Comparison of HER2 status by fluorescence *in situ* hybridisation and immunohistochemistry in gastric cancer

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Aim of the study: To investigate the differences and relevance, and to evaluate the clinical significance of fluorescence *in situ* hybridisation and immunohistochemistry (IHC) in detecting HER2 in gastric cancer tissues.
Material and methods: The expression of HER2 protein and the amplification of the HER2 gene in 118 gastric cancer tissues were detected by immunohistochemistry and fluorescence *in situ* hybridisation, respectively.
Results: Using IHC, we found that in 40 cases (33.9%) the HER2 expression was at level 0, in 33 cases (28.0%) the HER2 expression was at level 1+, in 16 cases (14%) the HER2 expression was at level 2+, and in 29 cases (25.6%) the HER2 expression was at level 3+, respectively. Using the FISH test, 38 of 118 cases (32.2%) were judged as positive results. The concordance rate between the results of IHC and FISH in all cases was 85.6%. The concordance rate of IHC and FISH was high in cases of HER2 expression at level 0, 1+ and 3+ according to IHC, but low in cases of 2+ according to IHC (43.8%).
Conclusions: Immunohistochemistry cannot predict HER2 gene amplification accurately. The FISH test should be executed in IHC 2+ cases.

Key words: immunohistochemistry, fluorescence *in situ* hybridisation, gastric cancer, human epidermal growth factor receptor-2.

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Introduction

Gastric cancer is a major global health concern as 1.4 million new cases of gastroesophageal and gastric cancer are diagnosed per year [1]. Most patients are diagnosed at an advanced stage, or already have metastatic disease. The combination of conventional chemotherapeutic agents such as 5-fluorouracil, cisplatin, epirubicin and docetaxel have been of limited success and the 5-year survival rate is less than 20% [2]. Therefore, novel and more effective treatments are urgently needed. In recent years, efforts have been focused on identifying better molecular targets for treatments that interfere with the signalling cascades involved in cell differentiation, proliferation and survival [3, 4].

The HER2 proto-oncogene is located on chromosome 17q21 and encodes a 185-kDa transmembrane tyrosine kinase receptor HER2 (also known as HER2/neu, ERBB2, p185). The HER2 protein is a member of the epidermal growth factor receptor (EGFRs) superfamily, which plays a role in the development and progression of many human cancers and has been associated with poor prognosis when activated by ligand binding; it also dimerises and regulates intracellular signal transduction through the mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways [5]. The final target of these pathways is the regulation of gene expression for various proteins that play roles in a multitude of cellular processes such as differentiation, proliferation and survival.

Trastuzumab is an antibody targeting the HER2/neu protein, which induces the antibody-dependent cytotoxicity in malignant cells, inhibits HER2-mediated signalling and prevents cleavage of extracellular domain of HER2 [6, 7]. HER2/neu was introduced as a predictive biomarker for the treatment of breast cancer with trastuzumab and had been proven to enhance survival rates in both primary and metastatic HER2-positive breast cancer patients [8, 9]. The efficacy of trastuzumab in breast cancer patients prompted studies to evaluate the potential clinical benefits of this drug in patients with other HER2-positive cancers, including gastric cancer. Recently, the Trastuzumab for Gastric Cancer (ToGA) study demonstrated that trastuzumab combined with chemotherapy improves the median overall survival rate of patients with HER2-positive advanced gastric or gastro-oesophageal junction cancer [10, 11].

An accurate assessment of HER2 status is essential in selecting the patients who would most benefit from targeted therapy with trastuzumab, among patients with gastric cancer [12, 13]. In this study, we used immunohistochemistry (IHC) and fluorescence *in situ* hybridisation (FISH) to assess HER2 status in specimens from patients with gastric cancer.

Table 1. Clinical and pathological characteristics of patients

Patients (*n*)	118
Age (years)	
Mean ± SD	64.3 ±10.5
Median	63.7
Gender (*n*)	
Men	106
Women	12
Laurén phenotype (*n*)	
Intestinal	72
Diffuse	20
Unclassified	8
Mixed	18
TNM classification (*n*)	
T-stage	
T1a	2
T1b	11
T2	16
T3	47
T4a	33
T4b	9
N-stage	
N0	35
N1	16
N2	24
N3	43
UICC stage (*n*)	
IA	9
IB	6
IIA	16
IIB	11
IIIA	15
IIIB	18
III	22
IV	21

Material and methods

Study population

We retrieved data from patients who had undergone either total or partial gastrectomy for adenocarcinoma of the stomach or oesophago-gastric junction between 2009 and 2011 at the First Hospital of JingZhou, HuBei, China. Patients that had received adjuvant or neoadjuvant chemotherapy, or radiotherapy, either as a single therapy option or in a combination, were excluded.

Ethics statement

This study project was approved by the Clinical Research Ethics Committee of the First Hospital of JingZhou. All patients' records were pseudonymised before study inclusion.

Histology and TNM classification

Tissue specimens were fixed in formalin and embedded in paraffin. Deparaffinised sections were stained with haematoxylin and eosin. Tumours were classified according to Laurén classification [14]. The pTNM stage of all studied patients was determined according to the seventh edition of the UICC guidelines [15].

Immunohistochemistry

Immunohistochemistry was performed with a commercially available kit (Ultra Sensitive TM S-P kit; Maixin-Bio Co., Fuzhou, China) according to the manufacturer's guidelines. HER2 immunostaining was scored according to the recommendations by Hofmann *et al.* [16]. These recommendations were originally made for breast cancer and were modified in order to optimise the IHC scoring procedure for gastric cancer. Briefly, the scores were as follows: no positive staining or staining of only a part of the cell membrane in < 10% of cells (0, negative); barely visible or positive staining of only a part of the cell membrane in > 10% of cells (1+, ambiguously positive); weak-to-moderate, complete or basolateral positive staining in > 10% of cells (2+, weakly positive); and moderate-to-strong, complete or basolateral-positive staining in > 10% of cells (3+, strongly positive) (Fig. 1).

Fluorescence *in situ* hybridisation

Fluorescence *in situ* hybridisation (FISH) was performed with a commercially available kit (Dual-colour FISH kit; GP Medical Co., Beijing, China) according to the manufacturer's guidelines. HER2 gene amplification was considered positive when its exhibited ratio of HER2:CEP17 (centromeric probe 17) was ≥ 2 in a minimum of 60 counted cancer cell nuclei, or when an HER2 signal cluster was observed (Fig. 2).

Statistics

All statistical analysis was conducted using SAS 8.01 (SAS Institute Inc, Cary, NC, USA). The significance of correlation between clinicopathological parameters and HER2/neu status was tested using Fisher's exact test. For parameters of ordinal scale (T, N stage), we applied Kendall's tau test instead. Differences were considered statistically significant at P value < 0.05.

Results

In this study a total of 118 patients fulfilled all study criteria. Demographic and clinical-pathological characteristics of patients are presented in Table 1. Patients had a median age of 63 years (range 33-86 years). There were 12 female and 106 male patients. According to Laurén classification, intestinal type GC was found in 72 patients (61.0%), diffuse type in 20 patients (16.9%), unclassified type in 8 patients (6.8%) and mixed type in 18 patients (15.3%).

Her2 protein overexpression

A total of 118 gastric cancer specimens were evaluated using IHC. We found that in 40 cases (33.9%) the HER2 expression was at level 0, in 33 cases (28.0%) the HER2 expression was at level 1+, in 16 cases (14%) the HER2 expression was at level 2+ and in 29 cases (25.6%) the HER2 expression was at level 3+, respectively. Of the 118 surgically resected tumour specimens, 45 (2+, 3+ cases; 38.1%) of the tumours were found to exhibit HER2 protein overexpression [95% confidence interval (CI): 29.3–46.9%]. There was no association between HER2 protein overexpression and age, gender, T-stage, N-stage, M-stage or pathology stage (p > 0.05).

Table 2. Concordance between the results of IHC and FISH in surgically resected tumours

IHC score	FISH amplified	FISH non-amplified	Concordance
0 (n = 40)	2	38	95.0% (38/40)
1+ (n = 33)	3	30	90.1% (30/33)
2+ (n = 16)	7	9	43.8% (7/16)
3+ (n = 29)	26	3	89.7% (26/29)
Total (n = 118)	38	80	85.6% (101/118)

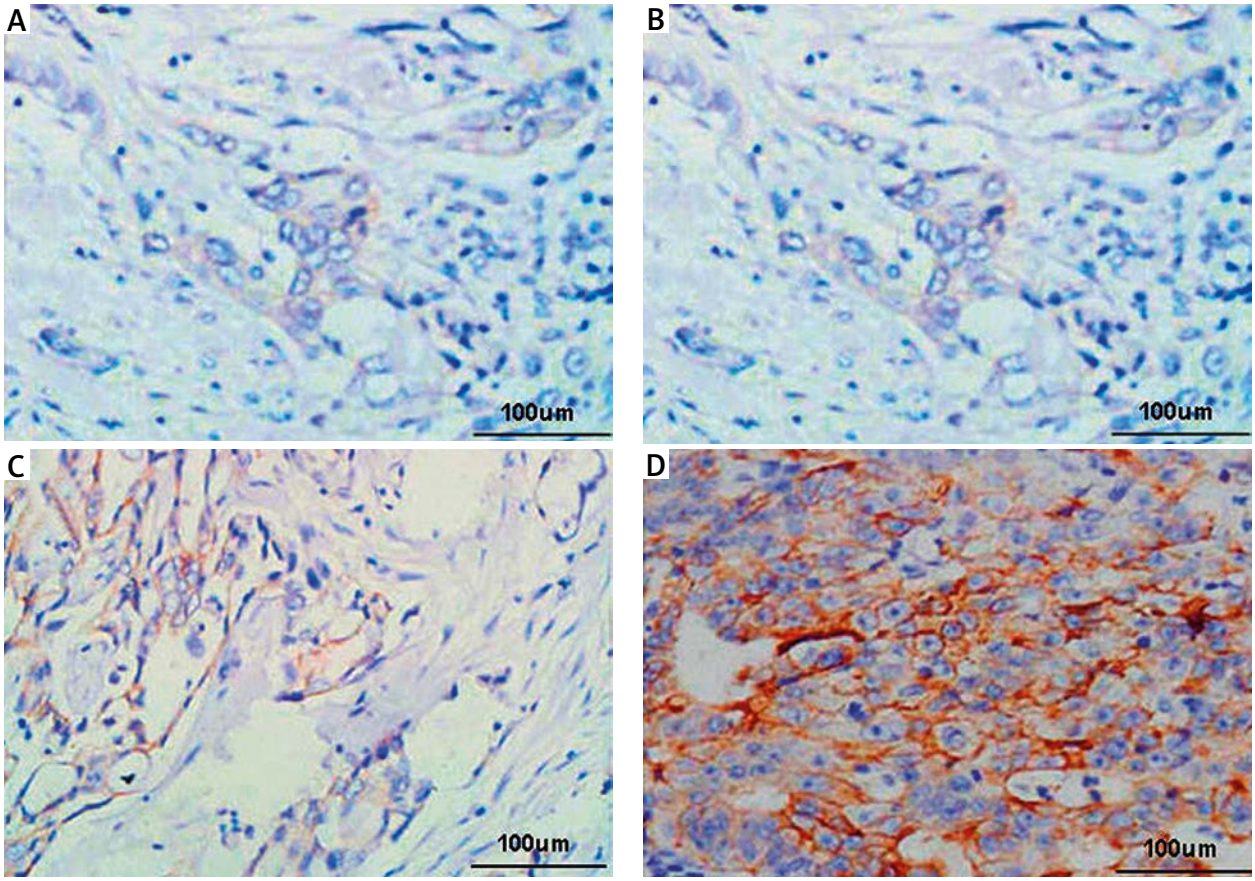

Fig. 1. HER2 protein expression detected by IHC results using the 0 to 3+ scale: scores of 0 (**A**) or 1+ (**B**) were considered negative; 2+ (**C**) was weak positive; and 3+ (**D**) was strong positive for HER2 overexpression

HER2 gene amplification

Thirty-eight of the 118 cases (32.2%) were judged as positive by the FISH test (95% CI: 23.8–43.6%). HER2 was amplified in 25 of 72 intestinal-type cancer specimens (34.7%), in 5 of 20 diffuse specimens (25%), in 3 of 8 unclassified specimens (37.5%) and in 5 of 18 mixed-type specimens (27.8%). We found no association between *HER2* gene amplification status and age, gender, T-stage, N-stage, M-stage or pathology stage ($p > 0.05$).

Concordance between the results of immunohistochemistry and fluorescence *in situ* hybridisation

The concordance rate between the results of IHC and FISH in all cases was 85.6% (95% CI: 79.3–91.9%) (Table 2). In the 73 cases with negative HER2 protein (0 and 1+) according to IHC, 68 cases did not show amplification with FISH and its concordance rate was 93.1%. In the 45 cases with positive HER2 protein (2+ and 3+) according to IHC, 33 cases showed amplification with FISH and its concordance rate was 73.3%.

Discussion

Many studies have examined the idea that HER2 status could be a predictor of the survival rate in patients with gastric cancer. A study by Andreas *et al.* showed that there was significant association between the level of expression of HER2 and quicker achievement of patients being cancer-free and overall survival [17]. Brien *et al.* used multivariate analysis in their study and showed that the

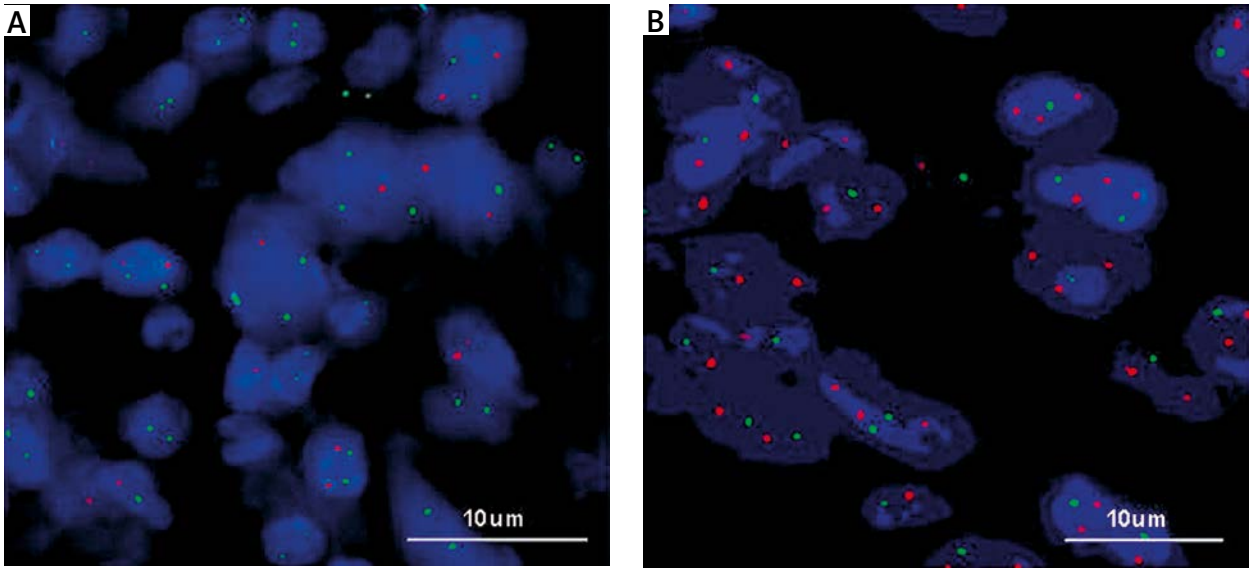

Fig. 2. The *HER2* gene amplification detected by FISH. A ratio of HER2 (red): CEP17 (green) < 2.0 was considered non-amplified (E, negative) and ratios ≥ 2.0 were considered amplified (F, positive)

pathological stage and *HER2* gene amplification are independent prognostic factors of survival [18].

Targeted therapy with trastuzumab combined with chemotherapy have proven more beneficial in patients with higher levels of HER2 expression [19, 20]. However, patient-individualised treatment aims to avoid unnecessary medication in patients who are unlikely to respond to therapy. On the contrary, targeted therapy should reach every patient eligible for the treatment. For this reason, accurate assessment of the HER2 status of patients before they are treated with trastuzumab is very important.

Immunohistochemistry and fluorescence *in situ* hybridisation are the most widely used methods to evaluate HER2 status. Immunohistochemistry is easier to perform, relatively inexpensive and is used more often. However, the sensitivity and specificity of the assay can vary significantly depending on the commercial antibody used. By contrast, the FISH method is more standardised and less variable and has, therefore, emerged as a "gold standard" for assessment of HER2 status [21, 22].

In this study, HER2 protein overexpression was demonstrated in 38.1% of formalin-fixed paraffin-embedded specimens of surgically resected gastric cancers, and *HER2* gene amplification was demonstrated in 32.2%. The concordance rate between the results of IHC and FISH in all cases was 85.6%. The concordance rate of IHC and FISH was high in cases that were 0, 1+ and 3+ according to IHC, but low in cases that were 2+ according to IHC (43.8%). These results suggest that IHC cannot predict HER2 gene amplification accurately and that the FISH test should be executed in IHC 2+ cases.

In our study we did not find any association between *HER2* gene amplification status or Her-2 overexpression and age, gender, T-stage, N-stage, M-stage or pathology stage. Our results were similar to those of Andreas *et al.* [19]; however, Kim *et al.* found that gastric carcinomas with HER-2 overexpression were associated with well differentiated or moderately differentiated histology by WHO classification and with the intestinal type by Lauren classification; no significant relation was found between *HER-2* protein status and pathologic stage, age or sex [23]. The main reason for these discrepancies may be that all of the study populations were low. The clinical association between HER2 gene amplification status or Her-2 overexpression and gastric cancer pathology stage or classification require further studies.

The authors declare no conflict of interest.

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