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ORIGINAL ARTICLE

Association between high initial CEA, CA 19-9 levels and HER-2 status and their prognostic values on overall survival in metastatic gastric cancer

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Summary

Purpose: *This study aimed to investigate the correlations between baseline levels of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) and immunohistochemical (IHC), Human epidermal growth factor receptor (HER-2) expressions; and question their prognostic values in patients with metastatic gastric cancer.*

Methods: *Gastric cancer patients were retrospectively analyzed. Demographic information, clinical stages, immunohistochemical HER-2 expressions and serum CEA, serum CA 19-9 levels were evaluated at the time of diagnosis. The correlations between HER-2 IHC expressions and the initial marker levels were assessed, and survival analyses were performed.*

Results: *A total of 411 patients were included in the study. Median age of patients was 58 years (range: 22-90); males: 297 (72.3%); females: 114 (27.7%). Median overall survival (OS) was 24 months (range: 19-29). Patient HER-2 IHC expression 0, 1, 2, 3 ratios were 43, 22, 16, and 19%, respectively. At the time of diagnosis, the median value of CEA was 4 (range: 3-5), and the median value of CA 19-9 was 18 (range: 14-22). The increase in CEA and CA 19-9 levels were correlated with the increase of IHC levels (p=0.0001). OS of patients with high initial CEA levels (>5 ng/mL) were significantly shorter than those with low initial CEA levels (<5 ng/mL).*

Conclusion: *Significant positive correlations were shown between HER-2 IHC expressions and CEA, CA 19-9 levels. Baseline CEA, CA 19-9 levels predicted HER-2 positivity and this directly affected treatment and OS.*

Key words: *gastric cancer, CEA, CA 19-9, HER-2, IHC, survival*

Introduction

Gastric cancer still portends a dismal outcome despite innovations in the treatment. More than half of the patients present initially with metastasis [1].

Patients have been shown to have prolonged survival with effective combination therapies and anti-HER-2 treatments [2]. In the TOGA study, the addition of trastuzumab to chemotherapy in patients with HER-2 positive gastric and gastroesophageal junction tumors has been shown to significantly improve survival [3]. Apart from gastric cancer HER-2 expression has been proven to be predictive and prognostic in other types of cancer, especially in breast cancer. Overexpression of HER-2 is associated with shorter survival in gastric cancer, suggesting a potential survival benefit from anti-HER-2 treatments. HER-2 testing should be performed by validated IHC assay, and positive (3+) or negative (0 or 1+) HER-2 IHC results do not require further

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ISH/FISH testing. HER-2 positivity in gastric cancer ranges from 7 to 34% [4,5]. HER-2 IHC results showing 2+ (equivocal) expressions require further ISH/FISH methods [4,6] and cases with HER-2:CEP17 ratio ≥2 or an average HER-2 copy number ≥6.0 signals/cell are considered positive by ISH/FISH.

There is no specific data regarding use of markers, such as CEA and CA 19-9, in metastatic gastric cancer follow up. However, persistent elevation of CEA and CA 19-9 levels may predict poor prognosis. Additionally, high baseline marker levels at diagnosis may predict response to treatment [7]. CEA, an adhesion molecule of the immunoglobulin superfamily, is overexpressed in various malignancies, especially colon cancer. It consists of 29 genes, 18 of which are normally expressed in humans. CEA antigen-related cell adhesion molecules (CEACAM) 1-8, 16, 19, 20 and 21 are those that are most frequently encoded in humans. CEACAMs and HER-2 may coexist in the plasma membrane and show interaction. It was already concluded that this coexistence predicted response to trastuzumab treatment [8,9].

CA 19-9, a carbohydrate antigen, plays an important role in cancer cell adhesion. Both CEA and CA 19-9 are tumor markers for gastrointestinal malignancies, such as colorectal, gastric, biliary tract and pancreatic cancer [10], and since they are members of intercellular adhesion molecules, expression of these molecules may be associated with metastasis. CA 19-9, a specific ligand for ELAM-1, is expressed in activated endothelial cells and human cancer cells and associated with vascular invasion, metastasis and thus prognosis [11].

On the other hand, human epidermal growth factor receptor 2 (HER-2) belongs to the epidermal growth factor receptor (EGFR) family [12]. Ligand binding and/or receptor overexpression result in homo- or hetero dimerisation of HER-2, transphosphorylation of the kinase domains, and subsequent activation of downstream signalling [13,14].

In this study, we questioned the correlations between CEA and CA 19-9 levels and HER-2 IHC expressions and survival of patients with metastatic gastric cancer. To the best of our knowledge this is the first and only study that suggests clinical and laboratory correlations between CEA and CA 19-9 levels and HER-2 levels.

Methods

A total of 411 metastatic gastric cancer patients treated at the medical oncology outpatient clinic between 2013 and 2018 were included in this study. IHC HER-2 expressions and baseline CEA and CA 19-9 levels were determined at the time of diagnosis. Overall survival (OS) durations of patients were calculated and the correlations between CEA, CA 19-9 levels and HER-2 IHC expressions were targets of the survey analysis.

The following patient characteristics were collected from patients' charts after written informed consent was taken from patients or their relatives: age, gender, HER-2 IHC levels, CEA and CA 19-9 levels, OS duration. The Local Ethics Committee of our hospital approved the study. All patients received treatments in our outpatient clinic and were evaluated at regular follow-up visits.

Tumor marker analysis

The CEA and CA 19–9 values of the patients at the time of diagnosis and following treatment were determined with the two-sided radio-immunometric assay by using the IRMA-mat CA 19–9 and IRMA-coat CEA kits (Byk Sangtec Diagnostica GmbH & Co. KG, Dietzenbach, Germany). The normal value for CEA was 0.52–6.3 ng/mL for males and 0.42–4.8 ng/mL for females among smokers and 0.37–3.3 ng/mL for nonsmokers. The normal value for CA 19–9 was 0–37 IU/mL. Calculations were made by classifying CEA and CA 19-9 levels.

Human epidermal growth factor receptor 2 (HER-2) - IHC

All histologic sections were obtained from formalin-fixed, paraffin-embedded gastrectomy and lymph node dissection specimens. Gastric resection specimens and positive lymph nodes were simultaneously studied by IHC and SISH for HER-2 overexpression. HerceptTest was used for IHC. No reactivity or no membranous reactivity in <10% of invasive tumor cells was assessed as IHC 0; faint or barely perceptible membranous reactivity in ≥10% of cancer cells that are reactive only in part of their membrane was assessed as IHC 1+; weak to moderate complete, basolateral, or lateral membranous reactivity in ≥10% of invasive cancer cells were assessed as IHC 2+; and strong complete, basolateral, or lateral membranous reactivity in ≥10% of invasive cancer cells were assessed as IHC 3+.

Statistics

Statistical analyses were performed using SPSS software 17.0 (SPSS Inc., Chicago, IL, USA). X^2 test and Fisher's exact test were used to analyze the relationships. Survival analysis and curves were calculated and drawn using the Kaplan-Meier method. Groups were compared by the log-rank test. OS was defined as the time from diagnosis to the date of the patient's death or loss to follow-up. Univariate and multivariate analyses were performed using the Cox proportional hazards model. Multivariate p values were used to characterize the independence of these factors. The 95% confidence interval (CI) was used to quantify the relationship between survival time and each independent factor. All significance tests were two-sided at a significance level<0.05.

Results

A total of 411 patients participated in this study. Of 411 patients, 114 (27.7%) were female and 297 (72.3%) male, with a median age of 58 years (range: 22-90). All patients had metastatic disease at the time of diagnosis. Distribution of baseline

CEA levels and patient numbers (percentages) were as follows: 0-3: 47 (11.4%); 3-5: 64 (15.6%); 5-7: 95 (23.1%); 7-10: 107 (26.0%); >10: 98 (23.8%). Distribution of baseline CA 19-9 levels and patient numbers (percentages) were as follows: <15: 141 (34.3%); 15-35: 208 (50.6%); >35: 62 (15.1%). And distribution of baseline HER-2 IHC expressions and patient numbers (percentages) were as follows: 0: 32 (7.8%); 1: 147 (35.8%); 2: 119 (29.0%); 3: 113 (27.5%). Increasing HER-2 IHC expressions were positively correlated with increasing CEA and CA 19-9 levels (Table 1 and 2;p=0.0001). This correlation of CEA and CA 19-9 levels with the HER-2 IHC expressions was found to have direct relationships with OS. As the marker levels increased, IHC positivity also increased and OS duration decreased (p=0.0001;Table 3). Patient OS were correlated with marker levels and HER-2 IHC expressions. Overall survival curves according to CEA, CA 19-9 and HER-2 (IHC) levels are shown in Figures 1, 2 and 3, respectively.

Discussion

CEA, a oncofetal glycoprotein, belongs to the immunoglobulin superfamily and plays role in cell-cell adhesions. CEACAM5 gene that is overexpressed in various cancers, such as gastrointestinal, respiratory, genitourinary and breast cancers, encodes CEA protein. Overexpression of CEA promotes cancer proliferation via several mechanisms, such as initiation and facilitation of abnormal cell differentiation and growth, prevention of apoptosis and development of resistance to therapeutic agents. The overall functions of the CEA are yet to be determined, yet its close relationship with cancer aggressiveness has been identified long ago. Especially in gastric cancer patients receiving neoadjuvant chemotherapy normal values before treatment are correlated with favorable prognosis [15], however higher preoperative CEA levels are associated with aggressive cancer and lower patient survival rates [16]. Since its expression abounds in

Table 1. The relationship between carcinoembryonic antigen level and IHC

	IHC 0 *n (%)*	*IHC 1* *n (%)*	*IHC 2* *n (%)*	*IHC 3* *n (%)*	*p*
CEA 0-3	0 (0)	39 (26.5)	8 (6.7)	0 (0)	
CEA 3-5	10 (31.2)	35 (23.8)	19 (16.0)	0 (0)	
CEA 5-7	9 (28.1)	33 (22.4)	32 (26.9)	21 (18.6)	0.0001
CEA 7-10	12 (37.5)	39 (26.5)	56 (47.1)	0 (0)	
CEA > 10	1 (3.1)	1 (0.7)	4 (3.4)	92 (81.4)	

Immunohistochemical (IHC), carcinoembryonic antigen (CEA)

Table 2. The relationship between CA 19-9 level and IHC

	IHC 0 *n (%)*	*IHC 1* *n (%)*	*IHC 2* *n (%)*	*IHC 3* *n (%)*	*p*
CA 19-9 (< 15)	17 (53.1)	86 (58.5)	18 (15.1)	20 (17.7)	
CA 19-9 (15-37)	13 (40.6)	41 (27.9)	79 (66.4)	75 (66.4)	0.0001
CA 19-9 (> 37)	2 (6.2)	20 (13.6)	22 (18.5)	18 (15.9)	

Immunohistochemical (IHC), Carbohydrate antigen 19-9 (CA 19-9)

Table 3. Survival according to CEA, CA 19-9 and IHC

CEA	CA 19-9	IHC level
0-3 vs 3-5, p= 0.3	0-15 vs 15-37, p= 0.0001	0 vs 1, p= 0.8
0-3 vs 5-7, p= 0.02	0-15 vs > 37, p= 0.0001	0 vs 2, p= 0.4
0-3 vs 7-10, p=0.0001	15-37 vs > 37, p= 0.0001	0 vs 3, p= 0.4
0-3 vs > 10, p= 0.0001		1 vs 2, p= 0.07
3-5 vs 5-7, p= 0.2		1 vs 3, p= 0.03

Immunohistochemical (IHC), Carcinoembryonic antigen (CEA), Carbohydrate antigen 19-9 (CA 19-9)

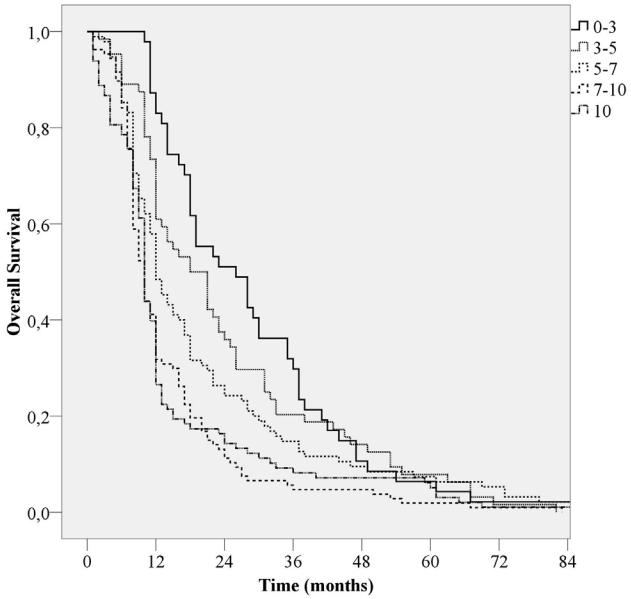

Figure 1. Overall survival according to CEA.

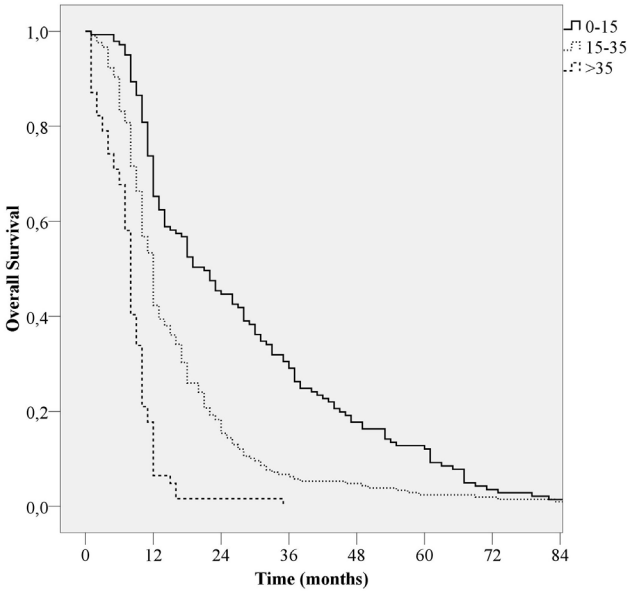

Figure 2. Overall survival according to CA 19-9.

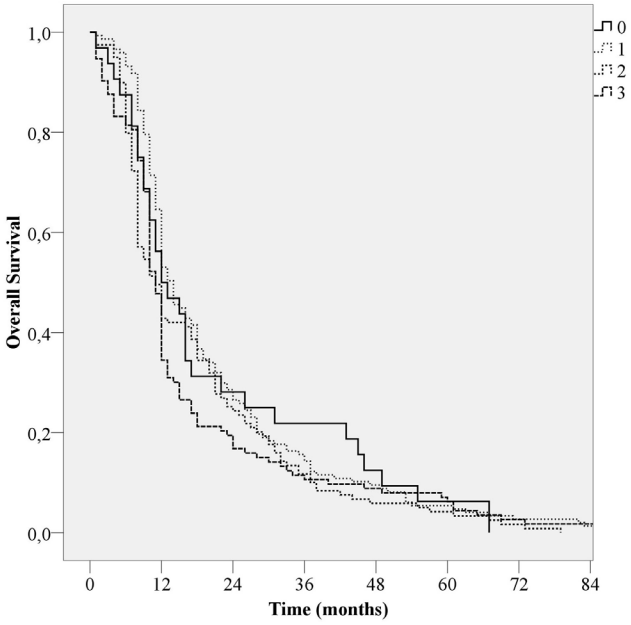

Figure 3. Overall survival according to HER-2 (IHC).

cancer cells and it is secreted in great amounts to serum, CEA levels have been widely used as serum tumor marker.

HER family has important and specific involvements in the pathogenesis of various human cancers. HER-2, a proto-oncogene, encodes a transmembrane protein with tyrosine kinase activity and is involved in signal transduction pathways, leading to cell growth and differentiation [17]. Amplification of HER-2 gene and overexpression of HER-2 protein are found in 15-20% of patients with gastric and gastroesophageal junction cancers and are associated with poor prognosis and a more aggressive disease.

It was reported that both CEACAM and HER-2 are found in plasma membrane. Iwabuchi et al showed interaction between CEACAM6 and HER-2 in BT474 cell lines while studying the predictability of trastuzumab treatment [14,18]. Knockdown of CEACAM decreased the sensitivity of trastuzumab in cell lines. CEACAM6 is thought to be involved in tumor progression in HER-2 positive breast cancer but its mechanism is unknown [14]. HER-2 modulates cancer cell progression through the activation of PI3K/Akt and MAPK pathways. Similarly, CEACAM6 overexpression has been shown to activate the Akt pathway in pancreatic and gastric cancer [19,20]. To elucidate the full mechanism of the correlation between CEA/CEACAM and HER-2 and understand its prognostic and predictive values fully in order to adapt novel treatment modalities, new molecular and clinical studies are required. In this study we clearly demonstrated a clinical relationship between HER-2 and CEA levels. Serum CEA levels are measured routinely and the determination of CEACAM level is technically not cost-effective. We acknowledge the fact that analyzing the relationship between CEACAM and HER-2 via measuring the serum CEA levels due to financial constraints was one of the limitations in our study, yet we believe that our results might encourage studies questioning CEACAM/HER-2 correlation clinically and also at molecular levels.

İn addition, the fact that the treatment modalities patients received, comorbidity factors, metastasis sites and other prognostic variables and de-

tails that will affect OS were not evaluated in detail, and this was another limitation of this study.

Our study showed significant positive correlations between IHC expressions and CEA and CA 19-9 levels, which were correlated with prognosis. In conclusion, baseline CEA and CA 19-9 levels predicted HER-2 positivity and this in turn directly affected treatment and OS. To the best of our knowledge this is the first and only study that suggests clinical and laboratory correlations between CEA and CA 19-9 levels and HER-2 levels.

The following patient characteristics were collected from patients' charts after written informed consent was taken from patients or their relatives: age, gender, HER-2 IHC levels, CEA and CA 19-9 levels, OS duration. The Local Ethics Committee of our hospital approved the study.

Conflict of interests

The authors declare no conflict of interests.

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