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Discordances in HER2 status between primary gastric cancer and corresponding metastatic sites

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Abstract

Objective: Determination of human epidermal growth factor receptor-2 status in advanced gastric cancer is important in clinical decision making. In the trastuzumab for GC trial, trastuzumab-based therapy demonstrated a significant overall survival benefit in patients with human epidermal growth factor receptor-2-positive advanced gastric cancer. Human epidermal growth factor receptor-2 discordance in gastric cancer primary and its metastases has been long debated. The aim of the study was to evaluate the rate of human epidermal growth factor receptor-2 discordance and its effect on treatment decisions in advanced gastric cancer.

Methods: A total of 74 patients with advanced gastric cancer were included in the study. Both immunohistochemical staining and dual-color silver *in situ* hybridization were performed in all patients to evaluate the human epidermal growth factor receptor-2 status of the primary lesion and paired metastasis.

Results: The assessment of human epidermal growth factor receptor-2 status with the immunohistochemical staining method and dual-color silver *in situ* hybridization revealed a discordance rate of 9.5 and 16.2%, respectively. However, this discordance was clinically meaningful in only one patient leading to a change in treatment decision. While this patient had a human epidermal growth factor receptor-2-negative status in primary tumor (immunohistochemical = 0, dual-color silver *in situ* hybridization = negative), the human epidermal growth factor receptor-2 status was positive for liver metastasis (immunohistochemical = 2+, dual-color silver *in situ* hybridization = positive). Trastuzumab was added to the chemotherapy regimen.

Conclusions: In this study, we found a higher rate of human epidermal growth factor receptor-2 discordance between primary gastric tumor and metastatic lesions compared with the rates reported in previous studies. Detection of a human epidermal growth factor receptor-2-positive metastasis with a human epidermal growth factor receptor-2-negative primary tumor suggests that investigation of human epidermal growth factor receptor-2 is also required for the metastatic lesion and that trastuzumab could be administered in the case of a positive result.

Key words: HER2, gastric cancer, discordance, IHC, SISH

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Introduction

Gastric cancer (GC) is the second leading cause of cancer mortality worldwide (1). The majority of the patients are diagnosed at an advanced stage and palliative chemotherapy plays an important role in improving the quality of life and prolongs the survival. The survival of patients with advanced GC remains poor, median overall survival (OS) is 7–10 months (2).

Human epidermal growth factor receptor-2 (*HER2*/neu) is a proto-oncogene located on chromosome 17q21 and a member of human epidermal growth factor receptor (EGFR) family (3). Amplification of the *HER2* gene and overexpression of HER2 protein have been identified in many human epithelial malignancies, including breast cancer and GC (4). In breast cancer, *HER2* gene amplification is reported with frequency of 20–25% and has been identified as a prognostic and predictive marker. HER2 is an important target for GC. Previous studies showing *HER2* amplification or overexpression in 8–54% of GC, especially in intestinal type and distal GC (5). In our recent study, HER2 positivity was 13.7% in Turkish patients (6)

Trastuzumab is a humanized monoclonal antibody directed at the HER2 (7). In the trastuzumab for GC (ToGA) trial, trastuzumab-based therapy demonstrated a significant OS benefit in patients with HER2-positive advanced GC (8). GC samples should be accurately tested for HER2 status as assessed routinely by primarily immunohistochemistry (IHC) and only patients with IHC3+ or IHC2+ tumors and confirmatory *in situ* hybridization (ISH)-positive results are eligible for trastuzumab therapy in metastatic GC.

HER2 testing with IHC and ISH are standard methods in breast cancer. However, HER2 evaluation in GC is different from breast cancer with more heterogenic tumor and the occurrence of basolateral and incomplete membrane staining. Recent studies have shown discordance in HER2 status between primary and metastatic tumors in patients with breast cancer (9). However, it remains unknown in advanced GC. This issue has been addressed in six previous studies showing a high concordance rate in HER2 status between primary tumors and corresponding metastases. (10–13).

The aim of this study was to evaluate the rate of HER2 discordance in primary tumors and metastatic sites and its effect on treatment decisions.

Patients and methods

Study population

A total of 74 patients with advanced GC who received treatment between May 2007 and June 2013 were included in the present study. This study was approved by the local ethics committee of Gazi University. Patients with either biopsy of metastatic sites or surgery of metastases were identified. HER2 status in primary tumors were evaluated in endoscopic biopsies ($N = 12$) and gastric resection specimens ($N = 62$). Fifty-one patients (68.9%) had synchronous, 23 patients (31.1%) had metachronous cancer. Demographics, tumor characteristics, treatment characteristics were retrospectively obtained from patient's reports. Both immunohistochemical staining (IHC) and dual-color silver *in situ* hybridization (SISH) was performed in all patients to evaluate the HER2 status of the primary lesion and the site of metastasis.

HER2 assessment

Immunohistochemistry

Sections with thickness of 4–5 μm, obtained from the paraffin-embedded tissues were transferred onto a polylysine-coated slide and deparaffinized by keeping in a 56°C incubator for 12 h. After deparaffinisation and blocking of endogenous peroxidase, HER2 immunostaining was performed using 'Ab-17 (Lot MS-730-PO), monoclonal, clone: e2 4001+3B5, Thermo Scientific, Cheshire, UK'. Clarification performed in xylene. Tissue sections were coated with Entellan (Shandon Counsul-Mount, Thermo Scientific). HER2 assessment in gastric carcinoma samples was performed in line with the literature references (5,8). Accordingly, scores of 0, 1+, 2+ and 3+ were assigned. Cases where HER2 status showed difference between the primary tumor and metastasis site as assessed by IHC as negative (0 and 1+), equivocal (2+), positive (3+) were considered as discordance.

Dual-color silver *in situ* hybridization (SISH)

HER2 amplification was performed on formalin-fixed, paraffin-embedded tissue using automated Ventana INFORM HER2 Genomic probe platform (Tucson, Arizona, USA). Ventana ultra-View detection kit and Ventana Inform Her2 DNA probe (LOT: A00959) were used for SISH. *HER2* and CEN17 were counted and the copy number was averaged per cell. In each case, *HER2*/CEN17 ratio was assessed. In the SISH assessment, the *HER2* signals and the chromosome 17 signals were estimated by counting in at least 20 cells (14). The samples with a *HER2*/CEN17 ratio ≥2 as detected by SISH method were considered *HER2* positive. The term '*HER2* cluster' was used for cases which contained innumerable *HER2* signals in the tumor nuclei.

Evaluation by IHC and SISH was conducted on primary and metastatic specimens of all patients to assess the HER2 concordance in the sites of primary GC and corresponding metastasis. The rate of HER2 discordance in primary tumors and metastatic sites by SISH method was evaluated.

Statistics

Descriptive statistics were calculated for the baseline demographics and clinicopathologic characteristics. Statistical assessment was performed using SPSS version 21 (SPSS Inc., Chicago, IL). The HER2 positivity was evaluated by using the samples obtained from the primary tumor and the samples from the most distant metastatic site. The change in HER2 status between the primary disease and metastatic disease was compared with the McNemar test. P value <0.05 was considered as a statistically significant.

Results

Patient characteristics

The median age was 61 (range 27 to 80) years. There were 47 males (63.5%) and 27 females (36.5%). Baseline patient characteristics and demographics are shown in Table 1. Fifty-one patients (68.9%) were detected to be at a metastatic stage at the time of diagnosis, 6 (8.2%) had Stage I–II, and 17 had (23%) Stage III disease. The rate of histopathological adenocarcinoma was 47.3%, while the rates of signet-cell adenocarcinoma and adenocarcinoma involving signet cells (mixed) were 27 and 21.6%, respectively. The sites of metastases for which HER2 status was evaluated and outlined in Table 1.

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The concordance between IHC and SISH

The concordance between the IHC and SISH methods for the primary gastric tumor was 89.2%. While eight patients had an IHC score of 0 or 1+, the SISH result was detected positive in primary lesions (Table 2). In primary gastric tumors, three of the six IHC 2+ patients had a positive SISH and three had a negative SISH. The rate of concordance between the IHC and SISH methods was 77.1% for metastatic lesions. Seventeen patients had a HER2 status of 0 or 1+ as assessed by IHC, and a positive result with the SISH method for the metastatic lesions. In metastasis of GC, 1 of the 2 IHC 2+ patients had a positive SISH and the other had a negative SISH. However, none of the patients had a negative HER2 status in the primary tumor and the metastatic site as assessed by the SISH method with an IHC 3+ (Table 3).

Table 1. Patient characteristics

	No. of patients ($N = 74$)	%
Gender		
Male	47	63.5
Female	27	36.5
Age, range	61 (27–80)	
Stage at initial diagnosis		
Stages 1–2	6	8.1
Stage 3	17	23
Stage 4	51	68.9
Primary tumor site		
Distal	38	51.4
Proximal	21	28.4
Linitis plastica	15	20.2
Histological type		
Adenocarcinoma	35	47.3
Signet-cell adenocarcinoma	20	27
Mixed	16	21.6
Adenosquamous	3	4.1
Metastatic sites		
Peritoneum	28	37.8
Liver	16	21.6
Ascitic fluid	5	6.7
Pancreas	4	5.4
Colon	4	5.4
Subcutaneous fat tissue	4	5.4
Distant lymph nodes	3	4.1
Brain	2	2.7
Ovarian	2	2.7
Other sites	6	8.1

Table 2. HER2 status in primary gastric cancer assessed by IHC and SISH

	IHC primary tumors				
	Negative		Equivocal	Positive	Total
	0	**1+**	**2+**	**3+**	
SISH−	52	3	3	0	58
SISH+	8	0	3	5	16
Total	60	3	6	5	74

HER2, human epidermal growth factor receptor-2; IHC, immunohistochemical staining; SISH, dual-color silver *in situ* hybridization.

The discordance between the primary and metastatic lesions by IHC

The results for the IHC staining of the primary and metastatic lesions are presented in Table 4. In primary GC, IHC assessment of the HER2 status revealed a score of 2+ in four patients, and 3+ in one patient while the IHC score was 0 for metastasis. While the IHC score was 2+ in the metastatic site in one patient, the IHC score was 0 for the primary tumor (discordance 9.5%) ($P = 0.231$)

The discordance between the primary and metastatic lesions by SISH

While the HER2 status determined by SISH was negative for primary GC in 56 patients (75.7%), it was positive in 18 patients (24.3%). In metastatic lesions, the HER2 status by the same method was negative in 52 patients (70.3%) and positive (29.7%) in 22 patients. Four of the patients (5.4%) had a HER2-positive primary tumor and HER2-negative metastasis, while 10.8% ($N = 8$) had a negative primary tumor and positive metastasis. In 16.2% of the patients ($N = 12$), a HER2 discordance was detected between the primary lesion and the metastatic lesion with the SISH method (Table 5) ($P = 0.388$). Five and three of the patients had *HER2* cluster amplification in the primary GC and the metastasis, respectively.

The role of HER2 discordance in the treatment of GC

When the IHC staining for primary GC and metastasis was assessed along with the SISH method, discordance was detected in 16 patients

Table 3. HER2 status in metastatic sites assessed by IHC and SISH

	IHC metastatic sites				
	Negative		Equivocal	Positive	Total
	0	1	2	3	
SISH−	49	2	1	0	52
SISH+	17	0	1	4	22
Total	66	2	2	4	74

Table 4. IHC staining of the primary and metastatic lesions

	IHC metastatic sites				
	Negative		Equivocal	Positive	Total
HER2 primary tumors	0	1+	2+	3+	
Negative					
0	57	1	1		59
1+	4				4
Equivocal (2+)	4	1	2		7
Positive (3+)	1			3	4
Total	66	2	3	3	74

Table 5. HER2 SISH status of the primary and metastatic lesions

	HER2 metastatic sites (SISH)		
HER2 primary tumors	SISH−	SISH+	Total
SISH−	48	8	56
SISH+	4	14	18
Total	52	22	64

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(Table 6). Of these 16 patients two had metachronous cancer. We observed that investigation of HER2 for the primary tumor and the metastasis would lead to a difference in administering trastuzumab treatment in only one patient. While this patient had a HER2-negative status in primary tumor (IHC = 0, SISH = negative), the HER2 status was positive for liver metastasis (IHC = 2+, SISH = positive) (Fig. 1). This patient had positive conversion and received trastuzumab with chemotherapy. Only one case exhibited negative-to-positive conversion (IHC 0–2+/SISH negative to IHC 3+ or to IHC 2+/SISH positive). A positive-to-negative conversion in HER2 status (IHC 2+/SISH positive to IHC 0–2+/SISH negative or IHC 3+ to IHC 0–2+/SISH negative) was not observed in our study.

Table 6. Discordance of HER2 status assessed by both IHC and SISH on samples obtained from primary and metastatic sites

Patient no.	Histologic subtype	Primary site IHC	Primary site HER2/CEN17	Primary site SISH	Metastatic sites IHC	Metastatic site HER2/CEN17	Metastatic sites SISH	Metastatic sites
1	Mixed	2	3.00	Positive	0	3.25	Positive	Liver
2	Mixed	0	1.00	Negative	0	2.64	Positive	Ovary
3	Adenocancer	1	1.00	Negative	0	2.82	Positive	Liver
4	Signet cell	0	1.75	Negative	0	1.75	Negative	Peritoneum
5	Adenocancer	0	1.00	Negative	0	2.00	Positive	Liver
6	Mixed	2	25.00	Positive	0	2.80	Positive	Peritoneum
7	Adenocancer	0	1.00	Negative	0	4.43	Positive	Liver
8	Adenocancer	0	1.00	Negative	2	7.50	Positive	Liver
9	Adenocancer	2	2.25	Positive	0	2.40	Positive	Liver
10	Adenocancer	3	30.00	Positive	0	4.17	Positive	Peritoneum
11	Signet cell	0	1.00	Negative	0	2.12	Positive	Peritoneum
12	Adenocancer	0	1.00	Negative	0	2.40	Positive	Colon mesentery
13	Mixed	2	1.50	Negative	1	1.00	Negative	Ascitic fluid
14	Adenocancer	0	2.40	Positive	0	1.00	Negative	Peritoneum
15	Signet cell	2	1.00	Negative	0	1.00	Negative	Pleural fluid
16	Signet cell	0	3.95	Positive	0	1.00	Negative	Peritoneum

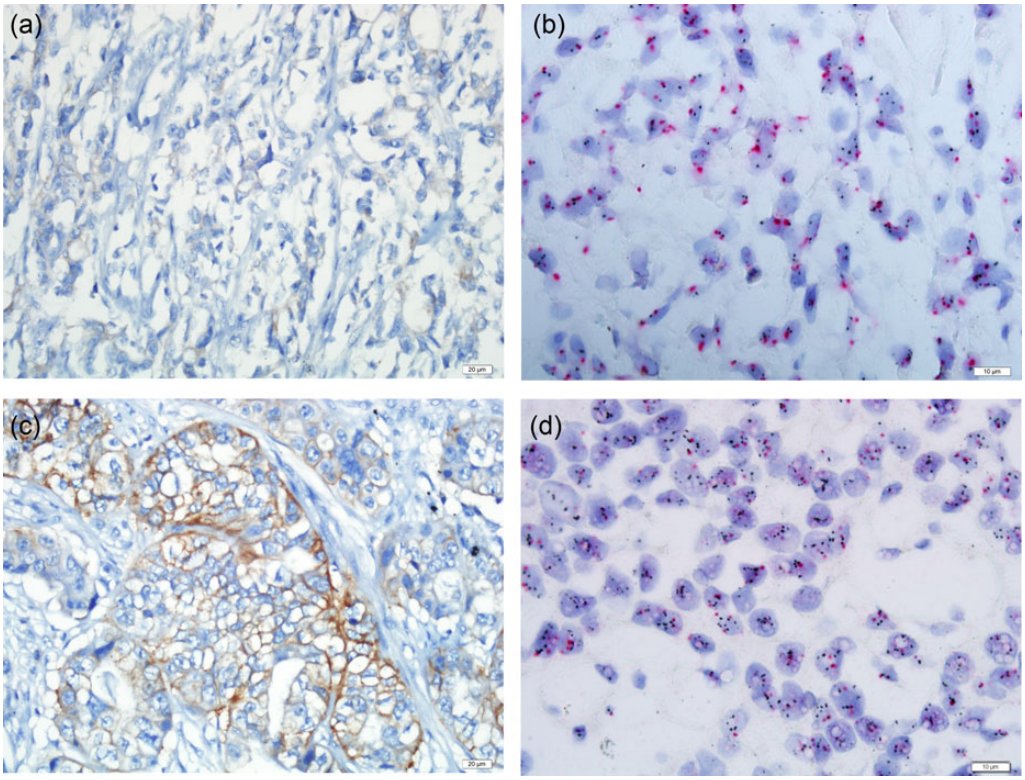


Figure 1. (a) Human epidermal growth factor receptor-2 (HER2) immunohistochemistry in the primary tumor. The score is 0. There is no membranous staining; ×400 magnification. (b) *HER2* dual-color silver *in situ* hybridization in the primary tumor. The signal ratio was 1.0 and there was no amplification; ×1000 magnification. (c) HER2 immunohistochemistry in the liver metastasis. There is membranous staining with moderate intensity corresponding to a score of +2; ×400 magnification. (d) HER2 SISH in the liver metastasis. The signal ratio was 7.5; thus indicating the presence of *HER2* gene amplification; ×1000 magnification.

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Discussion

Amplification of the *HER2* gene and overexpression of HER2 protein in GC was first described in a GC cell line in 1986 (15). HER2 positivity in GC varies widely from 8.2 to 53.4% (5). The reason of this may be the differences in the study populations, the difference of HER2 expression validated HER2 scoring systems. HER2 positivity was 22.1% of all patients in the ToGA trial, and 13.7% of Turkish patients (6,8).

A significant discordance in HER2 status between primary cancer and metastatic sites has been reported in the breast cancer, bladder cancer and prostate cancer (16–18) The concordance between IHC and fluorescence in situ hybridization (FISH) method is less consistent than that observed in breast cancer (19) In the ToGA trial, the concordance between HER2 positivity detected by IHC and FISH was 87% and the differences were largely due to FISH-positive cases (8). In our study, for primary gastric tumors, the rate of concordance between the IHC and SISH method was 89.2% and this was similar to the rate detected between FISH and IHC in previous studies. In a study by Yano et al. (20) the rate of concordance between IHC and FISH was 86.9%. In our study, the rate of discordance in metastatic lesions between IHC and SISH was 22.9%. The higher rate of discordance observed between the two methods (IHC and SISH) in the metastatic site relative to that detected for the primary tumor was an important finding.

The discordance in gastric adenocarcinoma has been evaluated in only six previous studies (10–12,21–23). Marx et al. reported a study in which tissue microarray sections of 166 gastric adenocarcinomas and 69 lymph node metastases were analyzed to determine HER2 overexpression and amplification. They found that HER2 status was identical in 49 primary tumors and the corresponding lymph node metastases by FISH technique (10). Kim et al. (21) found that the incidence of concordant amplification for primary versus metastasis was 97.2%. Bozzetti et al. assessed HER2 status in primary and paired metastatic sites in patients with GC. The rate of concordance was evaluated by FISH method in 68 primary and matched metastatic sites. In this study, the concordance rate was 98.5% and only one patient showed discordance between the primary tumor and metastasis. The concordance of IHC results evaluated in 39 patients was 94.9% (11). In their study, Kochi et al. (12) demonstrated a HER2 concordance rate of 90.2% between the primary gastric and the corresponding lymph node metastasis in 102 patients. Cho et al. (22) found that discrepancies in HER2 status between primary and paired metastatic lymph nodes were in 11% by IHC, 7% by SISH. In another study published in 2014, the HER2 was positive at a rate of 52% for the primary GC, the positive patients had 93 and 100% positivity for the paired lymph node and liver metastases, respectively (13).

In contrast to the studies evaluating HER2 status in primary GC and the paired metastatic site, another study investigated the HER2 status in the biopsy and the paired resection materials of 128 patients with GC. Eighteen patients (14%) were detected to have a positive HER2 status both in the biopsy and the resection material (concordance: 96.1%) (23). In our study, we found a higher rate of HER2 discordance in the primary GC and the paired metastasis compared with the rates reported in previous studies. This may be attributed to the absence of standard and validated scoring systems to evaluate HER2 in GC and the HER2 heterogeneity in the tumor. In breast cancer, *HER2* amplification and expression is highly homogeneous, but in GC HER2 heterogeneity has not been investigated extensively. (21,24,25). Hofmann et al. (5) reported a heterogeneity rate of 4.8% in GC, while Lee et al. (26) detected a rate of 50%. The impact of the intratumoral HER2 heterogeneity on the response to trastuzumab treatment remains unknown in GC. The underlying mechanisms of intratumoral heterogeneity of HER2 amplification were assessed in previous studies. First, it is controversial whether the biopsy is representative of the entire lesion in GC. Biopsies may have better antigen presentation than resection specimens because of smaller size and more rapid formalin fixation. Lee et al. reported that only 57.6% of HER2 positive biopsy cases had HER2 reactivity in all tumor fragments. Another important issue that has been reported previously was HER2 status in gastric dysplasia. There was HER2 positivity in dysplastic epithelium and not in invasive areas in 4.3% cases (27). At the present time, there is neither a definition nor a cutoff value of intratumoral heterogeneity of HER2 amplification in GC. Tajiri et al. (28) used 50% cutoff value and found 41% intratumoral heterogeneity of HER2 amplification. Defining intratumoral heterogeneity in GC and establishing guidelines on detecting this aspect has recently become a significant subject of investigation.

In ToGA trial, median OS for patients whose tumors had low HER2 amplification (IHC 3+ and FISH negative) was 17.5 months in those assigned to trastuzumab plus chemotherapy compared with 17.7 months in those assigned to chemotherapy alone (HR:0.83). The addition of trastuzumab to chemotherapy did not provide benefit in low-level HER2 amplifier cases (IHC 3+ and FISH negative) (8). In our study, no SISH-negative IHC-positive cases were seen. There is no report comparing the outcomes of low- and high-level amplification irrespective of IHC results.

In our study, 16.2% of the patients ($N = 12$) were detected to have a HER2 discordance between the primary lesion and the paired metastatic lesion as assessed by the SISH method. In our study, in metastatic disease positive conversion occurred in eight (10.8%) patients and negative conversion in four (5.4%) patients by SISH and positive conversion was higher than negative conversion. A similar result was observed in Kim et al.'s study; positive conversion was more frequent than negative conversion (2.4 vs. 0.4%) (21). However, negative conversion was more frequent than negative conversion (3 vs. 31%) in Cho et al.'s study (22).

In contrast to our study, where the SISH method was used, the FISH method was used in other relevant studies. The correlation between the FISH method and the SISH method in GC was first assessed in 2011 in a study involving 166 patients. The concordance rate was 96.4%. (23). Park et al. (29) detected a 98.3% rate of concordance between the SISH and FISH methods ($\kappa = 0.927$). Yan et al. (30) showed a 100% concordance between the SISH and FISH methods in 119 specimens. SISH is a bright-field methodology and therefore allow rapid identification of HER2-positive tumor foci within a heterogeneous sample. For this reason, SISH method was reported to be superior compared with other methods in showing GC gene amplification (31,32).

The fact that HER2 was negative in the primary tumor and positive in the metastasis suggested that investigation of the HER2 status also in the metastatic site could lead to administration of trastuzumab treatment. In the ToGA study, trastuzumab was recommended for patients with IHC3+ or IHC2+, confirmed to be positive by the FISH method since trastuzumab is not effective in IHC negative (0 and 1+) patients with HER2 amplification as detected using the FISH method (8). We observed that investigating the HER2 status in the primary tumor and the metastasis could lead to a difference in administering trastuzumab only in patient no. 8, as specified in Table 6. This patient had a negative HER2 status in the primary tumor (IHC = 0, SISH = negative) and a positive status in the liver metastasis (IHC = 2 +, SISH = positive). Trastuzumab was added to the chemotherapy regimen. In our study, only one case showed positive conversion, and no case exhibited negative conversion in HER2 status.

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Being the third study to compare the HER2 status in primary GC and the metastatic sites other than lymph node metastases, our study contributes to the current literature. The rate of discordance found to be higher in the present study compared with those observed in the previous studies is a significant finding. The single-center design of the study and evaluation of the HER2 status by a qualified pathologist enhances the value of the study. The limitations of the study include the small sample size and the fact that the metastatic sites were peritoneum and ascites fluid in 33 patients.

In conclusion, there are limited number of studies investigating the HER2 status both in the primary and the metastatic lesions in metastatic GC. These studies rarely reveal discordance and the impact of this on treatment outcomes has not been studied previously. While detection of a HER2-positive metastasis with a HER2-negative primary tumor suggests that investigation of HER2 is also required for the metastatic lesion and that trastuzumab could be administered in the case of a positive result, it also shows that the further elucidation of this subject requires randomized prospective studies.

Conflict of interest statement

None declared.

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