26 (24%)	1.0	0.2	0.5	1.5	0.331
	Mixed	12 (24%)	1.0	0.4	0.2	1.8	
	Diffuse	12 (24%)	0.0	.	.	.	
Goseki gradus	I	23 (46%)	1.0	0.4	0.2	1.8	
	II	2 (4%)	1.0	.	.	.	
	III	18 (36%)	1.0	0.3	0.3	1.7	
	IV	7(14%)	0.0	.	.	.	
LVI	Negative	28 (56%)	1.0	0.2	0.6	1.4	0.073
	Positive	22 (44%)	2.0	0.3	1.5	2.5	
HER2	Negative	34 (68%)	1.0	0.3	0.5	1.5	0.941
	Positive	16 (32%)	1.0	0.3	0.4	1.6	

*Breslow test

as parameters related to the tumor (histological type according to the WHO classification, degree of tumor differentiation, tumor location, size and involvement of regional lymph nodes according to the TNM classification of AJCC/UICC) and immunohistochemical HER-2 expression.

In study, significant difference in overall survival was found in regard of tumor grade, e.i. patients with gastric cancer grade 1 had the longest median survival of 2.0 (95% CI 0.0 to 0.43) and patients with grade 4 had the shortest ($p<0.05$).

Regarding the tumor size, low-pT stages were associated with longer median survival time ($p<0.05$).

Concerning the nodal status, it was shown that node-negative tumors were associated with longer median survival time. Patients with pN0 tumors had median survival of 2.0 (95% CI 1.1 to 2.9) years, and shows statistically significant difference in survival regarding to patients with pN3 tumors ($p<0.05$). However, the survival distributions for other pN stages were not statistically significantly different.

Sex, age, tumor localization, histological type of gastric cancer (Lauren's classification) and Goseki grade did not show significant difference in median survival ($p>0.05$).

In study there was a statistically significant difference in survival distributions for the Borrmann's type III vs IV cancers. Patients with ulcerating growth of gastric cancer had shorter survival than other cancer types. Our results were similar with Li et al. (15), who showed that gender, Borrmann type and depth of invasion were all associated with the status of nodal involvement. Also, they found statistically significant differences in overall survival among patients with Borrmann type I and II tumors, Borrmann type III tumors, and Borrmann type IV tumors according to depth of invasion (pT) and nodal involvement (pN), except in pN3 tumors. Borrmann type is an independent prognostic factor for the patients with advanced gastric cancer (15).

In this study, we investigated the incidence of HER-2 positive expression in gastric cancers and its possible of impact on survival. The incidence of HER-2 positive tumors is different among studies. Differences could be caused by geographic localisation, tumor heterogenity, differences in scoring systems and pathologist expertise. In this study, precentage of HER-2 positive gastric cancers was 32% which is consistent with the literature, like the study of Yu et al. (16) who determined HER-2 expression rate of 28% by using immunohistochemistry and Western blot method, although this study included higher number of patients.

In our study from 2013.y. we found, that there was only a small subgrupe of intestinal type of gastric cancers, which potentially could respond to HER2 targeted therapy. The overexpression of HER2 protein was observed in 13,36% of intestinal-type of cancers (17).

Median survival time for patients with HER-2 negative and positive tumors was the same (1 year). We found no significant differences in overall survival between patients with HER-2 positive and HER-2 negative gastric cancers ($p>0.05$), which is consistent with results of Uprak et al. (9), Lee et al. (18) and Grabsch et al. (7). In two independent series, they showed, that HER-2 expression is not related to prognosis of patients with gastric cancer.

Kim et al., found no significant differences between the two groups in terms of overall survival while a significant difference was detected in disease-free survival time (8).

On the contrary, there are many studies that have found correlation between HER-2 expression and survival in gastric cancer patients, such as a study of Vizoso et al, who found high levels of c-erbB-2 significantly associated with shorter relapse-free survival time and overall survival in patients with resectable gastric cancer (19). Study of Park et al (6) showed similar results, e.i. patients with HER-2 positive gastric cancers had lower 5-year survival rate and median overall survival rate in regard to HER-2 negative patients.

Conclusion:

Results of this study confirmed the significance of standard clinicopathological parameters (tumor grade, size and involvement of lymph nodes) as prognostic factors in terms of overall survival.

HER-2 protein overexpression was not found as significant prognostic factor for survival, e.i. we found no statistically significant difference in survival between HER-2 positive and HER-2 negative gastric cancer patients.

Limitations of our study were small sample size, tumor heterogeneity, influence of geographic and ethnic differences, as well as the fact that data on survival time were presented in years. Further prospective studies, with larger sample size and usage of more sofisticated methods, will be necessary to determine the impact of HER-2 expression on survival of patients with invasive gastric cancer.

Declaration of interest

Authors declare no conflict of interest.

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