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ORIGINAL ARTICLE

Serum HER2 as an adjunct to assess HER2 status for advanced gastric cancer: A prospective multicenter trial (SHERLOCK)

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ABSTRACT

Background: Intratumoral human epidermal growth factor receptor 2 (HER2) heterogeneity of gastric cancer can be an obstacle to accurate HER2 assessment. Serum HER2, concentrations of the HER2 extracellular domain shed into the bloodstream, has a potential to compensate HER2 immunohistochemistry (IHC) but has not been scrutinized in gastric cancer. This study sought to explore the clinical utility of serum HER2 in gastric cancer.

Methods: We performed a prospective multicenter trial (SHERLOCK trial) involving patients with all-stage gastric or gastro-esophageal junction cancer. Serum HER2 was measured using direct chemiluminescence while tissue HER2 status was determined using IHC and fluorescent in situ hybridization. For stage IV cases, concordance between local and central laboratories in tissue HER2 assessment was also evaluated.

Results: Of 224 patients enrolled, both tissue HER2 status and serum HER2 levels were successfully determined in 212 patients and 21% (45/212) were tissue HER2-positive. Serum HER2 levels, ranged from 4.5 to 148.0 ng/ml (median 10.3), correlated with tissue HER2 status ($p = 0.003$). At a cut-off level of 28.0 ng/ml determined by receiver operating characteristics analysis, sensitivity, specificity, positive and negative predictive values of serum HER2 were 22.6%, 100%, 100% and 82.3%, respectively. All nine cases with elevated serum HER2 were tissue HER2-positive stage IV cases. Among 61 stage IV cases, the agreement rate for IHC scoring between the local and the central laboratories was 82% and tissue HER2 judgment was conflicting in five (8.2%) cases. Of these five cases, four were confirmed as false-negative and two of these four patients demonstrated elevated serum HER2.

Conclusions: Serum HER2 levels correlated with tissue HER2 status in gastric cancer. Although the low sensitivity is a drawback, serum HER2 might be a useful adjunct tool to detect tissue HER2 false-negative gastric cancer.

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The human epidermal growth factor receptor 2 (HER2) protein, which is a 185-kDa transmembrane tyrosine kinase (TK) receptor and a member of the epidermal growth factor receptor (EGFR) family, has important roles in cell growth, differentiation and survival [1]. HER2 is overexpressed mainly through gene amplification in a subset of breast cancers as well as in other solid tumors [2,3]. With regard to gastric cancer, HER2 overexpression rate varies from 7% to 34% [4–6] and it was 22% in a large scale international prospective trial (ToGA) [6]. Trastuzumab (Herceptin™; Genentech Inc., South San Francisco, CA, USA) is a humanized monoclonal antibody directly binding to HER2 extracellular domain (ECD). The study demonstrated adding trastuzumab to platinum-fluoripyrimidine chemotherapy improved time-to-progression and overall survival in patients with HER2-positive advanced gastric cancer [6]. Consequently, tissue HER2 assessment by

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immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) has become routine practice in the management of advanced gastric cancer. However, gastric cancer frequently exhibits heterogeneous HER2 overexpression [5,7,8] in contrast to breast cancer that generally shows homogenous pattern. Incomplete and basolateral membrane immunoreactivity also hampers accurate tissue HER2 assessment in gastric cancer [8]. Past studies demonstrated potential risk of false-negative in tissue HER2 assessment for unresectable gastric cancer [9,10]. Substantial discordance in HER2 assessments among laboratories [11] as well as pathologists [12] was also reported.

The HER2 receptor is composed of an intracellular domain with TK activity, a transmembrane domain and ECD. Serum HER2 is the circulating HER2 ECD shed from the surface of tumor cells, and thus it could be used as a diagnostic marker for tissue HER2 status. Numerous studies have investigated the clinical utility of serum HER2 analysis in breast cancer and recent reviews concluded that there is insufficient evidence to support the use of serum HER2 in the clinical management of breast cancer patients [13–15]. In contrast, only a small number of retrospective studies have investigated serum HER2 in gastric cancer to date and its clinical significance has not been determined.

SHERLOCK (Serum Her2/neu elevation in gastric cancer; a multicenter trial in Hokkaido, UMIN 000009773) was a prospective multicenter study aimed to evaluate the correlation between tissue HER2 status and serum HER2 levels and their relationship with clinicopathological parameters in gastric cancer. We also evaluated discrepancies in tissue HER2 assessment between local and central laboratories.

Material and methods

Study populations

This prospective multicenter trial was conducted at 14 hospitals in Hokkaido, Japan. Twenty years or older patients with histologically confirmed adenocarcinoma of the stomach or gastro-esophageal junction were included in the study. Major exclusion criteria were previous chemotherapy and other active malignancy or prior malignancy in the previous five years. Recorded clinical and pathological features for each patient included age, gender, histological classification, tumor stage according to the seventh edition of the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification, surgical treatment and adjuvant therapy, and chemotherapy. The protocol and consent form for this study were approved by the institutional review board at each participating hospital. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study.

Serum HER2 assay

Pretreatment blood samples were collected from all patients into sterile tubes. After centrifugation, serum samples were stored at −20 °C until analysis. Serum HER2 levels were measured using an ADVIA Centaur HER2/neu assay (Siemens Healthcare Diagnostic Co., Ltd., Tokyo, Japan), a sandwich immunoassay that employs direct chemiluminescence technology using two monoclonal antibodies against the ECD of the HER2 antigen.

Tissue HER2 analysis

Paraffin-embedded block or unstained 4-μm sections of tumor tissue were submitted for central laboratory testing for each patient. The central laboratory performed HER2 IHC using the HercepTest™ (Dako, Glostrup, Denmark) in strict accordance with the manufacturer's instructions. HER2 immunoreactivity was evaluated according to the scoring criteria specifically developed for gastric cancer [5], which were applied to the ToGA trial. In IHC 2 + tumors, FISH analyses were carried out using the PathVysion™ HER2 DNA probe kit (Abbott Laboratories, Abbott Park, IL, USA). The results were reported as the ratio of the average copy number of the *HER2/neu* gene to that of the chromosome 17 centromere. Specimens with a signal ratio of 2.0 or greater were considered as amplified. IHC 3 + cases or cases that were IHC 2 + and FISH-positive were considered as tissue HER2-positive.

For stage IV cases, tissue HER2 was first assessed by the local laboratories of each hospital using various anti-HER2 antibodies including the HercepTest™ (Dako), the PATHWAY HER2/neu (4B5) rabbit monoclonal antibody (Ventana Medical System, Tucson, AZ, USA), and the SV2-61γ monoclonal antibody (Nichirei Biosciences, Tokyo, Japan). The central laboratory then reassessed the same samples using HercepTest™ in a blinded manner. When IHC results were conflicting between the local and the central laboratories, the central laboratory performed additional IHC using SV2-61γ as well as FISH.

Statistical analysis

Chi-square and Mann-Whitney U-tests were used to assess associations among clinicopathological features, tissue HER2 expression and serum HER2 levels. Kruskal-Wallis test was used to assess correlation between serum HER2 level and IHC staining. All *p*-values were two-sided and results were considered significant when *p*-values were less than 0.05. Statistical analyses were carried out using statistical softwares EZR (http://cran.r-project.org) and GraphPad prism (La Jolla, CA, USA).

To determine efficient cut-offs and to evaluate their applicability, samples were randomly sorted into two groups in a 7:3 ratio (training set and test set) for validation analysis. Receiver operating characteristic (ROC) curves for tissue HER2-positive cases were constructed on the basis of serum HER2 levels, followed by calculation of the area under the curve (AUC). Serum HER2 cut-off levels were defined based on the shape of the ROC curves.

Results

Patient profiles and tissue HER2 status

A total of 224 patients were enrolled from 14 institutions from July 2011 to July 2013 (Figure 1). Twelve patients were

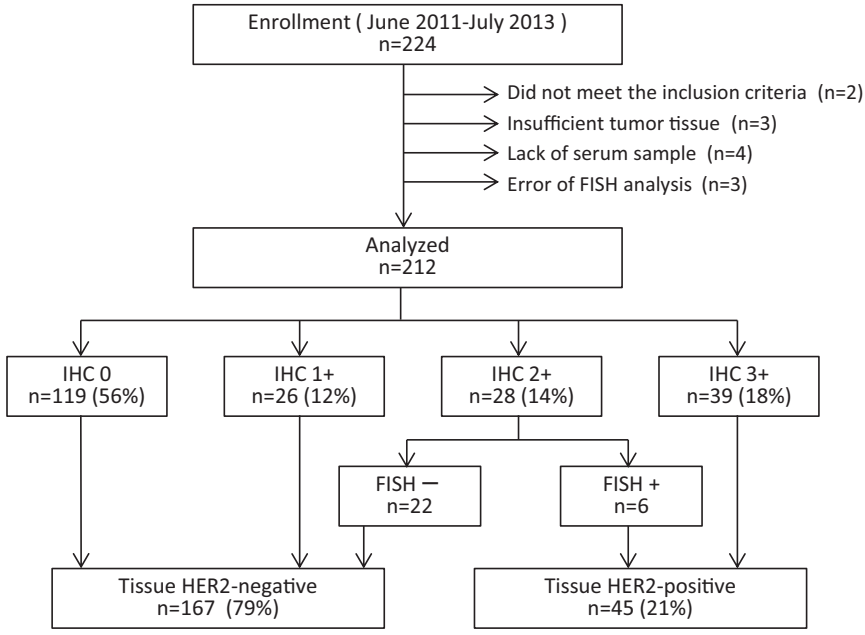


Figure 1. Study population and tissue HER2 status.

Table I. Clinical characteristics of tissue HER2-negative and positive patients.

All	Total ($n=212$)	tHER2-negative ($n=167$)	tHER2-positive ($n=45$)	p-value
Age (years)				
Median (range)	70 (33–90)	71 (40–90)	67 (33–88)	0.008
Gender				
Male	143 (67%)	106 (63%)	37 (82%)	0.020
Female	69 (33%)	61 (37%)	8 (18%)	
Tumor location				
Upper and EGJ	43 (20%)	30 (18%)	13 (30%)	0.182
Middle	75 (35%)	63 (38%)	12 (27%)	
Lower	94 (44%)	74 (44%)	20 (43%)	
Lauren classification				
Intestinal	113 (53%)	83 (50%)	30 (67%)	0.045
Diffuse and mixed	99 (47%)	84 (50%)	15 (33%)	
Stage				
I	115 (54%)	100 (60%)	15 (33%)	0.008
II	14 (7%)	11 (7%)	3 (6%)	
III	17 (8%)	12 (7%)	5 (11%)	
IV	66 (31%)	44 (26%)	22 (49%)	
Stage IV cases	Total ($n=66$)	tHER2-negative ($n=44$)	tHER2-positive ($n=22$)	p-value
No. of metastatic sites				
1	24 (36%)	18 (41%)	6 (27%)	0.416
>2	42 (64%)	26 (59%)	16 (73%)	
Liver metastasis				
Yes	27 (41%)	11 (25%)	16 (73%)	≤0.001
No	39 (59%)	33 (75%)	6 (27%)	
Lung metastasis				
Yes	7 (11%)	5 (11%)	2 (9%)	1.000
No	59 (89%)	39 (89%)	20 (91%)	
Peritoneum metastasis				
Yes	30 (45%)	22 (50%)	8 (36%)	0.432
No	36 (55%)	22 (50%)	14 (64%)	

excluded; two did not meet the inclusion criteria, seven had insufficient tumor tissue or serum samples, and three had errors in FISH analysis. The remaining 212 patients were fully evaluated. Of the 212 patients, 39 (18%) and 28 (14%) were IHC score 3 + and 2+, respectively. FISH revealed *HER2* amplification in six (21%) of the 28 IHC 2 + cases. Consequently, tissue HER2 was positive in 45 (21.2%) patients.

The clinical features of the 212 patients examined and the relationships between tissue HER2 status and clinicopathological characteristics are summarized in Table I. Of all patients, the median age was 70 years old (range, 33–90), 67% were male, 53% was Lauren's intestinal type. Half of the patients (54%) were at stage I while 31% were at stage IV. Compared with tissue HER2-negative cases, HER2-positive gastric cancer patients were younger, more male-predominant and more intestinal type-predominant, and tissue HER2-positive rates increased as the stage advanced ($p=0.008$). Tumor location had no correlation with tissue HER2 status. In stage IV patients, liver metastasis was observed more frequently in the HER2-positive than in the HER2-negative group ($p<0.001$), while the rates of lung and peritoneal metastasis were similar between the two groups.

Serum HER2 levels

The median serum HER2 level of the 212 patients was 10.3 ng/ml (SD = 13.8 ng/ml, range 4.5–148.0). The relationship between serum HER2 levels and tissue HER2 IHC scores is shown in Figure 2a. Kruskal-Wallis test demonstrated statistically significant associations between serum HER2 level and IHC staining ($p=0.028$) and the serum HER2 level of HER2 IHC 3 + patients was significantly higher than that of patients with a score 0 ($p=0.012$) using Steel's test. The mean serum HER2 levels of the tissue HER2-positive and negative groups were 21.8 ng/ml (SD = 27.7 ng/ml, range 6.5–148.0 ng/ml) and 10.6 ng/ml (SD = 3.4 ng/ml, range 4.5–27.2 ng/ml), respectively. There was a significant correlation between the serum HER2 level and tissue HER2 status ($p=0.003$) (Figure 2b).

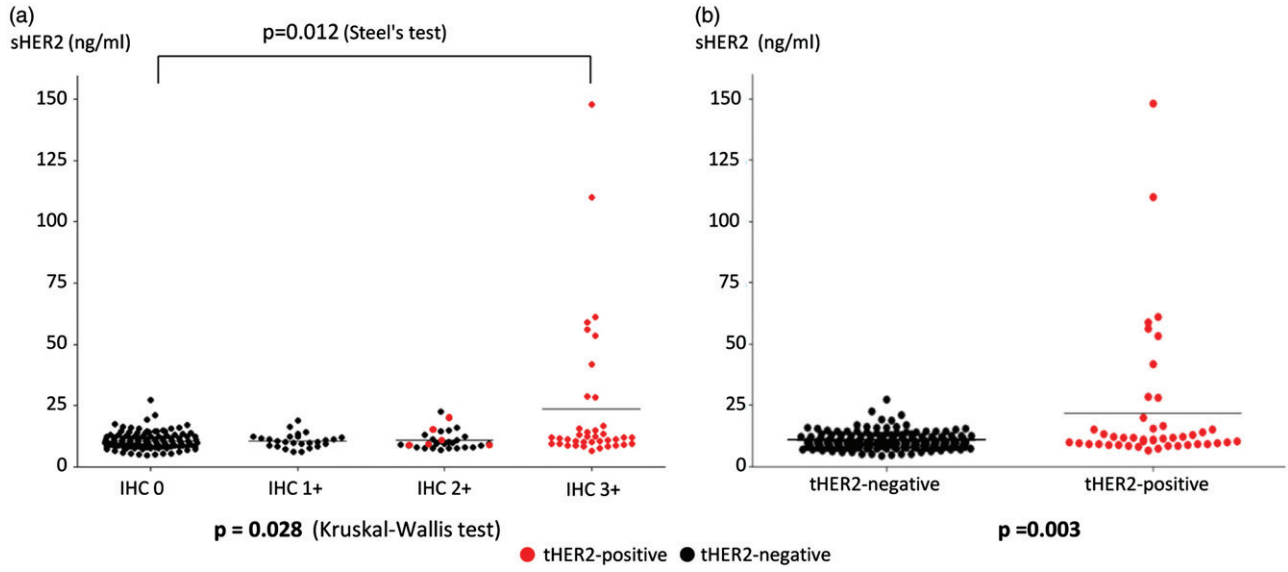


Figure 2. (a) Correlation between serum HER2 levels and tissue HER2 IHC scores. (b) Correlation between serum HER2 levels and tissue HER2 status.

Table II. Serum HER2 status in 45 tissue HER2-positive cases.

	sHER2 < 28 ($n = 36$)	sHER2 ≥ 28 ($n = 9$)	p-value
Age			
Median (range)	68 (33-88)	65 (56-81)	0.435
Gender			
Male	27 (75%)	9 (100%)	0.179
Female	9 (25%)	0 (0%)	
Tumor location			
Upper and GE junction	10 (28%)	3 (33%)	0.571
Middle	11 (31%)	1 (11%)	
Lower	15 (42%)	5 (56%)	
Lauren classification			
Intestinal	27 (75%)	5 (56%)	0.411
Diffuse and Mixed	9 (25%)	4 (44%)	
Stage			
I	15 (42%)	0 (0%)	0.001
II	3 (8%)	0 (0%)	
III	5 (14%)	0 (0%)	
IV	13 (36%)	9 (100%)	
Stage IV cases	sHER2 < 28 ($n = 13$)	sHER2 ≥ 28 ($n = 9$)	p-value
No. of metastatic sites			
1	4 (31%)	2 (22%)	1.000
>2	9 (69%)	7 (78%)	
Liver metastasis			
Yes	9 (69%)	7 (78%)	1.000
No	4 (31%)	2 (22%)	
Lung metastasis			
Yes	0 (0%)	2 (22%)	0.156
No	13 (100%)	7 (78%)	
Peritoneum metastasis			
Yes	4 (31%)	4 (44%)	0.662
No	9 (69%)	5 (56%)	

To determine the optimal cut-off level for serum HER2, we divided the subjects into two groups; a training set for cut-off determination, and a test set for validation. Two cut-offs were tentatively calculated by ROC analysis of the training set: a cut-off at 16.5 ng/ml yielded 94.8% specificity and 29.0% sensitivity to detect tissue HER2-positive gastric cancer while a cut-off at 28.0 ng/ml yielded 100% specificity and 22.6% sensitivity (AUC, 0.647; 95% CI 0.534–0.760) (Figure 3a). These results were validated by the test set that demonstrated similar trends (Figure 3b). When subjects were limited to stage IV cases, sensitivity and specificity of serum HER2 at a cut-off of 28 ng/ml were 41% and 100%, respectively.

Figure 4 shows the correlation among serum HER2 levels, tissue HER2 status and TNM stages. Using the cut-off value of 28.0 ng/ml, all elevated serum HER2 cases ($n = 9$) were tissue HER2-positive stage IV cancer. Even if 16.5 ng/ml was adopted as the cut-off value, 17 of the 18 (94.4%) elevated serum HER2 cases were at stage IV and 10 (55.6%) were tissue HER2-positive.

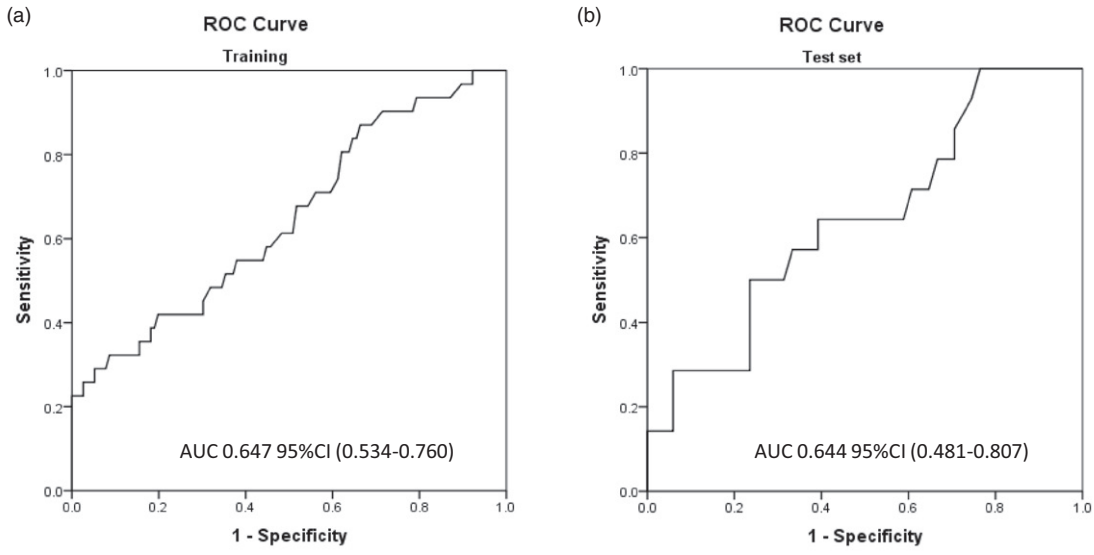
Serum HER2 levels in tissue HER2-positive patients

Using the cut-off level of 28.0 ng/ml, serum HER2 was elevated in nine of the 45 tissue HER2-positive cases while it was normal in the remaining 36. When these 45 tissue HER2-positive cases were divided into the elevated and normal serum HER2 groups, elevated serum HER2 was significantly associated with advanced tumor stages ($p = 0.001$). There were no differences in other parameters between the two groups (Table II).

Tissue HER2 assessment by local and central laboratories and serum HER2

For 61 stage IV cases, IHC was performed by both the local and the central laboratories using the same samples (Table III). The local laboratories used various anti-HER2 antibodies while the central laboratory performed IHC using HercepTest. The agreement rate between the local and the central laboratories for IHC scoring was 82.0%. When FISH analysis was added for IHC 2+ cases, the final judgments (tissue HER2-positive or negative) by the local and the central laboratory agreed in 56 (91.8%) while conflicted in five of 61 (8.2%) cases (Table III).

Details of the five cases with discordant results are shown in Table IV. Cases #1 to #4 were initially scored as IHC 0 or 1+ by the local laboratories but the central laboratory judged as 3+. Reevaluation using the SV2-61γ antibody and FISH proved



Cut-off	Negative	Positive
≤ 16.5	110	22
> 16.5	6	9

Sensitivity : 29.0%
Specificity : 94.8%

Cut-off	Negative	Positive
≤ 16.5	50	12
> 16.5	1	2

Sensitivity : 14.3%
Specificity : 98.0%

Cut-off	Negative	Positive
≤ 28.0	116	24
> 28.0	0	7

Sensitivity : 22.6%
Specificity : 100.0%

Cut-off	Negative	Positive
≤ 28.0	51	12
> 28.0	0	2

Sensitivity : 14.3%
Specificity : 100.0%

Figure 3. (a) The ROC curve of serum HER2 on a training set of 147 subjects. $n = 147$ (70% of all subjects) (Positive: 31, Negative: 116). (b) The ROC curve of serum HER2 on a test set of 65 subjects. $n = 65$ (30% of all subjects) (Positive: 14, Negative: 51).

tissue HER2-positive in all the four cases. Of note, cases #1 and #2 demonstrated elevated serum HER2 levels (61.2 and 53.3 ng/ml, respectively). Discrepant IHC findings for case #2 were shown in Figure 5. Initial diagnosis by the local laboratory was tissue HER2-negative (score 1+, Figure 5a,b) but IHC by the central laboratory showed a strong membranous staining (score 3+, Figure 5c,d) and serum HER2 was elevated (53.3 ng/ml). After tissue HER2-positivity was confirmed by IHC using two antibodies as well as FISH (Figure 5e), trastuzumab was added to the chemotherapy.

Discussion

Over the past two decades, many studies have investigated clinical utility of serum HER2 levels in breast cancer and they were recently reviewed. As summarized by Leyland-Jones et al. [15], 16 studies demonstrated significant association between serum HER2 levels and tissue HER2 status while 10 studies did not. The review therefore does not recommend serum HER2 level as a determinant of HER2 status in breast cancer. However, the target population as well as methodological approach for serum HER2 testing varied between the studies. Seven of the 10 studies showing negative results included significant number of stage I–III cases. In contrast, 12 of the 16 studies with positive results limited the subjects to metastatic breast cancer. These results suggest that serum HER2 level elevates when HER2-positive breast cancer metastasizes to distant organs.

To the best of our knowledge, only seven studies [16–22] to date have investigated the serum HER2 level in gastric cancer and results were conflicting like breast cancer: five studies [16,19–22] demonstrated significant correlation between serum and tissue HER2 levels while two [17,18] did not. Similar to the condition of breast cancer studies, the latter included only 13% [17] or no [18] metastatic gastric cancer. Kono and colleagues [16] reported a significant relationship between serum and tissue levels of the HER2/neu protein in 57 gastric cancers. They also commented that serum HER2 levels were higher in stage IV cases than in stage I–III cases. Our study also demonstrated significant correlation between serum and tissue HER2 levels and serum HER2 was elevated mostly in metastatic tissue HER2-positive cases (Figure 4). These findings suggest that it would be appropriate to limit serum HER2 test to metastatic gastric cancer patients who might be eligible for the HER2-targeted therapy.

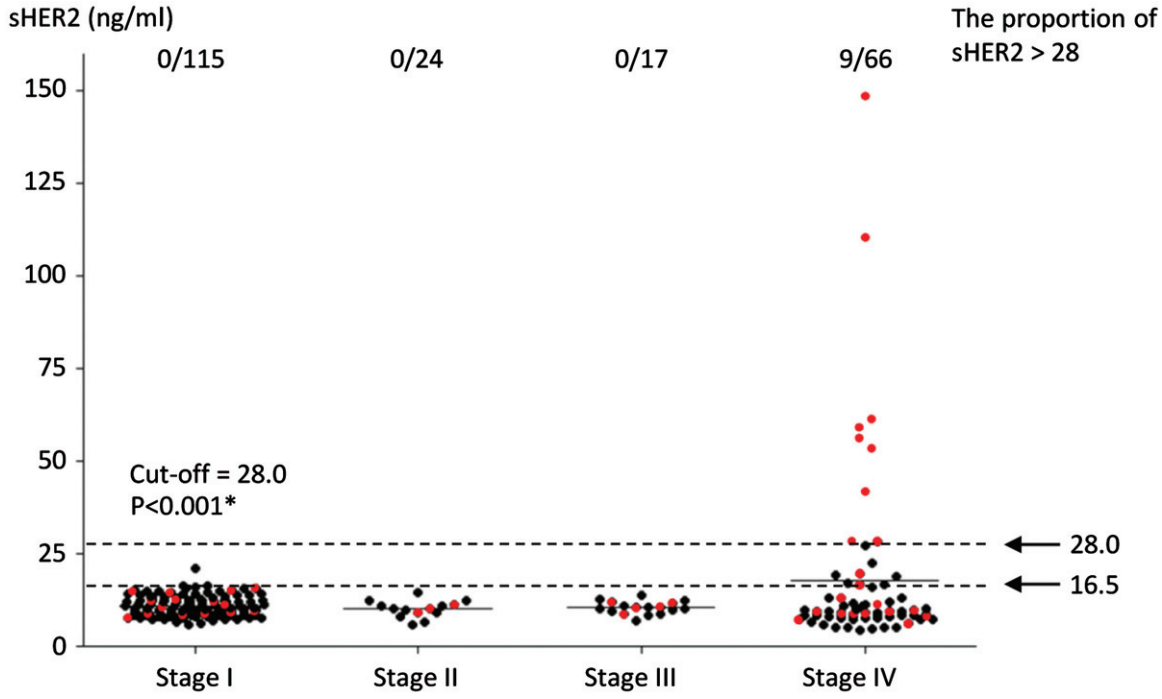


Figure 4. Serum HER2, tissue HER2 and TNM stages. *The proportion of each tumor stage group that was serum HER2 >28 ng/ml was compared using Fisher's exact test.

Table III. HER2 testing by local and central laboratories.

	Central Lab.							Agreement rate
	IHC 0/1+	IHC 2+	IHC 3+	Total	tHER2-negative	tHER2-positive	Total	
Local Lab.								
IHC 0/1+	32	1	4	37	-	-	-	82.0%
IHC 2+	3	3	0	6	-	-	-	
IHC 3+	1	2	15	18	-	-	-	
Total	36	6	19	61	-	-	-	
tHER2-negative	-	-	-	-	39	4	43	91.8%
tHER2-positive	-	-	-	-	1	17	18	
Total	-	-	-	-	40	21	61	

Table IV. Cases with a discordant IHC result.

No.	Histology	Liver metastasis	Local Laboratories	Central Laboratories			sHER2 (ng/mL)
				IHC score			
			IHC score (Anti-HER2 antibody)	HercepTest	SV2-61γ	FISH	
1	intestinal	Yes	0 (Pathway)	3+	3+	UE	61.2
2	mixed	Yes	1+ (Pathway)	3+	3+	+	53.3
3	diffuse	No	0 (HercepTest)	3+	3+	+	9
4	diffuse	No	0 (Pathway)	3+	0	+	8.7
5	intestinal	Yes	3+ (Pathway)	1+	2+	–	18.9

UE: unevaluable.

Cut-off value of serum HER2 has been set around 15.0 ng/ml in most studies of breast cancer [23,24] while it has not been validated in gastric cancer. Past studies of gastric cancer also set cut-off value at 15.0–16.5 ng/ml and low sensitivity (36.0–53.0%) was a common issue [19,20,22]. We selected two cut-off values (16.5 ng/ml and 28.0 ng/ml) from the ROC analysis but either cut-off value yielded low sensitivity (29.0% and 22.6%, respectively). Of the 45 cases with positive tissue HER2 status, only nine and 11 cases were positive for serum HER2 when the cut-off values were 28 and 16.5 ng/ml, respectively. Consequently, serum HER2 testing by itself is not suitable for screening purposes and cannot a substitute for IHC and FISH. However, 100% specificity and 100% PPV were obtained when the cut-off value was elevated to 28.0 ng/ml. Dai et al. also indicated a specificity of 100% with a cut-off value of 22 ng/ml [19]. Considering risks of tissue HER2

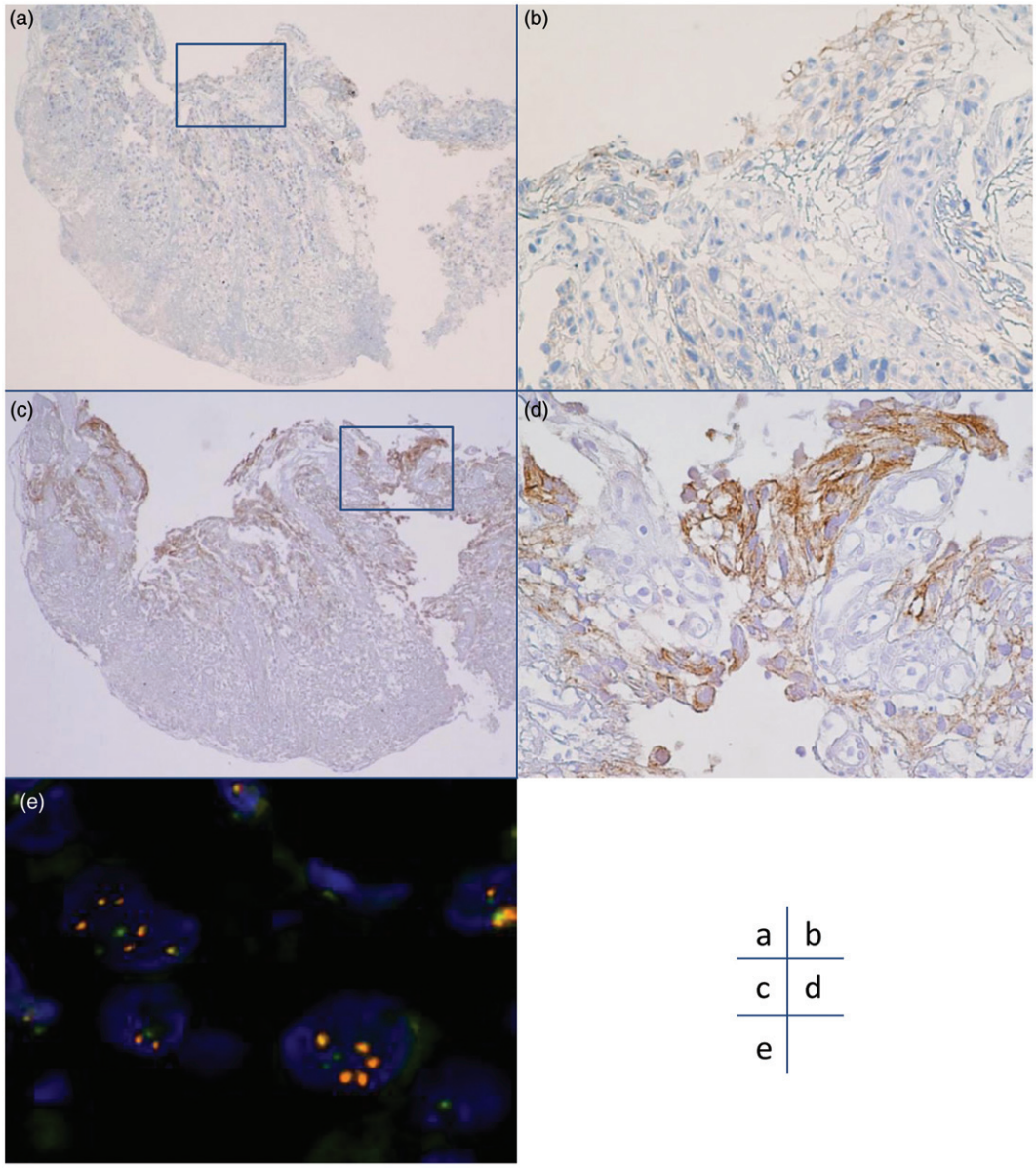


Figure 5. HER2 testing (Case #2). (a,b) The HER2 IHC assay (Pathway) at the local laboratory showed a faint, partial membrane staining (scored as 1+). (×40, ×400, respectively); (c,d) The HER2 IHC assay (Herceptest™) at the central laboratory revealed some tumor cell clusters with a strong complete membrane staining (scored as 3+). (×40, ×400, respectively); (e) Fluorescence in situ (FISH) analysis showed a high level HER2 amplification.

misjudgment, we would like to recommend 28.0 ng/ml as a cut-off value for advanced gastric cancer, limiting the purpose to identify tissue HER2 false-negative cases.

The inter-laboratory discrepancy of tissue HER2 assessment was substantial in our study, and it has also demonstrated in breast cancer [25,26]. Perez et al. evaluated diagnostic agreement rates of tissue HER2 status among local, central and reference laboratories in a large scale adjuvant trastuzumab trial for breast cancer [25]. The discordance rate was 12.0% (305/2535) between the local and the central laboratories while it was lower between the central and the reference laboratories. Huang et al. also demonstrated a similar trend in gastric cancer and they suggested that the main reason for this discrepancy was misinterpretation of the IHC score by local laboratories [11]. Methodological factors may also contribute to the discordance between laboratories, e.g. IHC testing can yield false-negative results when the HER2 epitope is destroyed through longer formalin fixation time. In addition, intratumoral HER2 heterogeneity might be a cause of misjudgment of tissue HER2 status in gastric cancer [9,10]. Although 6–8 biopsy samplings are recommended for accurate HER2 assessment in unresectable gastric cancer [8], this is not always feasible due to tumor bleeding. Tissue HER2 analysis by trained pathologists at experienced laboratories using sufficient number of samples is ideal but difficult in most clinical settings. In our study, of the four cases with false-negative tissue HER2 status determined by the local laboratories, two cases had elevated serum HER2 levels. Adopting higher cut-off value than for breast cancer, serum HER2 could be an adjunct to IHC in order to identify tissue HER2 false-negative gastric cancer.

There is another possibility of serum HER2 for advanced gastric cancer patients who have discordance of HER2 status

between primary and metastatic site. A meta-analysis concluded that HER2 discordance between paired primary and metastatic site is not rare, demonstrating 7% (95% CI 5–10%) of the pooled discordance rate [27]. As biopsy of metastatic site cannot be easy to be carried out, serum HER2 might be useful tool to find out HER2-positive metastases with negative primary gastric cancer patients.

HER2 testing algorithms in gastric cancer still vary among guidelines [28]. The guideline by the Japanese society of pathology as well as European society for medical oncology (ESMO) recommends IHC for the initial screening and FISH for IHC 2 + patients [29,30] while concurrent IHC and FISH test irrespective of IHC results is still common among National Cancer Institute (NCI)-designated cancer center [31]. Serum HER2 testing is less invasive, relatively inexpensive and quick manner, and our study demonstrated the potential of serum HER2 to detect false-negative results of tissue HER2 status. Thus serum HER2 testing in combination with tissue-based analysis might be a reasonable approach for unresectable gastric cancer. When serum HER2 are elevated in IHC 0 or 1 + cases, FISH analysis, IHC reevaluation or re-biopsy could be beneficial.

In conclusion, serum HER2 levels correlated with tissue HER2 status in gastric cancer. As trastuzumab has demonstrated significant positive impact on treatment for unresectable HER2-positive gastric cancer, efforts should be made to minimize HER2 false-negative cases. Although the low sensitivity is a drawback, analysis of serum HER2 might compensate for the aforementioned problems of tissue HER2 evaluation in advanced gastric cancer. Larger scale prospective studies are needed to confirm these findings.

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Declaration of interest

MS has received honoraria from Chugai and Takeda. KY has received research funding from Taiho and received honoraria from Chugai, Takeda and Ajinomoto. YA has received research funding from Astellas, AstraZeneca, Eizai and Takeda and received honoraria from Kyorin and Takeda. YH has received honoraria from Chugai. YS has received research funding from Pfizer, Novartis, Chugai and Daiichi Sankyo, and received honoraria from Eizai, Daiichi Sankyo, and Mitsubishi-Tanabe. All remaining authors have declared no conflicts of interest. The funding source of the study had no role in study design; in the collection, analysis, and interpretation of the data; or in the writing of this report.

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