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Original article

HER2 status in gastric cancer: A comparison of two novel in situ hybridization methods (IQ FISH and dual color SISH) and two immunohistochemistry methods (A0485 and HercepTest™)

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ABSTRACT

In contrast to breast HER2 testing, the optimal ISH method and antibody for gastric HER2 testing are unclear. The aim of this study was to find out gastric HER2 positivity rates in our institutional data, and to compare the two novel ISH methods with A0485 antibody and HercepTest™. IHC and ISH were carried out on gastrectomy specimens of 88 patients up to the standardly advised procedure protocols, and interpretations were also carried out up to widely accepted international protocols., HER2 expression was (−) in 65, (+) in 5, (++) in 6, and (+++) in 12 cases by A0485 IHC. IHC (+) 4 cases and (++) 3 cases were (−) by HercepTest™. One IHC (−) amplified case was (++) by HercepTest™. All A0485 and HercepTest™ (+++) 12 cases were amplified by ISH. HER2 amplification was detected in 18 (20.4%) and in 15 (17.2%) cases by SISH and FISH, respectively. Of the 18 cases, 4 showed focal heterogeneous low level amplification by SISH. Focal amplification was noted in only 2 cases by FISH. The HER2 status of our gastric cancer file is 17.2% by FISH, 20.4% by SISH. The concordance between HercepTest™/A0485 IHC and ISH is perfect in (+++) cases. Equivocal results (++) with any IHC method should be clarified by one of the molecular methods (SISH and FISH). Probably up to the higher level of heterogeneity of gastric carcinomas, there is a 4.5% dilemma of cases that are negative or weakly positive by conventional IHC methods. Therefore, regarding HER2 status in gastric carcinoma, the reliability of IHC methods should be checked.

Introduction

Identifying tumors that overexpress the human epidermal growth receptor 2 (HER2) protein has become an established guide for treating HER2-positive advanced stage metastatic gastric and gastroesophageal cancers, as humanized anti-HER2 monoclonal antibody trastuzumab (Herceptin® Genentech, South San Francisco, CA) plus chemotherapy caused a 2.4-month prolongation of the median survival based on the reports of a phase III randomized multicentric trial (ToGa study) [1].

Amplification of the HER2 gene appears to be the primary mechanism underlying overexpression of its gene product. Basically, there exist two approaches to determine the HER2 status: detection of the protein by immunohistochemistry (IHC) or genomic status by in situ hybridization (ISH). In most laboratories, IHC is preferred as a first step tool. In case of immunohistochemically equivocal results, an additional test is strongly recommended to clarify the HER2 status on the genomic level. The advantages and disadvantages of the techniques are well documented for breast carcinoma by several studies comparing HER2 testing with IHC, FISH, and SISH in the literature [2,3]. The concordance between IHC and ISH is about 90%; FISH and SISH is 96% for breast carcinomas [4]. However, an optimal method for IHC and ISH is still lacking for gastric carcinomas.

As incomplete basolateral membrane staining was observed frequently in gastric carcinomas, a modified HercepTest scoring system for gastric carcinomas was proposed by Hofmann et al. (Table 1). On biopsy samples (not surgical ones), strong complete/basolateral staining by IHC and/or FISH+ clones are considered as positive irrespective of the proportion of tumor cells [5].

While HER2 positivity rates in gastric cancers vary from 7.1 to 42.6% [6–9], most of the respectable studies demonstrate values of 15–25% [10–14]. Heterogeneity of the tumor cells as regards HER2 expression may be the cause of such a wide range of variation. As there are no universally admitted criteria (such as which part of the tumor and how many samples should be analyzed) for the detection of HER2 expression in heterogenous cases, the IHC results of such cases are still conflicting.

IHC methods are widely preferable in the detection of the HER2 status in the first step as they are cheap, and easily and quickly performed. IHC-stained slides can be stored for long periods and reassessed. Disadvantages of IHC are semiquantitative and

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Table 1
Consensus panel recommendations on HER2 scoring for gastric cancer (gastrectomy specimens).

IHC score	Reactivity characteristics
0/negative	No reactivity/membranous reactivity in <10% of cells
1+/negative	Faint/barely perceptible membranous reactivity in >10% of tumor cells
2+/equivocal	Weak/moderate complete or basolateral membranous reactivity in >10% of tumor cells
3+/positive	Moderate/strong complete or basolateral reactivity in >10% of tumor cells

subjective score interpretations, as well as the influence by the variations in preanalytic procedures. On the other hand, molecular detection methods (FISH and SISH) are more accurate regarding the detection of HER2 status, although they are expensive, time-consuming, and need more experience for interpretation. They are less affected by preanalytic procedures (fixation, tissue processing) as DNA is more stable than proteins. Recent studies have shown that FISH has the best performance on tissues with preanalytical problems [15]. Bright dual SISH is also a reliable method with high sensitivity and specificity, and it also produces distinct signals analyzable even in poorly preserved tissues [15].

The aim of this study was to find out gastric HER2 positivity rates in our institutional data, and to compare the two novel ISH methods with A0485 antibody and HercepTest™.

Materials and methods

Eighty-eight consecutive gastric cancer cases diagnosed at Izmir Tepecik Training and Research Hospital between 2007 and 2011 were selected for the study. All specimens were formalin-fixed, paraffin embedded, and processed according to the institute's standardized protocols. The entire material consisted of gastrectomy specimens. All cases were diagnosed according to the 2010 WHO classification [16], and clinicopathological characteristics are listed. The slides were prepared from archived paraffin blocks, and were processed in parallel for HE, FISH, SISH, and IHC (HercepTest™ and rabbit polyclonal antibody (A0485), Dako).

Immunohistochemistry

To determine the HER2 expression, the HercepTest™ and polyclonal rabbit HER2 antibody (clone: A0485, 1:300 dilution, Dako, Denmark) were used according to the manufacturer's protocol. The IHC procedure was performed on Autostainer Link 48 (Dako, Denmark). Immunostaining was scored according to the consensus panel recommendations on HER2 scoring for gastric cancer (Table 1) [5].

SISH (dual-color silver-enhanced in situ hybridization)

Automated SISH was performed on Ventana Benchmark XT (Ventana Medical Systems, Tucson, AZ, USA). Ultraview Inform HER2 DNA probe and Inform Chromosome 17 centromere (cen17) probe were visualized on the same slide. Assay conditions were modified to obtain optimal results. The whole assay procedure (deparaffinization, pretreatment, hybridization, stringency wash, signal detection and counterstaining) was fully automated. The HER2 probe was denatured at 95 °C for 20 min and hybridized at 52 °C for 6 h. The chromosome 17 centromere probe was denatured at 95 °C for 20 and hybridized at 44 °C for 6 h. Stringency washes were performed at 72°C for 8 min. The silver signal for HER2 was revealed by sequential silver reactions. The signal of the centromere was visualized with the RedISH Naphtol reaction. The tissues were counterstained with Hematoxylin II and Bluing Reagent.

FISH (instant quality fluorescence in situ hybridization)

The HER2 copy number was investigated by IQ FISH pharmDx Kit (Dako, Denmark). Paraffin sections were incubated at 60 °C for 60 min, deparaffinized in two series of xylol, and rehydrated with ethanol series. Slides were pretreated with pretreatment solution in a water bath at 99 °C for 10 min. Enzymatic digestion was carried out with ready-to-use pepsin for 4 min at 37 °C on hybridizer. After dehydration, 10 μl of HER2/cen17 probe mix was applied to each tissue section. The slides and probe were denatured at 66 °C for 10 min and hybridized at 45 °C for 120 min. After the hybridization period, the slides were washed with Stringent wash buffer at 63 °C for 10 min in a water bath. Then, the slides were dehydrated, and 10 μl of fluorescence mounting medium containing 4′,6-diamino-2-phenylindole (DAPI) was applied.

Interpretation of ISH methods

The entire tumor area on the slides was evaluated under 40× objective in the case of SISH and under 100× objective in the case of FISH. A fluorescence microscope (Olympus BX51) equipped with a DAPI/Spectrum Red/Spectrum Green filter set using a 100× oil immersion objective lens. SISH was scored with the use of a brightfield microscope (Olympus BX41) with 40× objective. HER2 amplification was considered to be positive when the HER2/cen17 ratio was ≥2 within 20 tumor cell nuclei [5,17]. Ratios between 2 and 5 were considered as low level, >5 as high level amplification.

Statistical analyses

The Pearson's chi-square test, the Mann–Whitney *U*, and Student's *T*-test were performed. The results were considered to be statistically significant when *p* values were less than 0.05. All statistical analyses were conducted using the SPSS 15.0 statistical software program (SPSS, Inc., IL, USA).

Results

The mean age of the cases was 61.2, and the mean tumor diameter was 5.8 cm. The most common histological subtype was tubular carcinoma (52.3%). Clinicopathological characteristics are detailed in Table 2.

HER2 overexpression (++ and +++) was observed in 15 of 88 (17%) cases by HercepTest™, and in 18 of 88 (20.4%) cases by A0485 (Table 3). We noted non-specific granular cytoplasmic staining in non-neoplastic areas with intestinal metaplasia, regenerative foveolar epithelium, and neoplastic areas with signet ring cells (Fig. 1).

HER2 gene was amplified in 18 of 88 (20.4%) and in 15 of 87 (17.2%) cases by SISH and FISH, respectively (Table 4). The result in one case was not interpretable by FISH but interpretable by SISH. When we checked the cause of this disconcordance, it seemed to be due to the faulty duration time of digestion.

Four of the 18 SISH-positive cases showed focal heterogeneous low level amplification. Two of the 15 FISH-positive cases were focally and heterogeneously amplified.

HER2 amplification rates were higher in gastroesophageal junction/cardia tumors than in gastric (corpus/antrum) ones (46% vs.