HER2-positive gastric cancer identified by serum HER2: A case report

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Abstract. Immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) are the current standards methods for the determination of tissue human epidermal growth factor receptor 2 (HER2) status in gastric cancer, as for breast cancer. However, HER2-positive gastric cancer occasionally exhibits heterogeneous tissue HER2 overexpression, raising concern regarding false-negative results in unresectable cases diagnosed by biopsy samples. Serum HER2, the concentration of the extracellular domain of HER2 protein shed into the bloodstream, has the potential to supplement the use of IHC or FISH to determine HER2 status. However, the clinical significance of serum HER2 has not been well studied in gastric cancer. The present study describes an illustrative case of metastatic gastric cancer initially diagnosed as HER2-negative (IHC score 1+). The patient exhibited an elevated serum HER2 level, which prompted a reevaluation of the tissue by IHC, using an alternative antibody, and FISH; re-biopsy analyses confirmed the case as HER2-positive, and trastuzumab was subsequently added to the combination chemotherapy with capecitabine and cisplatin. Serum HER2 may aid in avoiding false-negative diagnoses of HER2 gastric cancer.

Introduction

Since the international phase III trial demonstrating the survival benefit of trastuzumab for patients with human epidermal growth factor receptor 2 (HER2)-positive gastric cancer (1), tissue HER2 assessment has become a routine practice in patients with advanced gastric cancer. In contrast to HER2-positive breast cancer, which generally exhibits homogenous HER2 overexpression, gastric cancer frequently exhibits intratumoral heterogeneity of HER2 overexpression (2-5). In addition, recent studies have demonstrated substantial inter-laboratory, as well as inter-observer discordance in tissue HER2 assessments (6,7). This may be responsible for generating HER2 false-negative results, depriving the patient of the opportunity for anti-HER2 treatment.

Serum HER2 is the HER2 extracellular domain that sheds from the surface of cancer cells into the circulation, and may be quantified by chemiluminescence immunoassay (CLIA). The clinical significance of serum HER2 has been reported in breast cancer (8-13); however, few studies have investigated this in gastric cancer. The current report presents our experience with an illustrative case of HER2-positive metastatic gastric cancer that was initially diagnosed as HER2-negative and salvaged by serum HER2.

Case report

In October 2012, a 56-year-old male presented to Sapporo Medical University Hospital (Sapporo, Japan) with dysphagia and weight loss. The patient's past history was unremarkable, and physical examination revealed mild epigastric tenderness and a palpable liver. The baseline laboratory tests demonstrated elevated liver enzymes (aspartate transaminase, 148 IU/l, normal range, 11-39 IU/l; alanine transaminase, 89 IU/l, normal range, 5-40 IU/l; alkaline phosphatase, 1,292 IU/l, normal range, 110-370 IU/l; and lactate dehydrogenase, 2,976 IU/l, normal range, 119-229 IU/l) and elevated carbohydrate antigen 19-9 (131.9 U/ml; normal range, 0.0-37.0 U/ml). Upper gastrointestinal endoscopy revealed a Borrmann type III cancer (14) from the cardia to the lower esophagus (Fig. 1A), and computed tomography (CT) imaging revealed multiple liver and nodal metastases (Fig. 1B). The pathological diagnosis of three biopsy specimens was moderately to poorly differentiated tubular adenocarcinoma. Immunohistochemistry (IHC) for HER2 was performed on formalin-fixed, 4-μm thick, paraffin-embedded tissue sections (SurgiPath Paraplast; Leica Biosystems, Wetzlar, Germany) using a monoclonal rabbit PATHWAY anti-HER2 antibody (4B5; Bench Mark GX; Roche Diagnostics K.K., Tokyo, Japan). The tissue yielded a score of 1+ according to a scoring

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Key words: serum HER2, gastric cancer, intratumoral heterogeneity, HER2 overexpression, sampling error, false-negative

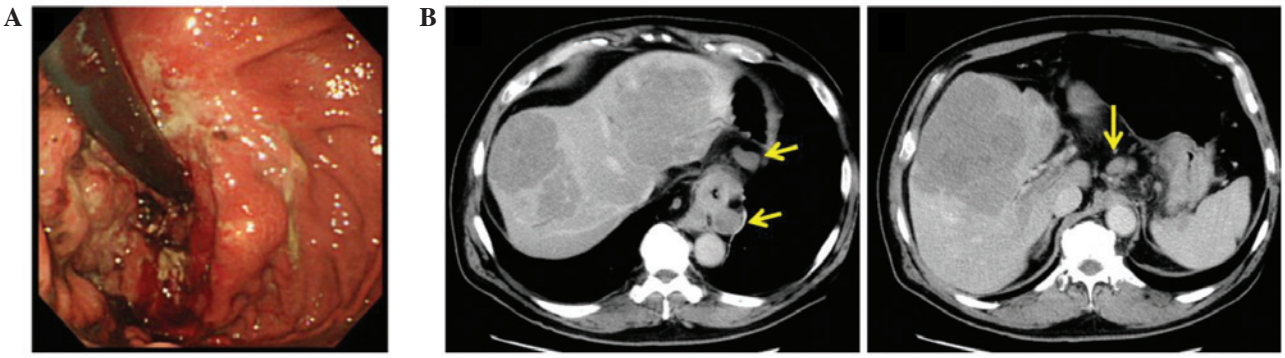

Figure 1. Imaging examinations. (A) Gastrointestinal endoscopy revealed a type 3 lesion in the cardia region. (B) Computed tomography imaging prior to chemotherapy revealed multiple liver and nodal metastases (arrows).

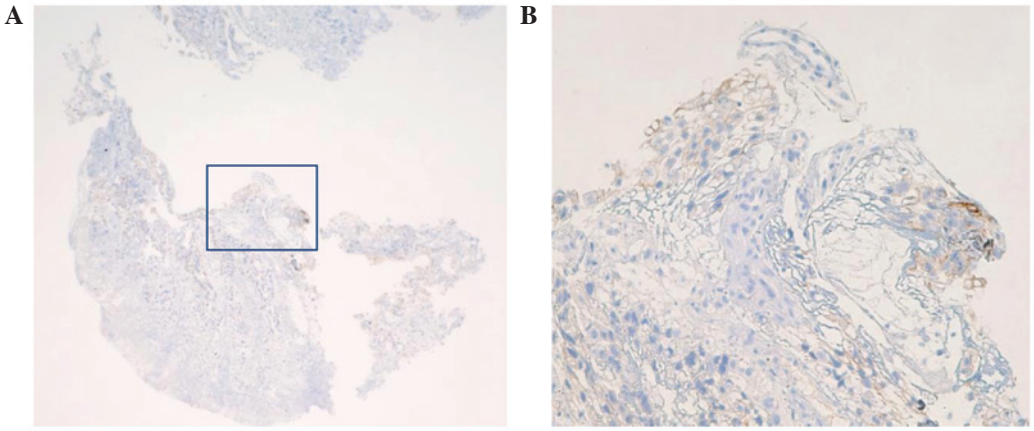

Figure 2. Initial human epidermal growth factor receptor 2 immunohistochemistry. Barely perceptible membrane staining (score 1+) was observed. (A) x40 and (B) x200 magnification.

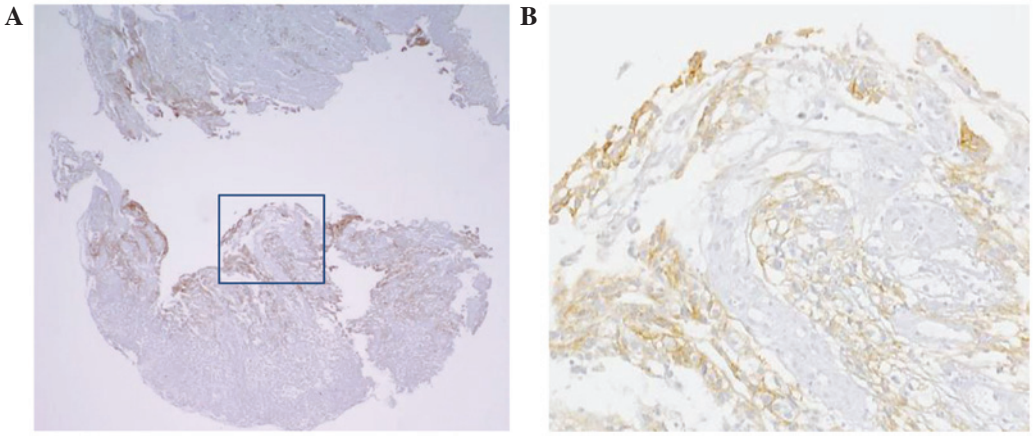

Figure 3. Second human epidermal growth factor receptor 2 immunohistochemistry. An alternative monoclonal antibody (HercepTest™) revealed numerous tumor cell clusters with strong complete membrane staining (scored as 3+). (A) x40 and (B) x200 magnification.

criteria specifically developed for gastric cancer (2). Staining was scored as follows: 0, no reactivity or no membrane staining; +1, tumor cell cluster with a faint/barely perceptible membranous reactivity; +2, tumor cell cluster with a weak to moderate complete, basolateral, or lateral membranous reactivity; +3, tumor cell cluster with a strong complete, basolateral, or lateral membranous reactivity. Tissues with a score of +3 or +2 in addition to fluorescence *in situ* hybridization (FISH) positivity were considered as HER2 positive. Thus, the patient was diagnosed as HER2-negative (Fig. 2). Based on this diagnosis, S-1 plus cisplatin combination chemotherapy (S-1, 40 mg/m^2, twice daily, days 1-21; cisplatin, 60 mg/m^2, day 8, every 5 weeks) was commenced.

The patient was enrolled onto our clinical trial investigating the association between serum HER2 and tissue HER2 status in gastric cancer (15). The serum HER2 level of this patient

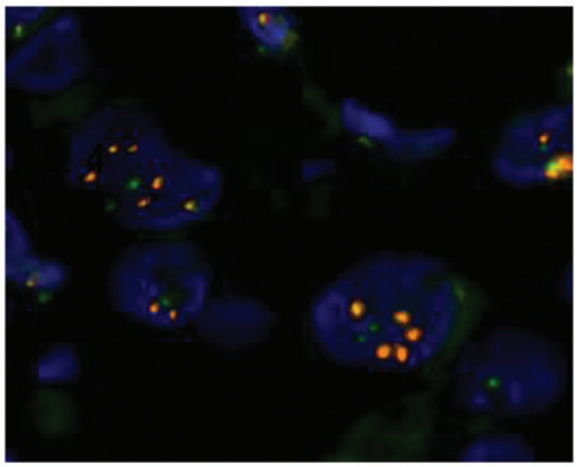

Figure 4. Fluorescence *in situ* hybridization analysis revealed a high level HER2 amplification. The ratio of HER-2/neu signal (orange) to centromere 17 signal (green) was 2:48.

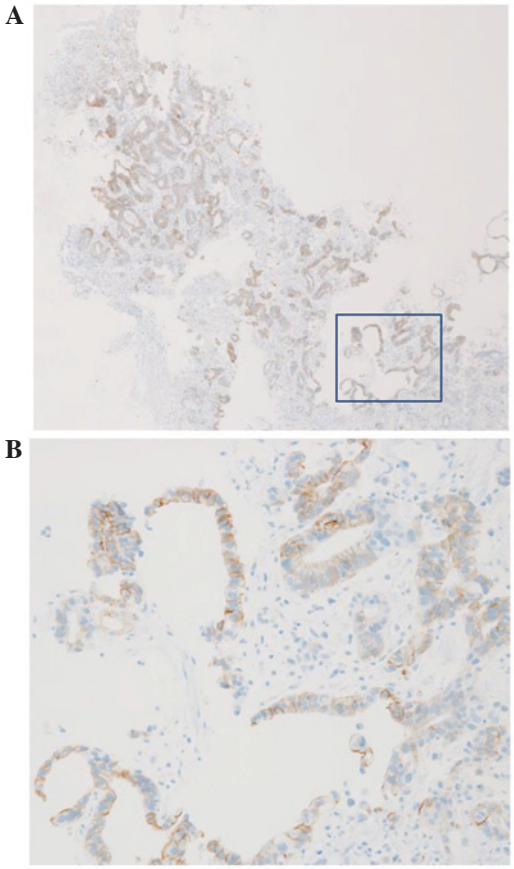

Figure 5. Immunohistochemical analysis of human epidermal growth factor receptor 2 in the re-biopsy specimens revealed strong complete membrane staining (scored as 3+). (A) x40 and (B) x200 magnification.

measured by CLIA (ADVIA Chemilumi-Centaur-HER2/neu assay™; Siemens Healthcare Diagnostics, Tokyo, Japan) was 53.3 ng/ml, which was higher than the upper limit of normal for breast cancer (15.2 ng/ml). In addition to the clinical features consistent with HER2-positive gastric cancer, such as the junctional location, differentiated histology and liver metastasis, the elevated serum HER2 level prompted the reevaluation of the tissue HER2 status.

The second IHC analysis, performed on formalin-fixed, 4-μm thick, paraffin-embedded tissue sections using an alternative polyclonal rabbit antibody from the HercepTest™ kit (Dako A/S, Glostrup, Denmark), demonstrated intensive membranous staining, judged to be HER2 score 3+ (Fig. 3). In addition, the HER2/chromosome 17 centromere ratio assessed by FISH was 2.48, also interpreted as HER2-positive (Fig. 4). Four biopsy samples were additionally taken at a follow-up endoscopy, and IHC for these re-biopsy specimens also confirmed a HER2 score of 3+ (Fig. 5). Based on the final diagnosis, the treatment protocol was changed from S-1 plus cisplatin to capecitabine, cisplatin and trastuzumab combination chemotherapy (XP+H; capecitabine, 2,000 mg/m^2, twice daily, days 1-14; cisplatin, 80 mg/m^2, day 1, every 3 weeks; trastuzumab, 8 mg/kg in the first cycle followed by 6 mg/kg, day 1) from the third course of chemotherapy. This regimen was well-tolerated, with grade 1 fatigue and grade 1 anorexia observed (16). The patient's symptoms were greatly relieved by the treatment, and CT imaging demonstrated regression of liver as well as nodal metastases. A partial response was maintained for four months; however, the disease progressed following 5 cycles of XP+H. Despite second and third-line treatment [paclitaxel (80 mg/m^2 on days 1, 8 and 15, every 4 weeks) and nab-paclitaxel (260mg/m^2, every 3 weeks) respectively], the patient succumbed to the disease 10 months after the initial presentation.

Discussion

Past studies have demonstrated that 7-34% of gastric cancer cases overexpress HER2 (17-19), and the rate was reported to be 22% in a recent large-scale international prospective trial (1). Since the introduction of trastuzumab, standardization and optimization of HER2 testing has been highlighted in the management of advanced gastric cancer. However, HER2 assessment by IHC and FISH present a substantial risk of false-negative results for gastric cancer, particularly when biopsy samples were used for the diagnosis (i.e. unresectable cases) (2-7). Serum HER2, a simple and less invasive method, assesses a different aspect of HER2 status from IHC and FISH. Although the clinical utility of serum HER2 in gastric cancer remains uncertain, a number of investigators have reported that serum HER2 level correlates with tissue HER2 status in gastric cancer (15,20-22). In the present case, the high serum HER2 level was the primary reason for the IHC reevaluation, and tissue HER2 positivity was eventually proven. Discrepancy of the IHC results may be due to use of a different primary antibody, as the same biopsy samples were used for the initial and second IHC.

There are numerous risk factors for diagnostic error of HER2 status in gastric cancer, including insufficient number of biopsy specimens, inadequate formalin fixation protocol, inexperienced laboratory staff and intratumoral HER2 heterogeneity (2-7). As trastuzumab has demonstrated a significant positive effect on treatment for unresectable HER2-positive gastric cancer, efforts must be made to minimize HER2 false-negative cases. Recent reviews concluded that serum HER2 is not useful for breast cancer management (11,23). However, serum HER2 may be useful to identify HER2

false-negative gastric cancer as HER2 overexpression in gastric cancer is frequently heterogeneous.

In conclusion, in the present case, serum HER2 was useful to identify a HER2-positive gastric cancer that was initially diagnosed as HER2-negative by IHC. This case suggests that serum HER2 may be useful to salvage tissue HER2 false-negative patients who are able to benefit from anti-HER2 treatment. Serum HER2 is expected to compensate for the aforementioned drawbacks of IHC in gastric cancer management. Large scale prospective studies are required.

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