ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

A Large Cohort of Consecutive Patients Confirmed Frequent HER2 Positivity in Gastric Carcinomas with Advanced Stages

Junhun Cho, MD[1], Jiyun Jeong, MD[1], Jiyoun Sung, MD[1], Chang Ohk Sung, MD[1], Kyoung-Mee Kim, MD, PhD[1], Cheol Keun Park, MD[1], Min Gew Choi, MD[2], Tae Sung Sohn, MD[2], Jae Moon Bae, MD[2], and Sung Kim, MD, PhD[2]

[1]Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea;
[2]Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South of Korea

ABSTRACT

Background. Trastuzumab in association with systemic cytotoxic chemotherapy is the standard of care for patients with advanced HER2-positive gastric carcinoma (GC). However, HER2 as a prognostic factor in GC remains controversial.

Methods. HER2 overexpression and amplification was evaluated by immunohistochemistry (IHC) and silver in situ hybridization (SISH) in 2,798 GCs obtained from 2,727 gastrectomy and 71 open/laparoscopic biopsy specimens from patients with peritoneal seeding. Regional heterogeneity was defined as the proportion of tumor cells showing membranous staining in 10–70 % of tumor cells. Genetic heterogeneity was determined by the existence of HER2/CEP17 ratio higher than 2.0 in >5 to <50 % of tumor cells.

Results. In IHC, 184 cases (6.6 %) were 3+ and 44 cases (1.6 %) were 2+. Of 44 HER2 2+ cases, SISH showed *HER2* gene amplification in 21 cases (47.7 %), chromosome 17 polysomy in six cases (13.6 %), and genetic heterogeneity in five cases (11.4 %). HER2 positivity found in 7.3 % of GCs was significantly associated with older age, male gender, intestinal histology, upper third in location, higher lymph node stage ($p < .002$), and advanced AJCC stage ($p = .033$). Regional heterogeneity of HER2 was closely associated with 2+ (70.5 vs 42.9 % in 3+, $p = .001$) and diffuse or mixed histologic type ($p = .005$).

Conclusions. Regional heterogeneity of HER2 expression was closely associated with weak HER2 overexpression (2+) and with diffuse or mixed histology. Polysomy of chromosome 17 would be an important cause of HER2 2+ in IHC. Frequent HER2 positivity observed in GCs with advanced stages suggests that HER2 may be involved in tumor progression and poor prognosis.

Gastric cancer (GC) is the fourth most commonly diagnosed cancer and the second leading cause of cancer-related mortality worldwide.[1] The incidence rates of GC have large regional variations, linked to H. pylori infection, socioeconomic status, smoking, and diet. Although, the incidence of GC has declined steadily in several Western countries, it remains common in many geographic regions, including Eastern Asia.[2] Actually, GC is the second most common cancer in Asia.[3] Moreover, GC is associated with poor prognosis and early diagnosis is challenging because most patients do not show symptoms until the later stages of disease.[4] The most effective treatment for localized GC is surgery; however, approximately half of all patients with advanced-stage disease experience recurrence following curative resection. The prognosis of patients with advanced or recurrent GC remains poor.[5]

Recently, the Trastuzumab for Gastric Cancer (ToGA) trial revealed the efficacy of the humanized monoclonal antibody against HER2 protein, trastuzumab, in patients with advanced GC or gastroesophageal junction cancer (GEJC).[4] In the ToGA trial, 22 % of the patients with metastatic gastric and gastroesophageal cancers were HER2 positive.[4] In recent years, the importance of *HER2* gene amplification in GC has increased; however, the

correlation of clinical and pathologic parameters with HER2-positive GC are still debated. Moreover, the prognostic significance of HER2 in GC as a predictor of poorer outcome and a more aggressive disease form has been controversial.[6–10]

In this study, we analyzed clinicopathologic features of HER2-positive GC in a large cohort of consecutive patients who underwent surgery and unveiled the genetic alterations underlying HER2 weak positive (2+) GCs.

MATERIALS AND METHODS

For this study, we retrospectively searched for patients who underwent surgery for GC from October 2009 to June 2011, and 2798 patients were retrieved from the pathology database of Samsung Medical Center, Seoul, Korea. The cases consisted of consecutive total/subtotal gastrectomy specimens ($n = 2,727$) and open/laparoscopic biopsy specimens from patients with peritoneal seeding ($n = 71$).

Clinicopathologic features recorded in surgical specimen included age, sex, pTNM stage (AJCC 7th edition), tumor location (lower, middle, upper third of stomach), and histologic type by Lauren.

IHC for HER2 (PATHWAY HER-2/neu (4B5) rabbit monoclonal antibody, Ventana Medical Systems, Inc., Tucson, AZ) were performed in all cases with a BenchMark XT automated stainer (Ventana Medical Systems, Inc., Tucson, AZ). The slides were examined by 2 pathologists (JC and KMK), and immunoreactivity was graded from 0 to 3+ (0, no staining or membrane staining in less than 10 % of the t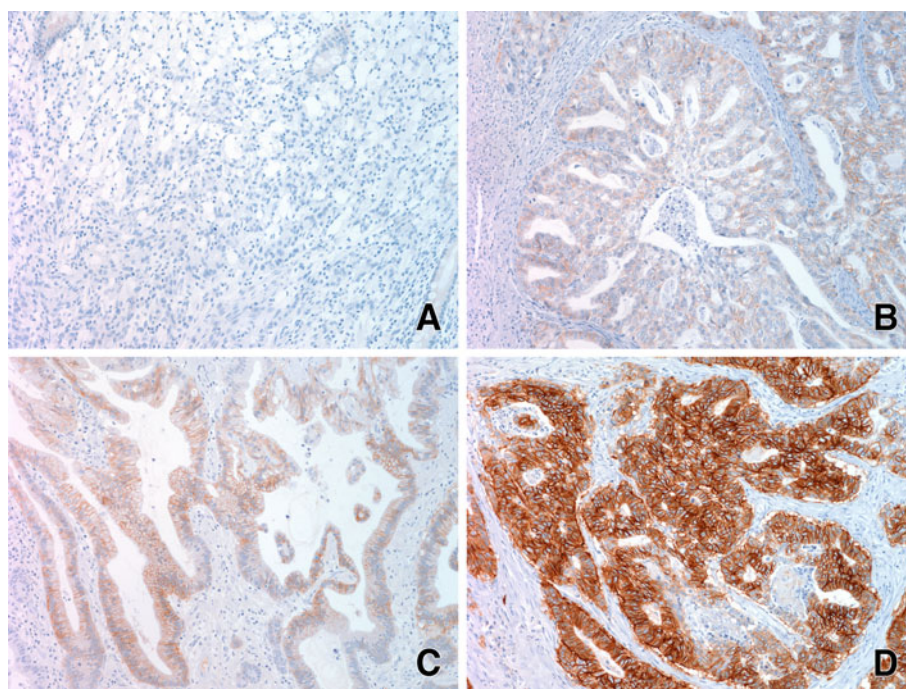umor cells; 1+, a faint/barely perceptible membrane staining in more than 10 % of the tumor cells; 2+, a weak to moderate complete membrane staining in more than 10 % of the tumor cells; 3+, a strong complete membrane staining in more than 10 % of the tumor cells), according to the criteria suggested by Hofmann et al. (Fig. 1).[11]

Silver in situ hybridization (SISH) for HER2 gene was performed on GCs with equivocal IHC result (2+). Automated SISH was performed on the Ventana Benchmark XT (Ventana Medical Systems, Inc., Tucson, AZ) according to the manufacturer's protocols for the INFORM HER2 DNA and Chromosome 17 probes. By counting 20 tumor cells on a high-power field ($\times 600$), mean HER2/CEP17 ratio were calculated. The HER2/CEP17 ratio ≥ 2.0 was considered to be HER2 gene amplified, while HER2/CEP17 ratio <1.8 was regarded as nonamplified. If the HER2/CEP17 ratio were 1.8 or more but less than 2.0, HER2/CEP17 ratio was calculated in 20 more tumor cells. In 40 tumor cells, when HER2/CEP17 ratio was 2.0 or more, the tumor was regarded as positive for HER2 gene amplification (Fig. 2). Polysomy of chromosome 17 was defined as more than 3 CEP17 signals on average.
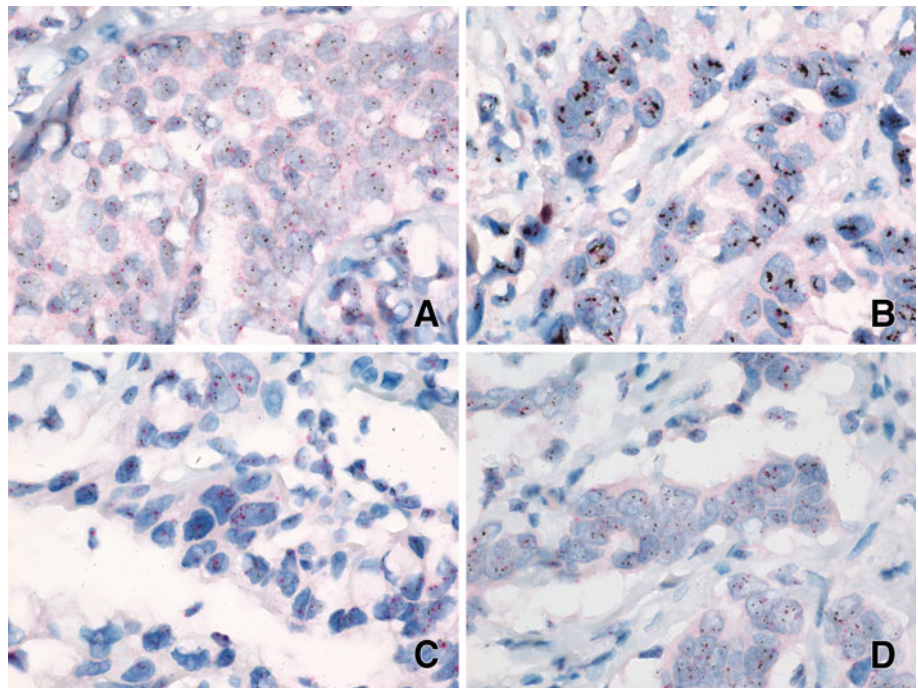
Regional heterogeneity was defined as the proportion of tumor cells showing membranous staining in 10–70 % of tumor cells in a representative slide from surgical specimens. Genetic heterogeneity was determined by the existence of more than 5 % but less than 50 % of tumor cells with a HER2/CEP17 ratio higher than 2.0.[12]

Results of HER2 IHC and SISH were correlated with clinicopathologic features. Statistical analyses were performed using SPSS 18.0 statistical software program (SPSS, Chicago, IL). The χ-square tests (Pearson χ-square test or χ-square test using linear-by-linear association)

FIG. 1 The immunohistochemical stains for HER2 in four gastric carcinomas. A. The tubular adenocarcinoma, poorlydifferentiated, with signet ring cells (diffuse-type) showed no immunoreactivity for HER2 (score 0). Theother three carcinomas were intestinal-type with different HER2 intensity (B, IHC score 1+; C, IHC score2+; and D, IHC score 3+)

FIG. 2 Silver in situ hybridization for HER2 gene in gastric cancers. A, no amplification of HER2 gene (HER2/CEP17ratio = 1.1). B, amplification of HER2 gene (HER2/CEP17 ratio = 6.0). C, Chromosome 17 polysomy withoutHER2 gene amplification (HER2/CEP17 ratio = 1.3; average CEP17 signals/nucleus = 4.05). D, Geneticheterogeneity observed in HER2 gene amplification-negative gastric carcinoma

were used to analyze correlations between HER2 IHC results and clinicopathologic parameters. The p values below .05 were considered statistically significant results.

RESULTS

Clinicopathologic Characteristics of Patients

The age of patients ranged from 23 to 90 years (median, 59 years). Among 2,798 patients, 179 patients were under the age of 41, 1,336 patients were from 41 to 60 years, and 1,283 patients were older than 60 years. There were more male patient ($n = 1,790$) than female patients ($n = 1,008$), with a male/female ratio of 1.76:1. The tumors were located in lower third in 1,270 (46.6 %), middle third in 1,030 (37.8 %), and upper third in 329 (12.1 %), and in 98 cases (3.6 %) the location was indeterminate mostly due to Borrmann type IV carcinoma involving more than 2 parts of the stomach. Clinicopathologic findings of patients are summarized in Table 1 in the online Data Supplement.

HER2 Immunohistochemistry in Gastric Carcinomas

Of 2,798 GC cases, 184 cases (6.6 %) were 3+, 44 (1.6 %) were equivocal (2+), 274 (9.8 %) were 1+, and 2,296 (82.1 %) were negative 0 for HER2 IHC (Table 1). HER2 positivity (IHC 3+ or IHC2+ and *HER2* gene amplification) was found in 200 of 2,727 (7.3 %) gastrectomy and 5 of 71 (7.0 %) open/laparoscopic biopsy specimens.

HER2 positivity was significantly frequent in older age, male gender, upper third in location, and intestinal

histologic type ($p < .001$). Although HER2 overexpression significantly correlated with higher lymph node ($p = .002$) and AJCC ($p = .033$) stages, depth of invasion (pT stage) did not correlate with HER2 overexpression ($p = .195$).

HER2 Gene Amplification in IHC 2+ Gastric Carcinomas

HER2 SISH was performed in 44 HER2 IHC 2+ cases. In SISH, the mean HER2/CEP17 ratio was 2.86 (0.9–12.0). The HER2/CEP17 ratio was less than 2.0 in 23 cases, greater than 4.0 in 8 cases, and in 13 cases the ratio was ≥2.0 and ≤4.0. *HER2* gene amplification was found in 21 of 44 cases (47.7 %), and 6 cases (13.6 %) were chromosome 17 polysomy. In these polysomy cases, the mean CEP17 signals per nucleus were 3.52 (3.20–4.05) (Fig. 2). When results of equivocal IHC cases had been revised according to HER2 SISH, the final HER2 positivity in all GC cases was 7.3 % (205 cases).

Heterogeneity of HER2

Regional heterogeneity of HER2 IHC was observed in 79 cases of 184 IHC 3+ cases (42.9 %) and 31 among 44 IHC 2+ cases (70.5 %). Regional heterogeneity of HER2 expression was closely associated with 2+ (70.5 vs 42.9 % in 3+, $p = .001$) and diffuse or mixed histologic type (68.4 and 70.0 vs 43.1 % in intestinal-type, $p = .016$) (Fig. 3). In all cases with regional heterogeneity, there was no difference in the staining pattern between superficial and deep portions of tumor.

TABLE 1 Correlations of clinicopathologic parameters with HER2 immunohistochemistry and regional heterogeneity

	HER2				P value	Regional heterogeneity				P value
	Negative		Positive			Absent		Present		
	n	(%)	n	(%)		n	(%)	n	(%)	
Age					<.001[a]					.338[a]
≤40	176	(98.3)	3	(1.7)		2	(40.0)	3	(60.0)	
41–60	1261	(94.4)	75	(5.6)		48	(57.8)	35	(42.2)	
≥61	1156	(90.1)	127	(9.9)		68	(48.6)	72	(51.4)	
Sex					<.001[b]					.836[b]
Male	1625	(90.8)	165	(9.2)		92	(51.4)	87	(48.6)	
Female	968	(96.0)	40	(4.0)		26	(53.1)	23	(46.9)	
Location					.001[b]					.441[b]
Lower	1164	(91.7)	106	(8.3)		56	(47.5)	62	(52.5)	
Middle	969	(94.1)	61	(5.9)		34	(53.1)	30	(46.9)	
Upper	296	(90.0)	33	(10.0)		24	(58.5)	17	(41.5)	
Other	98	(100.0)	0	(0.0)						
Histologic type by Lauren					<.001[b]					.005[b]
Intestinal	993	(85.9)	163	(14.1)		99	(56.9)	75	(43.1)	
Mixed	386	(93.7)	26	(6.3)		9	(30.0)	21	(70.0)	
Diffuse	1108	(99.0)	11	(1.0)		6	(31.6)	13	(68.4)	
Other	40	(100.0)	0	(0.0)						
pT stage					.195[a]					.913[a]
1	1496	(93.7)	100	(6.3)		56	(51.4)	53	(48.6)	
2	319	(89.6)	37	(10.4)		21	(48.8)	22	(51.2)	
3	373	(90.3)	40	(9.7)		24	(55.8)	19	(44.2)	
4	339	(93.6)	23	(6.4)		13	(46.4)	15	(53.6)	
pN stage					.002[a]					.663[a]
0	1714	(93.9)	111	(6.1)		61	(48.4)	65	(51.6)	
1	289	(90.0)	32	(10.0)		20	(58.8)	14	(41.2)	
2	217	(90.0)	24	(10.0)		14	(56.0)	11	(44.0)	
3	307	(90.3)	33	(9.7)		19	(50.0)	19	(50.0)	
AJCC stage					.033[a]					.435[a]
I	1636	(93.6)	112	(6.4)		62	(49.6)	63	(50.4)	
II	398	(91.7)	36	(8.3)		22	(55.0)	18	(45.0)	
III	465	(90.1)	51	(9.9)		29	(51.8)	27	(48.2)	
IV	94	(94.0)	6	(6.0)		5	(71.4)	2	(28.6)	

[a] χ-square test using linear by linear association

[b] Pearson χ-square test

Genetic heterogeneity of *HER2* gene amplification was observed in 5 of 44 SISH cases (11.4 %), in which 4 of them were amplification negative. There was no significant difference in clinicopathologic findings between patients with genetic heterogeneity of *HER2* gene and patients with *HER2* gene amplification ($p > .05$).
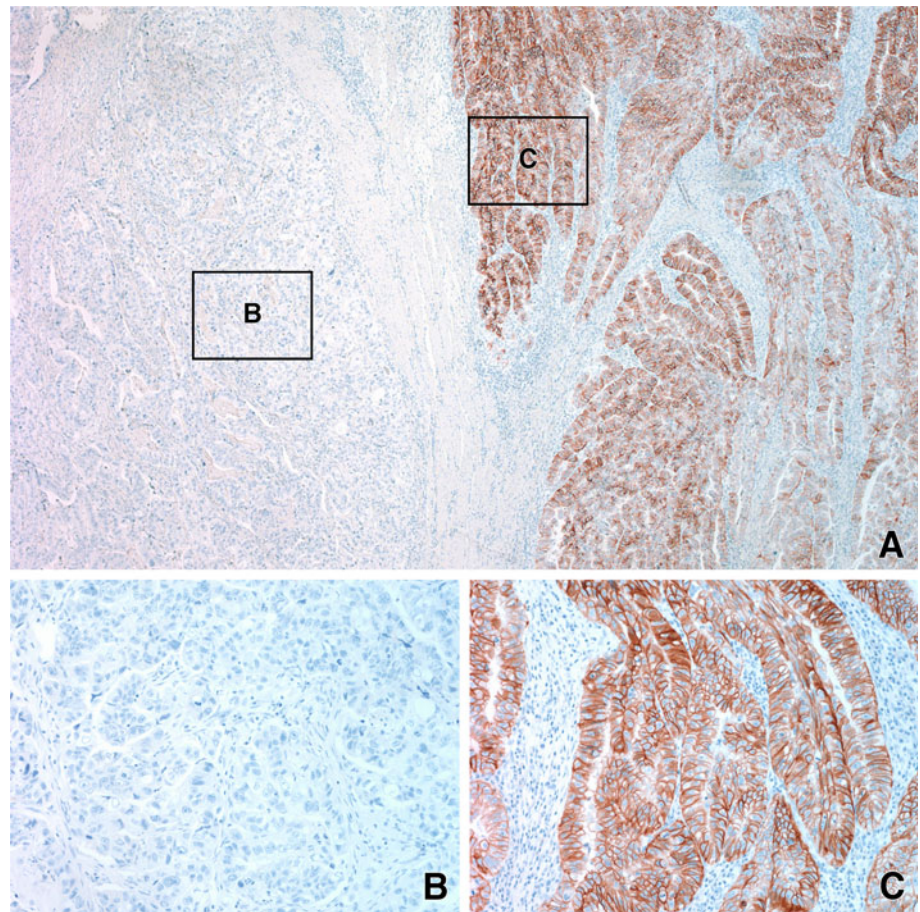
HER2 in Intestinal-Type GCs

Intestinal-type GCs were prevalent in older ages, male gender, frequent location in the lower 1/3, and lower pT, pN, and AJCC stages ($p < .001$) (Table 2 in the online Data Supplement). The most significant clinicopathologic characteristic influencing HER2 positivity in GC was histologic type by Lauren ($p < .001$). In intestinal-type GCs, HER2 positivity was 15.0 % (174 of 1,156) vs 1.7 % (19 of 1,119) in diffuse-type.

In subanalyses of intestinal-type GCs ($n = 1,156$), HER2 positivity revealed significant correlation with deeper invasion depth ($p = .009$), higher lymph node metastasis ($p < .001$), and higher AJCC stage ($p < .001$) (Table 2). However, HER2 overexpression showed no

FIG. 3 The immunohistochemical stains for HER2 in one gastric carcinoma. Regional heterogeneity of HER2 ingastric carcinoma (A) (original magnification, x50). The tumor cells on the right side showed strongmembranous immunoreactivity (C, score 3+) (original magnification, x200), while the tumor cells on the leftarea were negative for HER2 (B, score 0)

correlation with age ($p = .331$), sex ($p = 0.125$), and tumor location in those intestinal-type GCs ($p = .475$).

DISCUSSION

In a large cohort of consecutive patients who underwent surgery, we evaluated HER2 overexpression/amplification in 2,798 GCs. HER2 positivity was observed in 7.3 % of GCs and was significantly associated with older age, male gender, intestinal histology, upper third in location, frequent lymph node metastasis, and advanced clinical stages. Regional heterogeneity of HER2 expression was closely associated with 2+ and diffuse or mixed histologic type. Genetic heterogeneity of *HER2* gene was observed in 11.4 % of cases.

In many previous studies with IHC and/or FISH, HER2 positivity in GC was reported in a wide range from 7 to 38 %.[9,10,13–31] This variation is supposed to arise from inconsistent HER2 IHC scoring system or random selection of patients. In this study, we used HER2 scoring system suggested by Hofmann et al. showing high level of concordance (93.5 %) between IHC and FISH and the patients were consecutively selected.[11]

The higher positive rates of HER2 in intestinal-type GC than diffuse-type have been consistently reported, and our results were no exception.[14,16–18,20,23,28–32] On the other hand, the correlation of HER2 positivity with other clinicopathologic factors, including age, sex, and TNM stage has been controversial. Higher frequency of HER2 positivity significantly correlating with older age, male gender, upper location of tumor, lymphatic or vascular invasion, higher T stage, lymph node metastasis, distant metastasis, and higher TNM stage have been reported (Table 3 in the online Data Supplement).[10,15–18,20,24,26–28,31] However, contradictory results have also been reported.[13], [15–17,20,25,28–31] The positive relationship between pT stage and HER2 was found in a previous study, in which the number of patients was small ($n = 145$).[16] We confirmed that HER2 expression is related to deeper invasion depth, frequent lymph node metastasis, and advanced disease stages, suggesting HER2 as an indicator of tumor progression. In our previous study on advanced GCs, the incidence of HER2 positivity was high (13.8 %).[32] In ToGA trial, 22 % of the patients with metastatic gastric and gastroesophageal cancers were HER2 positive.[4] The results of IHC or FISH can be influenced by several factors

TABLE 2 Relationship between HER2 immunohistochemistry and clinicopathological parameters in patients with intestinal-type gastric cancer

	HER2 IHC				P value
	Negative		Positive		
	n	(%)	*n*	(%)	
Age					.331[a]
≤40	13	(100.0)	0	(0.0)	
41–60	370	(86.0)	60	(14.0)	
≥61	610	(85.6)	103	(14.4)	
Sex					.125[b]
Male	756	(85.0)	133	(15.0)	
Female	237	(88.8)	30	(11.2)	
Location					.475[b]
Lower	579	(86.4)	91	(13.6)	
Middle	282	(85.5)	48	(14.5)	
Upper	122	(83.6)	24	(16.4)	
Others	10	(100.0)	0	(0.0)	
pT stage					.009[a]
1	636	(87.7)	89	(12.3)	
2	146	(85.9)	24	(14.1)	
3	142	(80.7)	34	(19.3)	
4	69	(81.2)	16	(18.8)	
pN stage					<.001[a]
0	723	(88.2)	97	(11.8)	
1	131	(84.5)	24	(15.5)	
2	71	(79.8)	18	(20.2)	
3	68	(73.9)	24	(26.1)	
AJCC stage					<.001[a]
I	708	(88.0)	97	(12.0)	
II	158	(85.9)	26	(14.1)	
III	122	(75.8)	39	(24.2)	
IV	5	(83.3)	1	(16.7)	

[a] χ-square test using linear by linear association

[b] Pearson χ-square test

such as the time until fixing the tissues, the percentage of formalin, and duration of fixation. In this study, to avoid tumor ischemia induced by delays in formalin fixation, the gastric specimens were fixed no later than 2 h after removal in 10 % buffered formalin for 12–48 h. The histologic type of GC is one of the most powerful variables related to HER2 overexpression. Actually in our study, the ratios of intestinal type in all enrolled GCs were only 7.2 % in patients aged ≤40 years, while 55.6 % of GCs in patients >60 years were intestinal type. Moreover, intestinal-type GCs were prevalent in male gender, frequent location in the lower 1/3, and lower pT, pN, and AJCC stages. In this study, there was a tendency that the percentage of intestinal-type GC decreases and diffuse-type GC increases as pathologic disease stages increase. As a result, HER2 positivity can be decreased by younger age, female gender, and lower tumor location, while increased by higher pT stage, pN stage, and AJCC stage. So, when a researcher selects cases or interprets the results of HER2 on GC samples, lack of understanding of these clinicopathologic characteristics may lead to an inaccurate interpretation of the results. In this study, the number of patients with stage I was large (*n* = 1,748, 62.5 %) comprising more than half of cases. Moreover, small numbers of male patients (76 % in ToGA vs 64 % in this cohort), less-frequent intestinal type by Lauren (75 % in ToGA vs 41 %), and frequent location of carcinoma in the lower 1/3 may explain the low frequency of HER2 positivity.

Regional heterogeneity of HER2 IHC was frequently observed in GCs in 42.9 % of HER2 3+ cases and 70.5 % of HER2 2+ cases. Regional heterogeneity of HER2 expression was closely associated with HER2 2+ and

diffuse or mixed histologic type. In intensive slide review, there was no difference in the staining pattern between superficial and deep portions of tumor. Therefore, the determination of HER2 IHC result with endoscopic biopsy specimen seems to be unproblematic.

In this study, we first identified that 26.1 % of the 23 HER2 nonamplified cases were polysomy of chromosome 17. While the effect of gene amplification on HER2 protein overexpression has been well documented, the effect of chromosome 17-copy number on the expression of HER2 protein has not been well established even in the breast cancers.[33] According to the TOGA study data, polysomy (defined as ≥ 3 chromosome 17 signals) occurred in only 4.1 % of the entire screening population.[34] Chromosome 17 polysomy, through increased HER2 gene dosage, may impact *HER2* gene and protein expression; however, reports from the literature on this influence have been conflicting.[33] In the breast, in the absence of HER2 gene amplification, 27 % with chromosome 17 polysomy were scored as HER2 IHC 2+ and 3+ is rare (3 %). Moreover, genetic heterogeneity of *HER2* gene amplification was observed in 11.4 % of IHC 2+ cases. Although the number of examined cases is small, we first identified that genetic heterogeneity and polysomy of chromosome 17 would be an important underlying cause of weak HER2 overexpression (2+) in *HER2* gene nonamplified cases. Moreover, polysomy 17 was exclusively found in *HER2* gene nonamplified cases, suggesting that the mechanism of *HER2* amplification is independent of polysomy 17.

Although we did not perform survival analyses because of too short follow-up period, the correlation of HER2 positivity and advanced tumor stages of GC proposes a hypothesis that HER2 positivity may be related to disease progression and poor prognosis. In previous studies, despite some opposite reports, same as in breast cancer, HER2 positivity generally revealed an adverse outcome in GCs.[7–9,13,14,19,20,23,24,26–28,30]

In conclusion, regional heterogeneity of HER2 expression was closely associated with HER2 2+ expression and with diffuse histology. Polysomy of chromosome 17 would be an important cause of HER2 IHC 2+ in IHC. Frequent HER2 positivity observed in GCs with advanced stages suggests that HER2 may be involved in tumor progression and poor prognosis.

REFERENCES

1. Kelley JR, Duggan JM. Gastric cancer epidemiology and risk factors. *J Clin Epidemiol*. 2003;56:1–9.

2. Jemal A, Center MM, DeSantis C, Ward EM. Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev*. 2010;19:1893–907.

3. Sasako M, Inoue M, Lin JT, Khor C, Yang HK, Ohtsu A. Gastric Cancer Working Group report. *Jpn J Clin Oncol*. 2010;40 Suppl 1:i28–37.

4. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki 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;376:687–97.

5. Catalano V, Labianca R, Beretta GD, Gatta G, de Braud F, Van Cutsem E. Gastric cancer. *Crit Rev Oncol Hematol*. 2009;71:127–64.

6. Gravalos C, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Ann Oncol*. 2008;19:1523–9.

7. Garcia I, Vizoso F, Martin A, Sanz L, Abdel-Lah O, Raigoso P, et al. Clinical significance of the epidermal growth factor receptor and HER2 receptor in resectable gastric cancer. *Ann Surg Oncol*. 2003;10:234–41.

8. Allgayer H, Babic R, Gruetzner KU, Tarabichi A, Schildberg FW, Heiss MM. c-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor-associated protease systems. *J Clin Oncol*. 2000;18:2201–9.

9. Park DI, Yun JW, Park JH, Oh SJ, Kim HJ, Cho YK, et al. HER-2/neu amplification is an independent prognostic factor in gastric cancer. *Dig Dis Sci*. 2006;51:1371–9.

10. Yonemura Y, Ninomiya I, Ohoyama S, Fushida S, Kimura H, Tsugawa K, et al. Correlation of c-erbB-2 protein expression and lymph node status in early gastric cancer. *Oncology*. 1992;49:363–7.

11. Hofmann M, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, et al. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology*. 2008;52:797–805.

12. Vance GH, Barry TS, Bloom KJ, Fitzgibbons PL, Hicks DG, Jenkins RB, et al. Genetic heterogeneity in HER2 testing in breast cancer: panel summary and guidelines. *Arch Pathol Lab Med*. 2009;133:611–2.

13. Ananiev J, Gulubova M, Manolova I, Tchernev G. Prognostic significance of HER2/neu expression in gastric cancer. *Wien Klin Wochenschr*. 2011;123:450–4.

14. Begnami MD, Fukuda E, Fregnani JH, Nonogaki S, Montagnini AL, da Costa WL, Jr, et al. Prognostic implications of altered human epidermal growth factor receptors (HERs) in gastric carcinomas: HER2 and HER3 are predictors of poor outcome. *J Clin Oncol*. 2011;29:3030–6.

15. Im SA, Kim JW, Kim JS, Kim MA, Jordan B, Pickl M, et al. Clinicopathologic characteristics of patients with stage III/IV (M(0)) advanced gastric cancer, according to HER2 status assessed by immunohistochemistry and fluorescence in situ hybridization. *Diagn Mol Pathol*. 2011;20:94–100.

16. Yan SY, Hu Y, Fan JG, Tao GQ, Lu YM, Cai X, et al. Clinicopathologic significance of HER-2/neu protein expression and gene amplification in gastric carcinoma. *World J Gastroenterol*. 2011;17:1501–6.

17. Kim KC, Koh YW, Chang HM, Kim TH, Yook JH, Kim BS, et al. Evaluation of HER2 protein expression in gastric carcinomas: comparative analysis of 1414 cases of whole-tissue sections and 595 cases of tissue microarrays. *Ann Surg Oncol*. 2011;18:2833–40.

18. Moelans CB, Milne AN, Morsink FH, Offerhaus GJ, van Diest PJ. Low frequency of HER2 amplification and overexpression in early onset gastric cancer. *Cell Oncol (Dordr)*. 2011;34:89–95.

19. Yan B, Yau EX, Bte Omar SS, Ong CW, Pang B, Yeoh KG, et al. A study of HER2 gene amplification and protein expression in gastric cancer. *J Clin Pathol*. 2010;63:839–42.

20. Zhang XL, Yang YS, Xu DP, Qu JH, Guo MZ, Gong Y, et al. Comparative study on overexpression of HER2/neu and HER3 in gastric cancer. *World J Surg*. 2009;33:2112–8.

21. Yano T, Doi T, Ohtsu A, Boku N, Hashizume K, Nakanishi M, et al. Comparison of HER2 gene amplification assessed by fluorescence in situ hybridization and HER2 protein expression assessed by immunohistochemistry in gastric cancer. *Oncol Rep*. 2006;15:65–71.

22. Koeppen HK, Wright BD, Burt AD, Quirke P, McNicol AM, Dybdal NO, et al. Overexpression of HER2/neu in solid tumours: an immunohistochemical survey. *Histopathology*. 2001;38:96–104.

23. Jaehne J, Urmacher C, Thaler HT, Friedlander-Klar H, Cordon-Cardo C, Meyer HJ. Expression of Her2/neu oncogene product p185 in correlation to clinicopathological and prognostic factors of gastric carcinoma. *J Cancer Res Clin Oncol*. 1992;118:474–9.

24. Yonemura Y, Ninomiya I, Yamaguchi A, Fushida S, Kimura H, Ohoyama S, et al. Evaluation of immunoreactivity for erbB-2 protein as a marker of poor short term prognosis in gastric cancer. *Cancer Res*. 1991;51:1034–8.

25. Uchino S, Tsuda H, Maruyama K, Kinoshita T, Sasako M, Saito T, et al. Overexpression of c-erbB-2 protein in gastric cancer. Its correlation with long-term survival of patients. *Cancer*. 1993;72:3179–84.

26. Lee HR, Kim JH, Uhm HD, Ahn JB, Rha SY, Cho JY, et al. Overexpression of c-ErbB-2 protein in gastric cancer by immunohistochemical stain. *Oncology*. 1996;53:192–7.

27. Pinto-de-Sousa J, David L, Almeida R, Leitao D, Preto JR, Seixas M, et al. c-erb B-2 expression is associated with tumor location and venous invasion and influences survival of patients with gastric carcinoma. *Int J Surg Pathol*. 2002;10:247–56.

28. Shun CT, Wu MS, Lin JT, Chen SY, Wang HP, Lee WJ, et al. Relationship of p53 and c-erbB-2 expression to histopathological features, Helicobacter pylori infection and prognosis in gastric cancer. *Hepatogastroenterology*. 1997;44:604–9.

29. Tanner M, Hollmen M, Junttila TT, Kapanen AI, Tommola S, Soini Y, et al. Amplification of HER-2 in gastric carcinoma: association with Topoisomerase II alpha gene amplification, intestinal type, poor prognosis and sensitivity to trastuzumab. *Ann Oncol*. 2005;16:273–8.

30. Barros-Silva JD, Leitao D, Afonso L, Vieira J, Dinis-Ribeiro M, Fragoso M, et al. Association of ERBB2 gene status with histopathological parameters and disease-specific survival in gastric carcinoma patients. *Br J Cancer*. 2009;100:487–93.

31. Yu GZ, Chen Y, Wang JJ. Overexpression of Grb2/HER2 signaling in Chinese gastric cancer: their relationship with clinicopathological parameters and prognostic significance. *J Cancer Res Clin Oncol*. 2009;135:1331–9.

32. Cho EY, Srivastava A, Park K, Kim J, Lee MH, Do I, et al. Comparison of four immunohistochemical tests and FISH for measuring HER2 expression in gastric carcinomas. *Pathology*. 2012;44:216–20.

33. Downs-Kelly E, Yoder BJ, Stoler M, Tubbs RR, Skacel M, Grogan T, et al. The influence of polysomy 17 on HER2 gene and protein expression in adenocarcinoma of the breast: a fluorescent in situ hybridization, immunohistochemical, and isotopic mRNA in situ hybridization study. *Am J Surg Pathol*. 2005;29:1221–7.

34. Ruschoff J, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, et al. HER2 testing in gastric cancer: a practical approach. *Mod Pathol*. 2012;25:637–50.