Ann Med Res 2020;27(9):2234-9

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.02.156

Original Article

Results of concurrent HER2 (CERB-B2) staining in the primary tumor and lymph node metastasis in advanced stage gastric carcinoma

Ebru Akay[1], Fatos Tekelioglu[1], Saliha Karagoz Eren[2], Yunus Donder[2], Hatice Karaman[1]

[1]Department of Pathology, Kayseri City Training and Research Hospital, Kayseri, Turkey
[2]Department of General Surgery, Kayseri City Training and Research Hospital, Kayseri, Turkey

Abstract

Aim: We hypothesized that tumor cells with metastatic capacity and nodal metastasis would exhibit prominent HER2 overexpression in gastric cancer for which intratumoral heterogeneity is highly variable. This study purposed to investigate the mismatch for HER2 immunohistochemical staining between the primary tumor cells and the metastatic cells in patients with advanced-stage gastric cancer.

Material and Methods: A hundred and forty-four patients with advanced staged gastric cancer, and lymph node metastasis who underwent surgical resection were enrolled in this retrospective study. Primary tumor and lymph node metastasis underwent concurrent immunohistochemical staining for addressing the HER2 positivity. The concordance of HER2 positivity between the primary tumor samples and the lymph node metastases was investigated.

Results: There was a significant difference in HER2 positivity rate among the well, moderate, and poorly differentiated carcinomas, which was primarily driven by the high HER2 overexpression in well-differentiated subgroup. HER2 positivity was highly frequent in stage 3 tumors, whereas HER2 was negative in the majority of the stage 4 tumors. Tumor size was also significantly larger in subjects without HER2 overexpression compared to those with HER overexpression [6 (IQR=4.88) cm vs. 5.25 (IQR=3.5) cm, p = 0.037]. Concordance of primary tumors and the metastatic lymph nodes regarding HER2 positive IHC staining were 93.7%.

Conclusion: HER2 might be positive in lymph node metastasis samples even if the primary tumor is negative for HER2. We suggest that HER2 IHC staining of the lymph node metastasis should be considered if the primary tumor is signet ring cell carcinoma, moderately or poorly differentiated, and negative for HER2 in subjects with gastric carcinoma and lymph node metastasis

Keywords: Gastric carcinoma; HER2 (CerbB2); iymph node; metastasis

INTRODUCTION

Gastric cancers constitute 7-8% of all cancers and the second common cause of deaths due to cancer (1, 2). Five-year survival rate of gastric cancer is below 15% to 20% due to the aggressive nature, late diagnosis, and the limited treatment options in this kind of tumor (3).

Human epidermal growth factor receptor 2 (HER2) is proto-oncogene, which encodes tyrosine kinase receptors. Amplification and overexpression of HER2 results in a 10 to 100 times increase in the amount of the receptors located on the cell surface (4). Activated HER2 is responsible for uncontrolled cell proliferation, suppression of apoptosis, cell differentiation, and migration, which is in close relationship between the metastatic capacities. HER2 amplification or over expression has been detected in 7% to 34% of the gastric cancers and their effects on prognosis have been investigated since the introduction of the targeted therapy (5-7). HER2 inhibitors function through the induction of antibody-related cellular cytotoxicity and the inhibition of the HER2 related signal transmission. Thereby, treatment with HER2 inhibitors prevents HER2 amplification and consequently reactivates apoptosis. Prospective randomized phase III Transtuzumab for Gastric Cancer (ToGA) study has shown that the combination of HER-2 inhibition with chemotherapeutics compared to chemotherapeutics alone improves overall survival in HER-2 positive metastatic gastric cancer which is promising for such a poor prognosis malignancy (2,6,8).

Immunohistochemical staining of multiple tumor blocks with HER2 has been reported to increase the detection of the positive cases (9). However, whether the primary or the metastatic samples should be used

Received: 20.02.2020 **Accepted:** 21.08.2020 **Available online:** 17.09.2020
Corresponding Author: Ebru Akay, Department of Pathology, Kayseri City Education and Research Hospital, Kayseri, Turkey
E-mail: dreakay77@gmail.com

for immunohistochemical staining remains unclear. We hypothesized that tumor cells with metastatic capacity and nodal metastasis would exhibit prominent HER2 overexpression in gastric cancer for which intratumoral heterogeneity is highly variable.

The present study purposed to investigate the mismatch for HER2 immunohistochemical staining between the primary tumor cells and the metastatic cells in patients with advanced stage gastric cancer. This study also aimed to determine the rate of HER2 overexpression in gastric cancer and to assess its relation with clinical and pathological findings.

MATERIAL and METHODS

A hundred and forty-four patients with advanced staged gastric cancer, and lymph node metastasis who underwent surgical resection at Kayseri Education and Research Hospital between January 2010 and January 2018 were enrolled in this retrospective study. The study was approved by the Institutional Review Board (Erciyes University Clinical Research Ethical Committee 2018/609). Written informed consent was obtained from all participants.

After fixation with 10% formalin, all tissue samples underwent tissue processing and subsequently placed in paraffin blocks. Tissue paraffin blocks and specimens stained with hematoxylin & eosine were performed according to the Lauren classification (10). In cases with multiple metastatic lymph nodes, lymph nodes with greater tumor infiltration and those demonstrating metastatic areas with different differentiation from the primary tumor were selected for immune staining.

Appropriate paraffin blocks were selected for immunohistochemical staining, and slides with 3-4 µ thickness were placed on poly-L-lysin coated slides. HER2 (cerbB2) (PATHWAY, anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody) was applied as the primary antibody for a 35 minutes. Immunohistochemical staining was carried out through an XT ultraview DAB procedure (Ventana Bench Mark XT, Adriamed, Skopje, Macedonia). Following the application of alcohol and xylene all slides were covered with the appropriate coating solution. HER-2 positive breast invasive ductal carcinoma tissue was used as the positive control. All samples were evaluated by an experienced pathologist under a light microscope.

Immunohistochemical staining was evaluated according to the method described by Rüschoff and colleagues (11-13) . Clear staining at low magnification (x 2.5-5), x10 magnification, and x40 magnification were graded as +++, ++, and +, respectively. Staining > 10% of the tumor was taken into consideration in the evaluation of the resection material. +++ cases were regarded as appropriate for anti-HER2 treatment; thus were recorded these cases as positive. Confirmation with the Dual SISH method, one of the in situ hybridization methods, was carried out in ++ cases (13) . In our study, 6 cases were HER2 ++ and dual SISH analysis was performed on these cases. SISH analysis was performed using a PathVysion HER-2 DNA probe kit. Since the presence of Dual SISH (+) = HER2:Cep17 ≥2 indicates HER2 gene amplification, these cases were also recorded as positive (14) . In summary, IHC results were grouped as positive or negative, as described.

Statistical analysis

All analyses were performed on SPSS v22. Shapiro-Wilk test was used for the normality check. Data are presented as mean ± standard deviation or median (interquartile range) for continuous variables regarding normality. Normally distributed were analyzed with Student's t-test. Non-normally distributed variables were analyzed with the Kruskal-Wallis test. Mann Whitney U test was used for paired comparisons. Fischer's exact test of the Pearson chi-square test was used for comparison of categorical variables. A two-sided $p < 0.05$ was accepted as statistically significant.

RESULTS

A total of 144 cases were included in the study (mean age 63 ± 11 years, 63.9% male). 16.7% of the tumors were located at upper 1/3, 49.3% were located at middle 1/3, and 34% were located at lower 1/3 of the stomach. According to the Laurens classification, 78 subjects (54.2%) were intestinal type, 47 (32.6%) were diffuse type, and 19 (13.2%) were mixed type gastric carcinomas. Demographic features, clinicopathological features, and IHC of primary tumor and metastasis are given in Table 1.

Intestinal type carcinomas were well differentiated in 12 subjects (15.4%), moderately differentiated in 42 subjects (53.8%), and poorly differentiated in 24 subjects (30.8%). There was a significant difference in HER2 positivity among the well, moderate and poorly differentiated carcinomas, which was primarily driven by the high HER2 overexpression in well-differentiated subgroup (p=0,026).

There were no significant differences regarding the mean age between the primary tumors with and without HER2 overexpression (59 ± 10 vs. 63 ± 11, p = 0.088). However, tumor size was significantly larger in subjects without HER2 overexpression compared to those with HER overexpression [6 (IQR=4.88) cm vs. 5.25 (IQR=3.5) cm, p = 0.037]. HER2 positivity frequency was higher in stage 3 tumors than stage 4 tumors (p=0,043).

According to the IHC staining, 24 (16.7%) of the primary tumors were HER2 positive, and 120 (83.3%) were HER2 negative. On the other hand, 31 (21.5%) of the metastatic lymph nodes were HER2 positive, and 113 (78.5%) were negative.

Concordance of primary tumors and the metastatic lymph nodes regarding HER2 positive IHC staining were 93.7%. A different IHC staining pattern was observed in 9 of the 144 cases (6.3%) (Table 2). In 8 cases, the primary was negative while the lymph node had a positive transformation (Table 2, Cases 1,2,7). Three of these 8 cases had tumor differentiation in the lymph nodes.

Table 1. Comparison of demographic characteristics, clinical findings and pathologic features in primary tumor samples and the lymph node metastasis according to the HER2 positivity

		HER2-primary tumour		P value	HER2-Lymph node metastases		P value
		negative	positive		negative	positive	
Gender	Female	43	9	**0.87**	37	15	**0.108**
		35.8%	37.5%		32.7%	48.4%	
	Male	77	15		76	16	
		64.2%	62.5%		67.3%	51.6%	
Lauren Classification	Intestinal type	61	17	**0.071**	58	20	**0.207**
		50.8%	70.8%		51.3%	64.5%	
	Diffuse type	44	3		41	6	
		36.7%	12.5%		36.3%	19.4%	
	Mixed type	15	4		14	5	
		12.5%	16.7%		12.4%	16.1%	
Differantiation	Well	6	6	**0.026**	6	6	**0.036**
		9.8%	35.3%		10.5%	28.6%	
	Moderately	35	7		34	8	
		57.4%	41.2%		59.7%	38.1%	
	Poorly	20	4		17	7	
		32.8%	23.5%		29.8%	33.3%	
Stage	1	0	0	**0.043**	0	0	**0.028**
		0.0%	0.0%		0.0%	0.0%	
	2	6	3		5	4	
		5.0%	12.5%		4.4%	12.9%	
	3	28	10		26	12	
		23.3%	41.7%		23.0%	38.7%	
	4	86	11		82	15	
		71.7%	45.8%		72.6%	48.4%	
Borrman classification	Ulcerated	29	5	**0.287**	27	7	**0.411**
		24.2%	20.8%		23.9%	22.6%	
	Infiltrative	35	3		33	5	
		29.2%	12.5%		29.2%	16.1%	
	Fungate	49	14		47	16	
		40.8%	58.3%		41.6%	51.6%	
	Polypoid	7	2		6	3	
		5.8%	8.3%		5.3%	9.7%	
Localization	Upper 1/3	19	5	**0.594**	19	5	**0.222**
		15.8%	20.9%		16.8%	16.1%	
	Mid 1/3	58	13		51	20	
		48.4%	54.1%		45.1%	64.5%	
	Lower 1/3	43	6		43	6	
		35.8%	25.0%		38.1%	19.4%	
Vascular invasion	No	14	2	**1.00**	14	2	**0.52**
		11.7%	8.3%		12.4%	6.5%	
	Yes	106	22		99	29	
		88.3%	91.7%		87.6%	93.5%	
Perineural invasion	No	15	6	**0.12**	15	6	**0.39**
		12.5%	25.0%		13.3%	19.4%	
	Yes	105	18		98	25	
		87.5%	75.0%		86.7%	80.6%	

Table 2. Clinical and pathological findings of the subjects' demonstrating discordance in HER2 staining of the primary tumor and the lymph node metastasis

	Gender	Age	PNI	VI	Lauren classification	Stage	HER2 (Primary tumor)	HER2 (LN Metastasis)
1	Female	75	+	+	Diffuse	4	Negative	Positive
2	Female	74	-	+	Diffuse	4	Negative	Positive
3	Female	33	+	+	Diffuse	3	Negative	Positive
4	Female	62	+	+	Intestinal	3	Negative	Positive
5	Male	62	+	+	Intestinal	3	Negative	Positive
6	Female	53	+	+	Intestinal	4	Negative	Positive
7	Female	47	+	+	Intestinal	4	Negative	Positive
8	Male	71	+	+	Mixed	2	Negative	Positive
9	Male M	60	-	+	Intestinal	3	Positive	Negative

In 1 case, metastatic lymph node was HER2 negative while the primary tumor was HER2 positive. Nevertheless, when cases displaying different staining in primary tumor samples and metastatic lymph nodes were evaluated with regard to positive staining, HER2 positivity rate was 22.2% for all cases.

DISCUSSION

The rate of HER2 positive gastric carcinomas among all gastric carcinomas varies from 7% to 34% (5). While the variety in the prevalence of the HER2 positivity had been attributed to regional and racial differences in some of the previous reports, histological type and heterogeneity had been suggested as the cause of this variety in other studies (15-18). The HER2 positivity rate of primary gastric carcinomas in Turkey has been reported to range between 11.5% and 20%, which is highly correlated with our results indicating a HER2 positivity rate of 16.7% (16,18) .

As shown in previous studies, HER2 positivity is more common in intestinal-type gastric carcinomas compared to the diffuse-type gastric carcinomas. HER2 positivity has been reported in 16% to 34% of intestinal type gastric carcinomas, whereas it was reported to vary between 2% and 7% in diffuse type gastric carcinomas (2,6). In concordance with previous data, we found that HER2 overexpression was significantly higher in intestinal-type gastric carcinomas than that of the diffuse type gastric carcinomas (21.8% to 6.1%).

Despite the clear evidence concerning the association of HER2 overexpression with poor prognosis and more aggressive course in breast cancers, its role in prognosis and clinicopathological findings need to be clarified. While there are studies showing no significant relation between HER2 overexpression and age, gender, tumor location, stage, and survival, some recent evidence indicates that HER2 overexpression may be associated with tumor size, serosal, and lymphatic invasion, lymph node metastasis, and distant metastasis (19-22) . Our results indicate that tumor size, pathological stage, and differentiation are associated with HER2 overexpression. We showed that HER2 positivity was more frequent in well-differentiated and lower stage carcinomas than poorly differentiate and higher stage carcinomas. Our findings also reveal that gastric carcinomas with larger tumor sizes demonstrate less HER2 positivity than the small ones. However, we found no significant relationship between the tumor location and HER2 overexpression.

HER2 positivity rate up to 30% has been reported in cases with breast cancer. The discordance of HER2 receptor positivity in primary and recurrent/metastatic breast cancers has been shown in several retrospective studies (21). There are also a few studies indicating a discordance of HER2 receptor positivity in primary gastric carcinomas and lymph node metastasis (18,23) . It is obvious that the discordance of HER2 receptor positivity in the primary tumor and metastasis is critical for decision making in the management of patients with gastric carcinoma. In our study, while HER2 was positive in 16.7 of the primary tumors, the HER2 positivity rate of the lymph node metastasis was 22.2.%. The cause of the discordance of HER2 positivity in primary tumor and the metastasis has not been clarified yet. However, some of the previous studies indicate that it might be related to the biological predisposition of the tumor, which leads to the selection of a ne neoplastic cell clone (21). In our study, the primary tumor and the metastatic tissue underwent the same IC staining procedure; thus, we suggest that the HER2 positivity rate of the primary tumor ad the metastasis in our study was independent of the IHC procedure or the fixation method. In the study of Ieni and colleagues, HER2 positivity was concordant in primary gastric carcinoma and lymph node metastasis in 90% of the cases. Similar to the results of the Ieni et al., Bozzetti et al. reported that HER2 positivity was concordant in primary gastric carcinoma and lymph node metastasis in 94.9% of their study population (24) . The concordance in HER2 positivity rate of the primary tumor and the metastasis 88.9% in the study of Perrone et al. (25). The concordance in HER2 positivity between the primary tumor and the lymph node metastasis was 93.7% and was consistent with the previous data. In a previous study carried out by Kim and colleagues, the concordance in HER2 positivity rate between the primary tumor and the lymph node

metastasis was 21.8%. The authors concluded that this discordance was a consequence of the heterogeneity of the primary tumor (26). Previous data and our results indicate the same finding that inclusion of the metastasis into the IHC staining procedure of the primary tumor increases the frequency of the HER2 positive cases. It is also remarkable that vascular invasion and lymph node metastasis were more frequent in cases with different IHC staining patterns of the primary tumors and the lymph node metastasis. Moreover, while the primary tumor was negative, metastases were positive in diffuse-type gastric carcinoma cases.

The cause of the different HER2 scores in different regions of the same tumor has not been clearly elucidated yet. There was also heterogeneity in HER2 scores of the metastasis for which the primary tumor demonstrated heterogeneity. The discordance in HER2 positivity rate between the primary tumor and the lymph node metastasis, which has been obtained concurrently and underwent the same fixation on the same lamella, indicates that this discordance results from the tumors attitude rather than a mistake in microscopic evaluation or fixation technique.

CONCLUSION

In conclusion, when the role of the HER2 in proliferation, differentiation, and the migration of the tumor cells are taken into consideration, the higher HER2 positivity rate in the metastasis may explain the HER2 positivity of the neoplastic cell clone inclined to spread. The difference in the HER2 positivity between the primary tumor and the lymph node metastasis of the gastric carcinoma, for which novel treatment modalities are under research, is critical. We recommend HER2 IHC staining of the lymph node metastasis if the primary tumor is signet ring cell carcinoma, moderately or poorly differentiated, and negative for HER2. IHC staining of the metastatic lymph nodes in addition to the primary tumor may lead to an increased in the number of subjects who are a candidate for treatment may prevent the misjudgement of these subjects as negative for HER2.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: The kit for the primary antibody used in the study was funded by the Scientific Research and Development Support Program of the Kayseri Education and Research Hospital.

Ethical approval: The study was approved by the Institutional Review Board (Erciyes University Clinical Research Ethical Committee 2018/609). Written informed consent was obtained from all participants.

REFERENCES

1. Karimi P, Islami F, Anandasabapathy S, et al. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiol Biomarkers Prev. 2014;23:700-13.
2. Nadaf AS, Rani H, Dinesh US. Immuno-Histochemical Assessment of HER2NEU Expression in Gastric Adenocarcinoma in North Karnataka, India. Asian Pac J Cancer Prev 2018;19:1381-5.
3. Torre LA, Bray F, Siegel RL,et al. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87-108.
4. Abrahao-Machado LF, Scapulatempo-Neto C. HER2 testing in gastric cancer: An update. World J Gastroenterol 2016;22:4619-25.
5. Peng Z, Zou J, Zhang X, et al. HER2 discordance between paired primary gastric cancer and metastasis: a meta-analysis. Chine J Cancer Res 2015;27:163-71.
6. Bang Y-J, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010;28:687-97.
7. Jørgensen JT. Targeted HER2 treatment in advanced gastric cancer. Oncology 2010;78:26-33.
8. Sawaki A, Ohashi Y, Omuro Y, et al. Efficacy of trastuzumab in Japanese patients with HER2-positive advanced gastric or gastroesophageal junction cancer: a subgroup analysis of the Trastuzumab for Gastric Cancer (ToGA) study. Gastric Cancer 2012;15:313-22.
9. Huber AR, Buscaglia B, Koltz BR, et al. Impact of Specimen Type and Specimen Number on HER2 Status in Gastroesophageal Junction and Gastric Adenocarcinoma: More Is Better. Am J Clin Pathol 2019;151:461-8.
10. Chen Y-C, Fang W-L, Wang R-F, et al. Clinicopathological variation of Lauren classification in gastric cancer. Pathol Oncol Res 2016;22:197-202.
11. Rüschoff J, Dietel M, Baretton G, et al. HER2 diagnostics in gastric cancer—guideline validation and development of standardized immunohistochemical testing. Virchows Arch 2010;457:299-307.
12. Hofmann M, Stoss O, Shi D, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. Histopathology 2008;52:797-805.
13. Bartley AN, Washington MK, Ventura CB, et al. HER2 testing and clinical decision making in gastroesophageal adenocarcinoma: guideline from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. Arch Pathol Lab Med 2016; 140:1345-63.
14. Wolff AC, Hammond MEH, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. J Clin Oncol 2013;31:3997-4013.
15. Feizy A, Karami A, Eghdamzamiri R, et al. HER2 expression status and prognostic, diagnostic, and demographic properties of patients with gastric cancer: a single center cohort study from Iran. Asian Pac J Cancer Prev 2018;19:1721-5.
16. Yildirim S, Dandin O, Durmus M, et al. C-erb-B2 (Her2/neu) Expression Rate And its Association with Clinicopathologic Parameters in Gastric Cancer. International Journal of Hematology and Oncology 2012;28:156-62.

17. Emre H, Tekin SB, Dogan H, et al. Investigation of ErbB-2 overexpression on patients with gastric cancer in Eastern Anatolia of Turkey. Turk J Gastroenterol 2013; 24:211-7.
18. Selcukbiricik F, Erdamar S, Buyukunal E et al. Is her-2 status in the primary tumor correlated with matched lymph node metastases in patients with gastric cancer undergoing curative gastrectomy. Asian Pac J Cancer Prev 2014;15:10607-11.
19. Marx AH, Tharun L, Muth J, et al. HER-2 amplification is highly homogenous in gastric cancer. Hum Pathol 2009; 40:769-77.
20. Kataoka Y, Okabe H, Yoshizawa A, et al. HER2 expression and its clinicopathological features in resectable gastric cancer. Gastric Cancer 2013;1:84-93
21. Ieni A, Barresi V, Branca G, et al. Changes in human epidermal growth factor receptor 2 status between primary breast/gastric carcinomas and synchronous metastatic lymph nodes: how can we explain them? J Cancer Metastasis Treat 2015;1:21-6.
22. Jørgensen JT. Role of human epidermal growth factor receptor 2 in gastric cancer: biological and pharmacological aspects. World J Gastroenterol 2014;28;:4526-35.
23. Kim JH, Kim MA, Lee HS, et al. Comparative analysis of protein expressions in primary and metastatic gastric carcinomas. Hum Pathol 2009;40:314-22.
24. Bozzetti C, Negri F, Lagrasta C, et al. Comparison of HER2 status in primary and paired metastatic sites of gastric carcinoma. Br J Cancer 2011;26:1372-6.
25. Perrone G, Amato M, Callea M, et al. HER2 amplification status in gastric and gastro-oesophageal junction cancer in routine clinical practice: which sample should be used? Histopathology 2012l; 61:134-5.
26. Kim MA, Lee HJ, Yang HK, et al. Heterogeneous amplification of ERBB2 in primary lesions is responsible for the discordant ERBB2 status of primary and metastatic lesions in gastric carcinoma. Histopathology 2011;59:822-31.