

Prognostic and predictive role of HER2 expression in metastatic gastric cancer in Turkish patients

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Abstract: Background: In this study, we investigate the role of HER2 expression in prediction of the response to treatment combination including docetaxel, cisplatin and 5-FU which is used in metastatic gastric cancer in Turkish patients. Methods: Patients who were histopathologically diagnosed with metastatic gastric cancer at the Antalya Training and Research Hospital, Clinic of Medical Oncology from 2008 to 2010 were included in this study. Results: IHC assessment identified 4 patients (25%) 0 / negative, 5 patients (31.3%) 1+ / negative, 4 patients (25%) 2+ / suspected, and 3 patients (18.8%) 3+ / positive. The relationship between HER2 expression and the response using chi-square test was not statistically significant. Conclusions: We believe that identification of patients who will respond to chemotherapy combination is important to prevent complications. The important shortcomings in our study include covering a small number of patients and the use of FISH method. Therefore, we believe that further studies using FISH method with more patients are required to investigate the role of HER2 expression in predicting the response to DCF chemotherapy combination.

Keywords: Gastric cancer, Prognosis, HER 2, Chemotherapy, Docetaxel, Cisplatin

1. Introduction

HER2 is a member of the epidermal growth factor receptor, which is synthesized by *cerb-B2*, a proto-oncogene. Other members of the epidermal growth factor receptors are HER1, HER3 and HER4. HER2 is a transmembrane protein of 185 kD of weight, which has tyrosine kinase activity. When HER2 receptors are activated, they enable the intracellular signal transmission routes and proceed in the differentiation and proliferation pathways of the cell. HER2 gene amplification and overexpression have been identified in many types of cancer, and was primarily studied in breast cancer [1,2]. Positive HER2 has been identified in 15-25% of all cases of breast cancer [3,4]. In gastric cancer, HER2 positivity has an extensive variation in literature. Its positivity varies from 6.8 to 34% by immunohistochemical (IHC) method

and from 7.1 to 42.6% by Fluorescent in situ hybridization method (FISH) [5].

It is known that HER2 expression is a worse prognostic factor in gastric cancer, similar to the breast cancer [6,7]. Metastatic gastric cancer rapidly progresses after identification of the disease. Survival without chemotherapy is 3-5 months, whereas it can be extended to 8-12 months with combined chemotherapy [8]. Chemotherapy combination of docetaxel, cisplatin and 5-FU is frequently used in the metastatic period, and has severe adverse effects. Grade 3-4 toxicity was identified in 75-81% of the patients. During the term of the treatment, mortality rate in 60 days due to any reason varies from 7 to 9% [9]. Prediction of the response to this treatment combination will help to protect some patients from complications of the treatment and exitus of some others due to treatment-related reasons. Some clinical and

laboratory parameters identified during diagnosis may help to predict the prognosis of the disease and the response ratios to the planned treatment. Laboratory parameters can also be used in determining the intensity for treatments to be given to the patients. In this study, we investigated the prognostic role of HER2 expression in metastatic gastric cancer in Turkish patients and its role in predicting the response to DCF chemotherapy involving 24-hour-long constant infusion of docetaxel, cisplatin and 5-FU used in treatment.

2. Material and Methods

2.1. Patient Group

The study covered patients with metastatic gastric cancer, with performance status of ECOG 0-2, who have an objectively measurable disease, have sufficient reserve of bone marrow, and have normal hepatic and renal functions, as histopathologically diagnosed at the Antalya Training and Research Hospital, Clinic of Medical Oncology from 2008 to 2010. Patient files were retrospectively reviewed to gather data on the phase of the disease and treatments received. A chemotherapy protocol consisting of 24-hour constant infusion of docetaxel 75 mg/m² D1, cisplatin 75 mg/m² D1 and 5-FU 1000 mg/m² D1-5 was applied to the patients for at least one cycle. The patients who had an ECOG score of 3 and above were excluded from the study, as well as the patients whose treatments were started at other centers and continued at our clinic.

2.2. Immunohistochemical Investigation

All samples were obtained from tissues that were fixated with formalin and buried in paraffin. Paraffin blocks were cut at a thickness of 4 µm and were stained with hematoxylin and eosin for assessment. Following xylene deparaffinization of tissues, which were fixed in formalin in immunohistochemical staining and buried in paraffin, they were gradually rehydrated with ethanol and were processed for HER2.

2.3. Immunohistochemical Rating

Expression ratios of positive tumor cells in the samples were evaluated by a pathologist who was not familiar with the clinical properties of the patients. HER2 expressions were evaluated according to membrane staining of cells. The rating system suggested by Hofmann et al. was employed for rating [10]. (Table 1). 2+ cases, which were considered suspicious, were accepted as negative since they could not be processed with FISH.

2.4. Statistical Analysis

Statistical analysis was conducted on SPSS 13.0 software program. The relationship between HER2 positive and negative groups was evaluated by using the chi-square test. The relationship of each of the immunohistochemical

positive and negative results with survival was investigated using the Kaplan Meier survival analysis. Statistical variations were justified with the Log-rank test. The significant p value was considered as <0.05.

Table 1. Scoring HER 2 expression

Reactivity Properties	Score / Classification
Membranous reactivity or no reactivity in < 10% of cells	0 / negative
Pale/hardly perceptible membranous activity in > 10% of cells; reactivity only in part of the membrane in cells	1+ / negative
Weak or moderate complete or basolateral membrane activity in > 10% of tumor cells	2+ / suspected
Moderate or strong complete or basolateral staining in > 10% of tumor cells	3+ / positive
Biopsy samples with cohesive IHC 3+ and/or FISH + clones (not surgical material) are considered positive regardless of size; e.g.: < 10%	

3. Results

The study included a total of 16 patients, including 15 males (93.8%) and 1 female (6.3%). The mean age of the patients was identified as 61.3 (Table 2). The most common complaint for attending patients was stomach ache with 25%, followed by weight loss and loss of appetite. Metastatic disease was diagnosed in 30% of the patients using biopsy and imaging methods, and other patients were diagnosed after surgical exploration. The most common site of metastasis was the liver with 31.3%. Concurrent metastasis of the liver and peritoneum was identified in 18.8% of the patients. The other metastatic sites were the bone and peritone.

Table 2. General characteristics of patients

	Average, Standard Deviation	Median
Age	61,3±10,1	66
AST (U/L)	19.8±5,6	18
ALT (U/L)	19.1±10.1	19
ALP (U/L)	102.9±28.1	93
LDH (U/L)	241.4±94.5	196
WBC (10 ³ /mm ³)	5.6±4.1	5.9
HGB (g/dl)	11.2±1.4	10.9
PLT(10 ³ /mm ³)	347.3±157.1	346

ECOG score was 0 in 87.5% of the patients, and 1 in other patients. The patients underwent 4 cycles of chemotherapy on the average. One patient discontinued chemotherapy due to febrile neutropenia, and 2 patients went into exitus before completing the cycles of chemotherapy. Partial response was achieved in 37.5% of the patients. The median survival of the patients was found

as 13.1 months (Figure 1).

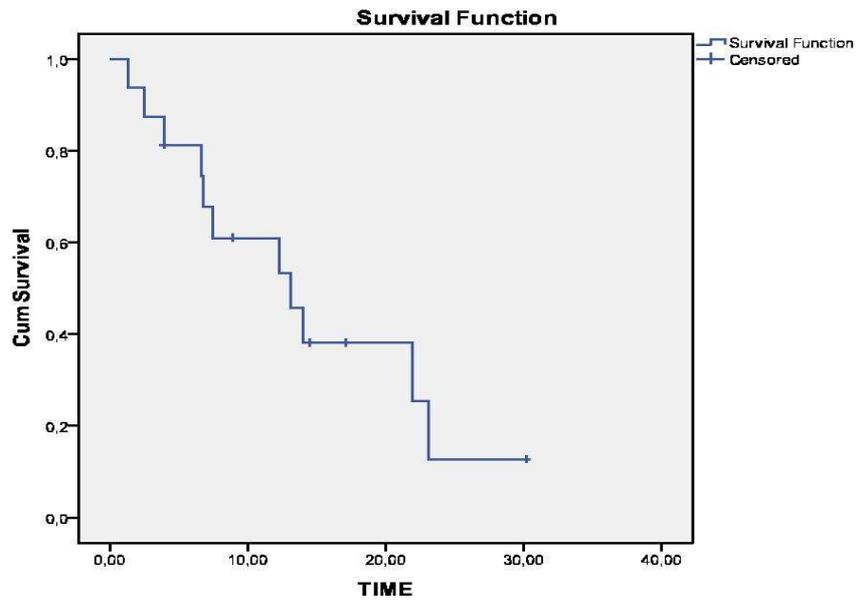


Figure 1. Kaplan –Meier Survival Chart

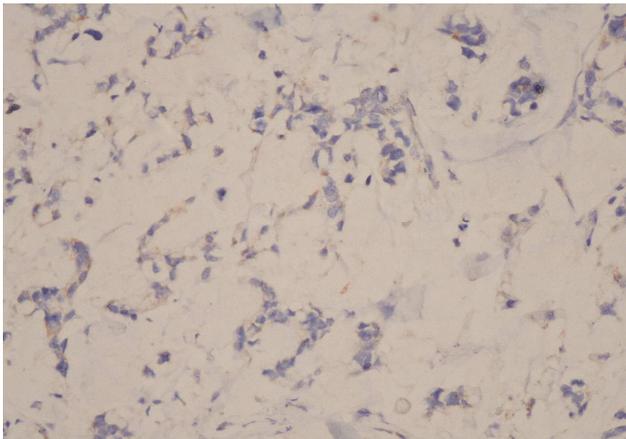


Figure 2. No staining (score 0), HER2 x200

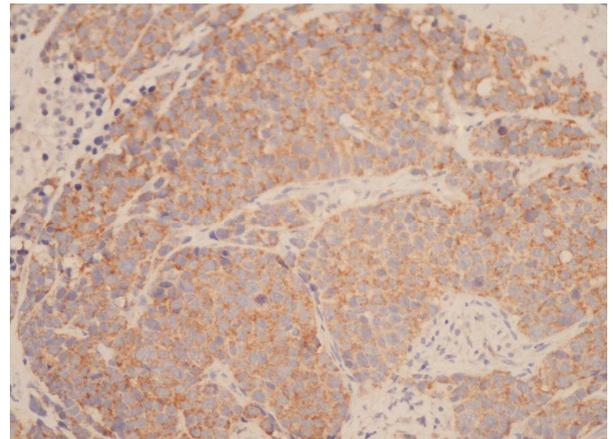


Figure 4. Complete membrane staining at least 10% of tumor cells, nonuniform and weak intensity (score 2+), HER2 x200

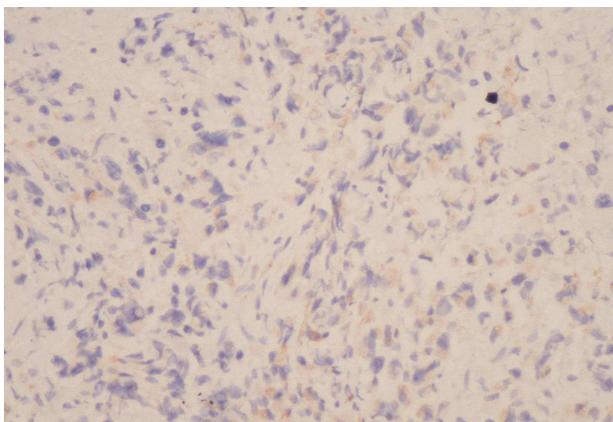


Figure 3. Weak and incomplete membrane staining of tumor cells (score 1+), HER2 x200

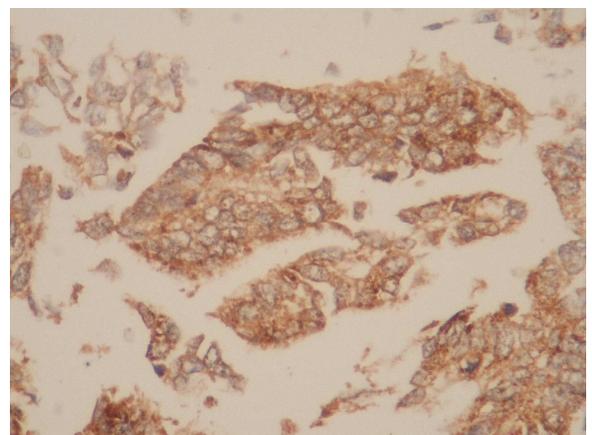


Figure 5. Uniform intense membrane staining of >30% of tumor cells (score 3+), HER2 x400

IHC was found as 0 / negative in 4 patients (25%), 1+ / negative in 5 patients (31.3%), 2 + / suspected in 4 patients (25%), and 3 + / positive in 3 patients (18.8%). (Figure 2-5) As suspected cases were considered as negative, in conclusion, 3 patients (18.8%) were considered as her2 positive, and 13 patients (81.3%) as negative. Evaluation of

the relationship between Her2 expression and the response by chi-square test did not identify a statistical significance, and the p value was found as 0.41. Evaluation of the relationship between Her2 expression and the survival time by long rank test did not yield a statistical significance as well (Figure 6).

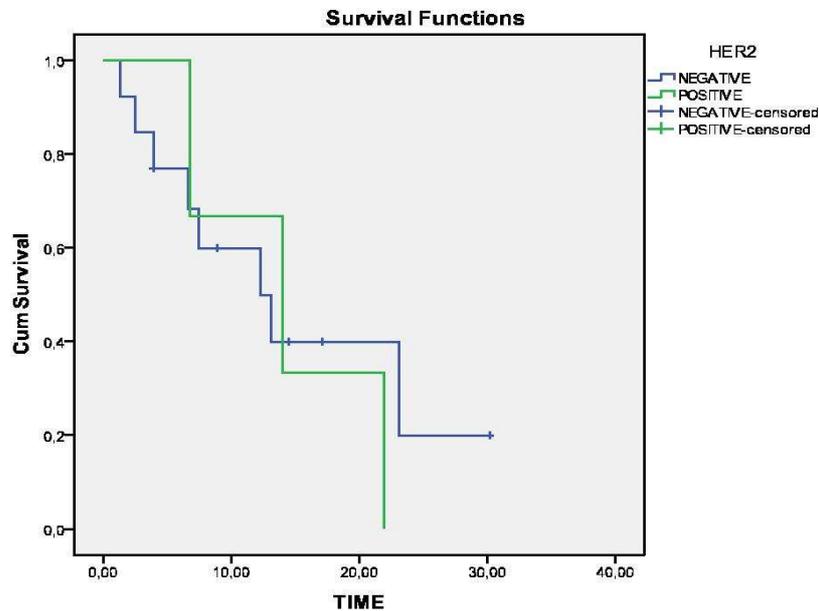


Figure 6. Relationship of Her 2 expression and survival time

4. Discussion

Despite early diagnostic and primary prevention efforts, gastric cancer is the second most common reason for exitus in the world [11]. Mucosal resection in the early stage and surgical resection in the local advanced stage are curative treatment methods. Despite adjuvant/neo-adjuvant chemotherapies or chemo-radiotherapies, many patients are lost due to recurrence. In the metastatic phase, the target of treatment is to extend the survival and quality of life. In treatments administered for this reason, it is important to determine which patient will be harmed by the treatment potentially.

In this study, we have identified the HER2 expression ratio in metastatic gastric cancer as 18.8% by IHC. Yano et al. found this ratio as 27.1% using FISH method on 200 Japanese patients who underwent surgery for gastric tumor [12]. Tanner et al. identified HER2 amplification in 12% of 131 cases of gastric adenocarcinoma using CISH method, and in 24% of 100 cases with gastroesophageal junction tumor [13].

Our study only used the IHC method and patients who were 2 positive were considered to have negative HER2 expression. It is known that some of the patients with breast cancer, who were 2 positive, had HER2 expression by FISH method. Therefore, HER2 expression ratio may differ from the values we have obtained. The important shortcomings in our study include covering a small number

of patients and the use of FISH method.

It is known that HER2 expression is an undesirable prognostic factor in breast cancer. Supportive findings were obtained in studies on gastric cancer. In a retrospective study where 108 cases were evaluated, HER2 overexpression was associated with adverse prognosis [14]. In another study, it was demonstrated that HER positive cases were associated with undesirable prognosis in 22 cases of early stage of gastric cancer with positive HER2 [15]. Nakajima et al. have demonstrated that HER2 positivity is the second most important prognostic factor in early stage gastric cancer, the first being nodal involvement [16].

HER2 overexpression was found to be correlated to lymph node metastasis, distant metastasis and high relapse. It was revealed that patients with HER2 overexpression had a significantly low survival with progression [17].

HER 2 pathway especially plays a role in the repair of DNA damage formed by platin analogues. A synergistic effect is obtained with the combination of treatments aiming Her 2 and chemotherapy [18]. In our study, we have determined that HER2 expression status does not constitute a statistically meaningful effect in terms of lifetime. Expressions of DNA repair proteins such as excision repair cross-complementing-1 (ERCC1) and breast cancer-1 (BRCA1) are bad prognostic factors for platin based chemotherapies [19]. However, BRCA1 expression demonstrates the docataxel sensitivity [20]. Trastuzumab is

a fully-humanized monoclonal antibody targeting the HER2 protein by directly binding to its extracellular domain. Majority of Trastuzumab's studies in combination with chemotherapy are combinations without platin based taxan [21].

Chemotherapeutical combination of docetaxel, cisplatin and 5-FU (DCF) is frequently used and effective in local advanced and metastatic gastric cancer. However, the combination has severe adverse effects (9). Two of our patients died during the cycles of chemotherapy. Yıldırım *et al* have demonstrated that in gastric cancer patients with Bcl-2 expression, the response to DCF chemotherapy was better [22].

We believe that identification of patients who will respond to this treatment may be important to avoid complications. Therefore, we believe that further studies using FISH method with more patients are required to investigate the role of HER2 expression in predicting the response to DCF chemotherapy combination.

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