World Journal of Gastroenterology

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DOI: 10.3748/wjg.v22.i19.4619

World J Gastroenterol 2016 May 21; 22(19): 4619-4625
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2016 Gastric Cancer: Global view

HER2 testing in gastric cancer: An update

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Author contributions: Abrahao-Machado LF performed research, developed and wrote the overall manuscript; and Scapulatempo-Neto C contributed by critically reviewing the manuscript for important intellectual content.

Conflict-of-interest statement: The authors declare no conflicts of interest.

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Received: January 19, 2016
Peer-review started: January 21, 2016
First decision: February 18, 2016
Revised: March 2, 2016
Accepted: March 30, 2016
Article in press: March 30, 2016
Published online: May 21, 2016

Abstract

Human epidermal growth factor receptor 2 (HER2) overexpression is increasingly recognized as a frequent molecular abnormality in gastric and gastroesophageal cancer. With the recent introduction of HER2 molecular targeted therapy for patients with advanced gastric cancer, determination of HER2 status is crucial in order to select patients who may benefit from this treatment. This paper provides an update on our knowledge of HER2 in gastric and gastroesophageal cancer, including the prognostic relevance of HER2, the key differences between HER2 protein expression interpretation in breast and gastric cancer, the detection methods and the immunohistochemistry scoring system.

Key words: Human epidermal growth factor receptor 2 testing; Gastric cancer; Immunohistochemistry; Scoring system; Trastuzumab

Core tip: It is clear that human epidermal growth factor receptor 2 (HER2) protein over-expression and gene amplification are much more heterogeneous in gastric cancer compared to breast cancer. Gastric and gastroesophageal tumors require a unique immunohistochemistry scoring system and interpretation expertise. We aimed to clarify the key differences in immunohistochemistry interpretation of gastric cancer, providing a practical update on HER2 testing and scoring.

Abrahao-Machado LF, Scapulatempo-Neto C. HER2 testing in gastric cancer: An update. *World J Gastroenterol* 2016; 22(19): 4619-4625 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i19/4619.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i19.4619

INTRODUCTION

Human epidermal growth factor receptor 2 (HER2), also known as CerbB-2 and ERBB2, is a proto-oncogene located on chromosome 17q21 that encodes a transmembrane protein with tyrosine kinase activity,

a member of the HER receptor family and is involved in signal transduction pathways, leading to cell growth and differentiation[1].

Amplification of the HER2 gene and overexpression of its product were first discovered in breast cancer and are significantly associated with worse outcomes[2]. Many studies have demonstrated that HER2 is also present in several other malignancies, including colorectal cancer, ovarian cancer, prostate cancer, lung cancer and, particularly, gastric and gastroesophageal cancer[3].

In gastric and gastroesophageal cancer, the frequency of HER2 overexpression varies widely in the literature; studies have yielded inconsistent findings regarding its prognostic relevance[4-12]. With the recent introduction of trastuzumab for the treatment of patients with advanced gastric cancer, the clinical demand for HER2 assessment is rapidly increasing. However, HER2 testing in gastric cancer differs from testing in breast cancer because of inherent differences in tumor biology, intratumoral heterogeneity of HER2 expression and incomplete membrane staining that are commonly observed in gastric tumors[13].

This paper aims to summarize the current evidence regarding HER2 in gastric and gastroesophageal cancer and to provide a practical update on HER2 testing and scoring that is essential for appropriate selection of patients who are eligible for treatment with trastuzumab.

RELEVANCE OF HER2 IN GASTRIC AND GASTROESOPHAGEAL CANCER

The frequency of HER2 overexpression in gastric and gastroesophageal cancer ranges from 4.4% to 53.4%, with a mean of 17.9%[4-14].

Although some small-scale studies have not demonstrated the prognostic properties of HER2[4,5,9,12], a larger number of studies indicate that HER2 is a negative prognostic factor, showing more aggressive biological behavior and higher frequencies of recurrence in HER2-positive tumors[1,6-8,11,14].

Given this controversy of HER2 prognostic values, a systematic review of a large number of studies was recently conducted in order to address this issue[14]. Forty-two publications with a total of 12749 patients were reviewed; the majority (71%) of the publications showed that a HER2-positive status was associated with decreased survival and clinicopathological features of tumor progression, such as serosal invasion, metastases and higher disease stage[14]. The results clearly set HER2 as a negative prognostic factor, suggesting that HER2 overexpression/amplification is a molecular abnormality that might be associated with the development of gastric cancer[7,14].

HER2 MOLECULAR TARGETED THERAPY

Trastuzumab is a monoclonal antibody directed against HER2; as one of the first molecular-targeted drugs to be developed, it was first introduced for the treatment of HER2-positive advanced breast cancer[2].

There is no consensus on the mechanism in which trastuzumab acts in cancer cells, but the evidence is that in addition to preventing dimerization of HER2 with other HER family members and stimulating endocytosis, it seems to induce cell mediated immunity and inhibit angiogenesis[15].

In the ToGA trial, patients with HER2-expressing unresectable gastric and gastroesophageal tumors were treated with chemotherapy and trastuzumab or with chemotherapy alone. A statistically significant increase in overall survival was observed in patients who received trastuzumab[16].

Although only a modest improvement of 2.7 mo in the median overall survival was observed in HER2-positive patients with the addition of trastuzumab, according to the ToGA trial, there was an improvement of 4.2 mo in the median overall survival in a post-hoc analysis[14,16-18].

Other molecular HER2-targeted agents have been tested or are currently being tested such as pertuzumab, lapatinib, the antibody-drug conjugate trastuzumab-emtansine (TDM-1)[19-23] and afatinib (NIH study trial registration number NCT01522768; ClinicalTrials.gov). However, the efficacy of these agents has been shown to be either unsatisfactory or as modest as trastuzumab[22,24]. Trastuzumab is the first molecular targeted agent approved as a standard treatment in gastric cancer, but it remains under investigation for more potent utilization.

Thus, it is imperative to determine the HER2 status in advanced gastric or gastroesophageal junction adenocarcinoma in order to select patients who may benefit from this promising treatment.

HER2 TESTING METHODS

HER2 status is mainly assessed by immunohistochemistry (IHC) or *in situ* hybridization (ISH) assays. Both methods can be done on formalin-fixed and paraffin-embedded biopsy tissues or surgical specimens and occasionally, cytological samples[25]. Fluorescent *in situ* hybridization (FISH) is regarded to be the gold standard; however, because of its higher cost and time consumption, as well as the need for a fluorescence microscope, generally only equivocal cases are subjected to this technique. Furthermore, the high concordance between FISH and IHC that is reported in the literature supports the use of IHC, the most familiar and readily accommodated method in most surgical pathology laboratories[26-29].

Thus, IHC should be used as the first screening method for HER2 evaluation and those cases with results considered equivocal for HER2 overexpression (2+) should be referred for FISH analysis or other alternative *in situ* hybridization method[28] (Figure 1). A simple and practical alternative to FISH for these

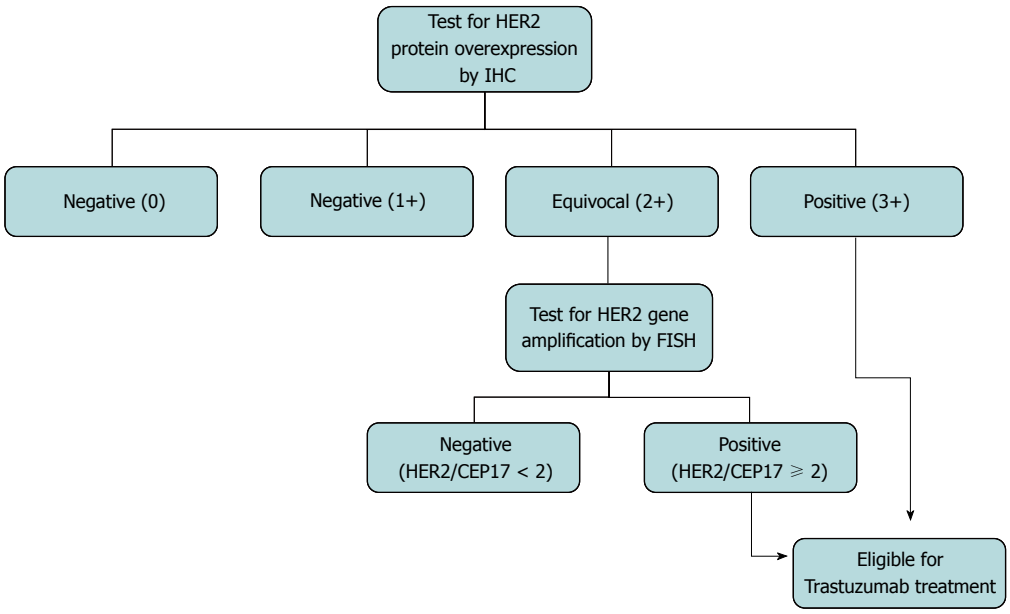

Figure 1 Human epidermal growth factor receptor 2 testing algorithm. HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry; FISH: Fluorescent *in situ* hybridization; CEP17: Chromosome 17.

Table 1 Advantages and disadvantages of the human epidermal growth factor receptor 2 testing methods

Method	Advantages	Disadvantages
IHC	Quick to perform; Most laboratories use fully automated processes; Widely used and familiar to all pathologists; Results can be viewed using a conventional bright-field microscope; Permits parallel viewing of tumor cell morphological features; Stained tissues do not degrade over time	Equivocal cases (2+) need another method for conclusion; Accuracy is more dependent on pre-analytic variables
FISH	Very objective and accurate; Actual copies of HER2 genes can be counted; Considered the golden standard of HER2 testing	Technically more demanding; Usually performed only in large laboratories/institutions; Costs are substantially high; Requires the use of fluorescence microscope and dark room; Comparatively more time consuming; Reagents degrade over time
SISH/CISH/ DDISH	Quick to perform; Very objective and accurate; Technique is fully automated; Results can be viewed using a conventional bright-field microscope; Permits parallel viewing of tumor cell morphological features; Slides can be stored because the signal is stable; Double-stranded probes labeled with two haptens can detect both markers on a single slide (DDISH)	More expensive than IHC; Unfamiliar to non-specialist pathologists

IHC: Immunohistochemistry; FISH: Fluorescent *in situ* hybridization; SISH: Silver *in situ* hybridization; CISH: Chromogenic *in situ* hybridization; DDISH: Dual-color dual-hapten *in situ* hybridization.

equivocal cases is provided by the employment of other *in situ* hybridization techniques such as silver *in situ* hybridization (SISH), chromogenic *in situ* hybridization and dual-color dual-hapten *in situ* hybridization. These three methods can be easily analyzed under a conventional bright field microscope and have shown excellent correlation with results obtained by FISH[30-32].

Because IHC is the easiest, least expensive and most widespread method of HER2 assessment, this paper focuses on IHC. Table 1 shows the different HER2 methods and their advantages and disadvantages.

Differences between HER2 expression in breast and gastric cancer

The key differences between HER2 expression in breast and gastric and gastroesophageal cancer are listed[17,30]: (1) the membranous distribution of the antibody in the neoplastic cells of breast cancer is

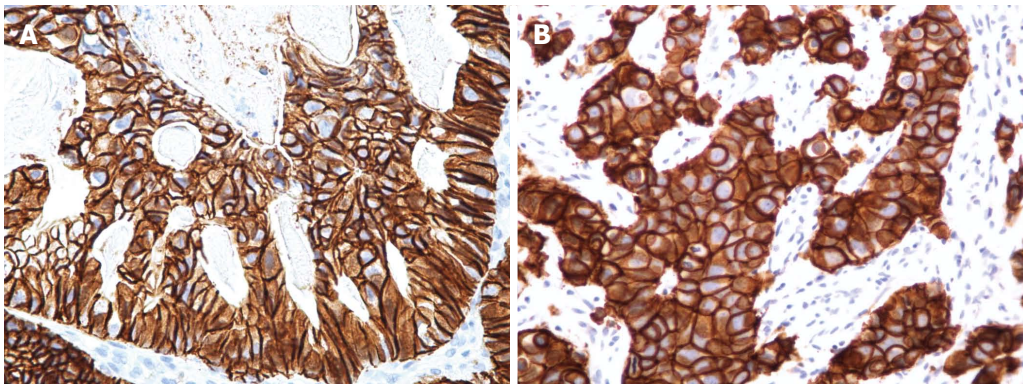

Figure 2 Human epidermal growth factor receptor 2 expression in gastric and breast tumors. A: A HER2-positive (3+) case of gastric adenocarcinoma; the cytoplasmic membranous immunostaining is incomplete and predominantly basolateral (× 400); B: A HER2-positive (3+) case of invasive ductal carcinoma of the breast; the cytoplasmic membranous staining is fully circumferential (× 400). HER2: Human epidermal growth factor receptor 2.

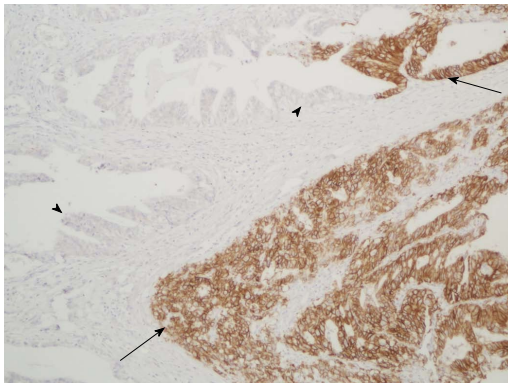

Figure 3 Representative image of the intratumoral heterogeneity of HER2 expression. Arrows indicate areas with strong continuous membranous staining (score 3+) and arrowheads indicate negative areas (score 0) (× 100). HER2: Human epidermal growth factor receptor 2.

predominantly circumferential, whereas in gastric cancer, it is generally incomplete, predominantly basolateral ("U"-shaped) or lateral (parallel lines) (Figure 2). Thus, unlike for breast cancer, circularity of IHC staining is not a criterion for HER2 IHC scoring in gastric cancer; (2) intratumoral heterogeneity, defined as the presence of areas with different HER2 scores within the same tumor, *i.e.*, focal or patchy positivity, is a common pattern encountered in gastric tumors but is only rarely seen in breast cancer (Figure 3). It may cause sampling errors when randomly sampled biopsies are examined (see below). Although the causes of intratumoral heterogeneity of HER2 expression are not yet fully understood, some studies indicate that it could be explained merely by tumor inherent genetic heterogeneity[33,34]. Since *Helicobacter pylori* (*H. pylori*) is widely accepted as the main causative agent of gastric cancer[35], we speculate whether among the diverse bacterial factors, concomitant infection with different strains and diverse host responses there could be a reasonable link with HER2 intratumoral heterogeneity. Interestingly, Tegtmeyer *et al*[36] showed that some *H. pylori* strains could in fact activate HER2, while infection with other strains suppressed HER2 activity. However, this correlation of the bacterium with HER2 intratumoral heterogeneity is still a matter of debate and requires further studies; and (3) variation of the incidence of HER2 expression with anatomic location does not occur in breast cancer, whereas it is more frequent in the proximal stomach, including the esophageal-gastric junction, than in the distal stomach. With the introduction of the seventh edition of TNM classification, a large number of tumors that were formerly categorized as gastric are now considered as esophageal and gastroesophageal junction tumors instead, with relatively high HER2-positivity rates in these primary neoplasms[37].

IHC score system

Given these differences between HER2 expression in breast and gastric cancer, an appropriate scoring system, exclusive for gastric tumors, was developed, because just transferring the breast cancer IHC scoring roles to gastric cancer could lead to a significant loss of patients. The system proposed by Hofmann *et al*[38] that has been assimilated by CAP and FDA, besides being specific for gastric tumors, also distinguishes biopsies from surgical specimens[17]. Table 2 shows the IHC score system for HER2 in gastric cancer and Figure 4 illustrates it.

Differences among samples

As mentioned above, mainly because of intratumoral heterogeneity, the size of the tissue sample might interfere in HER2 analysis. Although Hofmann's HER2 scoring system was formulated for evaluating HER2 status in biopsy and surgical specimens, discordant HER2 results in paired specimens were observed in a small percentage of tumors[39]. Intratumoral heterogeneity appears likewise to be the subject of conflicting results of HER2 expression in primary and metastatic tumor samples[33]. Moreover, in a previous study, we showed a significant difference in sensibility when analyzing HER2 expression in whole-tissue sections and in tissue microarrays[13]. Our personal

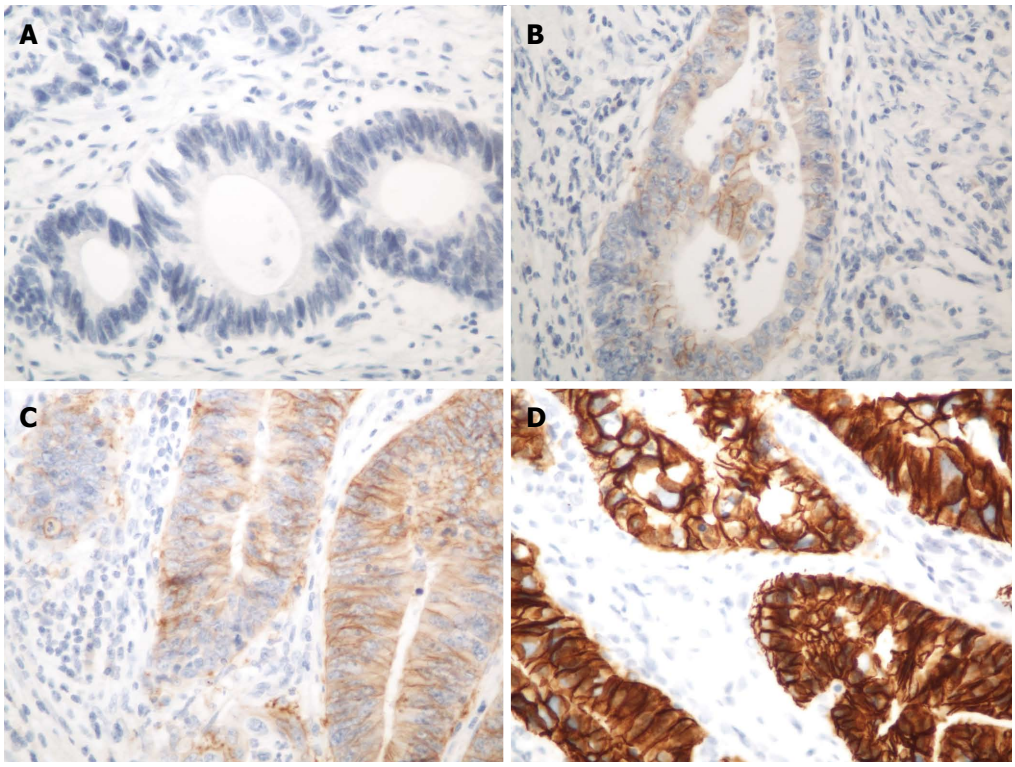

Figure 4 Human epidermal growth factor receptor 2 protein expression in gastric and gastroesophageal tumors. A: A negative (0) case; B: A negative (+1) case; C: An equivocal (2+) case; D: A positive (3+) case. HER2: Human epidermal growth factor receptor 2.

Table 2 Immunohistochemistry scoring for human epidermal growth factor receptor 2 expression in gastric and gastroesophageal junction cancer[17]

Score	Surgical specimen	Biopsy	HER2 overexpression assessment
0	No membranous staining or staining of < 10% of the tumor cells	No membranous staining or staining only in rare cells (less than 5 cohesive cells)	Negative
1+	Staining is weak or detected in only one part of the membrane in ⩾ 10% of the cells	Staining is weak or detected in only one part of the membrane of at least 5 cohesive cells	Negative
2+	Moderate/weak complete or basolateral membranous staining in ⩾ 10% of the cells	Moderate/weak complete or basolateral membranous staining of at least 5 cohesive cells	Equivocal
3+	Strong complete or basolateral membranous staining in ⩾ 10% of the neoplastic cells	Strong complete or basolateral membranous staining of at least 5 cohesive cells	Positive

HER2: Human epidermal growth factor receptor 2.

experience suggests that it is prudent to extend the evaluation to more than one sample and, if feasible, to also evaluate metastatic foci. In fact, testing all available specimens should be considered so that discrepancies can be excluded. When only biopsies are available, it is recommended to have at least four fragments containing tumor cells[40]. We also recommend that all surgical specimens from patients that previously obtained HER2-negative results in biopsies should also be tested to increase the chance of finding HER2-positive tumors.

IHC antibodies

The results of the HER2 test might differ according to the antibody used and, consequently, the antibody might considerably influence therapeutic decisions. An optimal IHC antibody should be adequately sensitive to select the greatest possible number of candidates for treatment and should have the lowest possible false-positive rate in order to avoid overtreatment.

The commercial antibodies currently available are the HercepTest and A0485 (Dako, Glostrup, Denmark), SP3 (Labvision; Thermo Fisher Scientific, Fremont, CA, United States), 4B5 (Ventana Medical Systems, Tucson, AZ, United States) and CB11 (Novocastra, Newcastle upon Tyne, England). Some studies have shown substantial divergence among the antibodies regarding the results of HER2 expression in gastric tumors[13,29,41]. Our previous study compared HercepTest, SP3 and 4B5. We observed that the 4B5 and SP3 antibodies showed similar good performance, with high NPV (negative predictive value) and AUC (area under the ROC curve) values that indicated higher accuracy compared to the HercepTest[13]. Based

on these results and on our personal experience, we believe that 4B5 and SP3 antibodies are more reasonable for first-line tests than the HercepTest in gastric tumors.

CONCLUSION

Given the recent introduction of trastuzumab for the treatment of patients with advanced gastric cancer, assessment of HER2 status is now mandatory for selecting patients eligible for this treatment. Although the development of automated platforms and image analysis should broaden the availability of *in situ* hybridization technologies, immunohistochemistry continues to play an essential role in HER2 status assessment. The overall reliability of HER2 evaluation by IHC, however, can be affected by diverse pre-analytical, analytical and post-analytical variables. Therefore, gastric and gastroesophageal cancer requires a unique scoring system, but above all, it requires expertise in interpretation.

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P- Reviewer: Coccolini F, Guo JM **S- Editor**: Ma YJ **L- Editor**: A **E- Editor**: Wang CH

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ISSN 1007-9327