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HER2 EXPRESSION IN GASTROINTESTINAL TUMOR. A FOCUS ON DIAGNOSTIC ALGORITHM OF HER2 STATUS FROM GASTRIC TO INTESTINAL CANCER

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Abstract: *Human epidermal growth factor receptor 2 (HER2) is a transmembrane growth factor receptor with tyrosine kinase activity regulating cell growth and survival. HER2 amplification is responsible for protein overexpression with a negative impact on prognosis, but HER2-overexpressing patients could be treated with anti-HER2 therapy. In gastric cancer HER2 overexpression is observed in approximately 22% of patients, but prognostic significance in this context is not clear. In colon adenocarcinoma, HER2 gene amplification is reported in a wide range from 0% to 83%. The wide range of reported series could be essentially attributable to a difference in scoring systems for HER2 protein expression. The use of testing score, although measuring the same protein, is different from gastric cancer to colorectal cancer. Herein the different scoring models are reported.*

KEYWORDS: *HER2, Gastric cancer, Colorectal cancer, Fluorescent in situ hybridization, Immunohistochemistry.*

INTRODUCTION

In 1998 the introduction of trastuzumab, an anti-HER2-targeting antibody, in the treatment of breast cancer (BC) patients, on the basis of immunohistochemical score and Fluorescent In Situ Hybridization (FISH), opened the way of predictive pathology in BC patients, i.e. the tool allowing the selection of patients for trastuzumab-based target therapy. Afterwards anti-HER2 targeting antibody was introduced to upper gastroesophageal carcinomas (GEC)[1]. In the last years, we are observing a possible extension of such therapy to other neoplasms, such as colorectal cancer (CRC)[2]. Human epidermal growth factor receptor 2 (HER2) is a transmembrane growth factor receptor protein encoded by a protooncogene located on chromosome 17q21[3]. The receptor includes an intracellular tyrosine kinase activity promoting cell growth and survival[3]. HER2 aberrant amplifications or rarely point mutations in breast cancer and the subsequent protein overexpression impact on prognosis, showing HER2-overexpressing BC patients, a poor prognosis[4]. But the HER2-overexpressing BC patients can be treated with anti-HER2 therapy, with a significant improvement of survival[5]. HER2 overexpression has been demonstrated in other malignancies, mainly in gastric (GC) and gastroesophageal cancer (GEC), but also in CRC, ovarian cancer, prostate cancer and lung cancer[5]. In GC/GEC, the incidence of HER2 overexpression was detected in about 22% of gastric cancer patients, but prognostic significance was controversial[6,7], although most series demonstrated HER2 as a negative prognostic factor, being related to serosal invasion, metastases and higher disease stage[8,9]. In colon adenocarcinoma, HER2 gene amplification and the subsequent product overexpression have been variously reported, with a range rate from 0% to 83%[10-12]. The wide range of reported series could be attributable to a substantial difference

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TABLE 1. Comparison of Her2 scoring in GC and CRC. Y/N eligibility to anti-Her2 Therapy depends upon FISH results, being eligible in cases with Her2 amplification

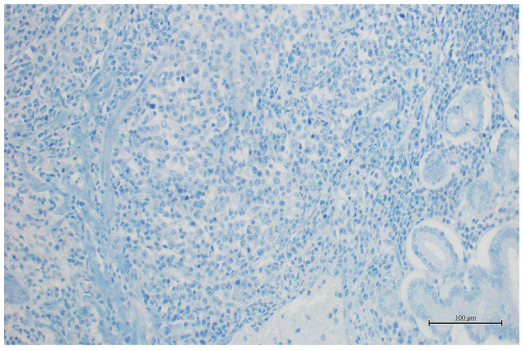
	IHC score	*IHC pattern of positivity*	*FISH requirement*	*Eligibility to anti-her2 therapy*
GC Biopsy	0	Absent positivity	NO	NO
	1+	Weak staining or partial staining of the membrane in at least 5 cohesive cells	NO	NO
	2+	Complete or basolateral moderate/weak membranous staining in at least 5 cohesive cells	YES	
	3+	Strong complete or basolateral membranous staining in 5 cohesive cells	NO	YES
Surgical sample	0	Absent positivity	NO	NO
	1+	Weak staining or partial staining of the membrane in ≥ 10% of the neoplastic cells	NO	NO
	2+	Complete or basolateral moderate/weak membranous staining in ≥ 10% of the cells	YES	Y/N
	3+	Strong complete or basolateral membranous staining in ≥ 10% of the neoplastic cells	NO	YES
CRC	0	Absent positivity	NO	NO
	1+	Faint, segmental or granular membranous staining of any cellularity	NO	NO
	2+ <50%	Any membrane positivity in less of 50% of neoplastic cells	NO	NO
	2+ >50%	Moderate circumpherential, basolateral and lateral positivity in more than 50% of neoplastic cells	YES	Y/N
	3+<10%	Intense circumpherential, basolateral and lateral positivity in 10%<> 50% of neoplastic cells	NO	NO
	3+>10%	Intense circumpherential, basolateral and lateral positivity in more than 50% of neoplastic cells	YES (PREFERABLE)	YES

in scoring systems for HER2 protein expression, a difference of technical approach, sample size and heterogeneity of the study population. When using an accepted scoring system, the rate of HER2 membranous and cytoplasmic positivity were observed in approximately 5% and 30%, respectively[13]. Thus, in this view trastuzumab may be effective only in HER2 membranous positive CRC in a low percentage of cases[14]. Also for CRC the impact of HER2 overexpression on prognosis is controversial[15-18]. In this review we focus the different scoring system through the different laboratory technique.

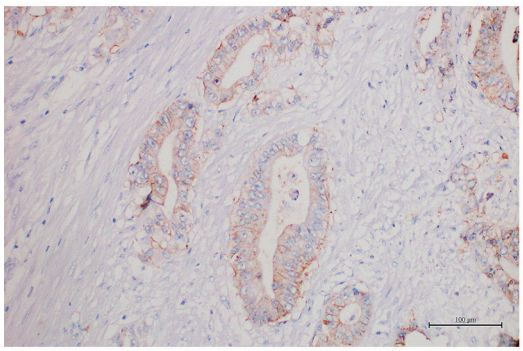
TECHNIQUE OF HER2 STATUS DIAGNOSIS IN GC/GEC

Immunohistochemistry (IHC) and *in situ* hybridization (ISH) in BC are two of the most commonly used techniques for the identification of HER2 status. Their main advantage is the possibility to use both methods on formalin-fixed and paraffin-embedded tissues. Although FISH is considered the gold standard, the higher cost addresses to this method, only equivocal cases from IHC staining, being very high the concordance of negative and positive cases between these two methodologies, with 87-98% concordance rates[19]. The scoring system used for BC has not applicable to GC. Thus, the scoring system proposed by Hofmann et al, assimilated by the College of American Pathologist (CAP) and Food and Drug Administration (FDA), is currently used[20]. In addition it is different when a bioptic sample or a surgical specimen are evaluated[21]. Thus 0 score is applied when no cell is positive at IHC in surgical specimen or rare staining is observed in less than 5 neoplastic cells, 1+ when staining is weak or detected in only one part of the membrane in ≥ 10% of the cells or in at least 5 cohesive cells, 2+ or equivocal when moderate/weak complete or basolateral membranous staining is present in ≥ 10% of the cells or in at least 5 cohesive cells and 3+ when strong complete or basolateral membranous staining is detected in ≥ 10% of the neoplastic cells or in 5 cohesive cells[20]. Differently from BC, circumpherential positivity is not observed in GC/GEC, being positivity mainly basolateral or lateral, as the physiological prevalence of growth factor receptors is concentrated at these sites[22-24]**.** In addition the site of cancer is related to Her2 positivity, being more frequently observed in the proximal stomach, including the esophageal gastric junction (33% of cases), than in the distal stomach (21% of cases)[25,26]. The Her2 deregulation is also related to the histotype, being Her2 positivity almost exclusively observed in intestinal-type carcinoma, the more frequent histotype in GEC[24] (Figure 1).

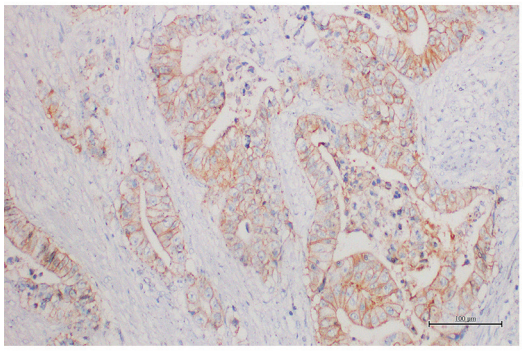
ERBB2 protein expression by VENTANA 4B5 in Gastric Cancer.



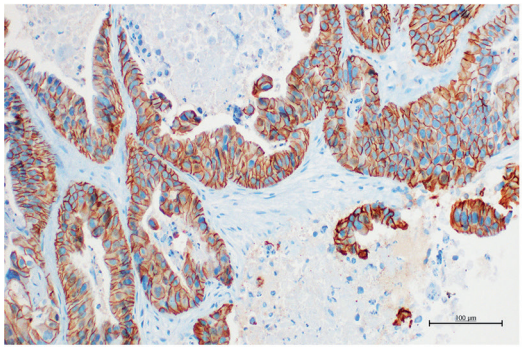
HER2 score = 0



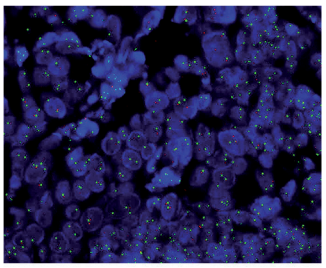
HER2 score = 1+



HER2 score = 2+



HER2 score = 3+



FISH: non-amplified

Equivocal: ERBB2 gene copy number by fluorescent in situ hybridization (FISH)

FISH: amplified

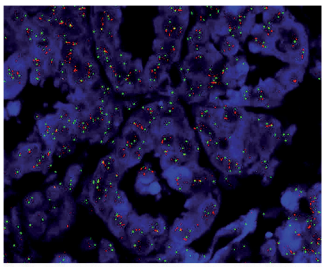


Fig. 1. Immunohistochemistry and fluorescent *in situ* hybridization score for HER2 receptor in gastric cancer. ***A***, no membranous staining of neoplastic cells (score = 0, HER2 overexpression is negative); ***B***, faint or barely perceptible membranous staining in 10% or more of tumor cells (score = 1+, HER2 overexpression is negative); ***C***, weak to moderate complete, basolateral or lateral membranous staining in 10% or more of tumor cells (score = 2+, HER2 overexpression is equivocal and confirmatory FISH testing is recommended); ***D***, strong complete, basolateral or lateral membranous staining in 10% or more of tumor cells (score = 3+, HER2 overexpression is positive); ***E***, HER2: CEP17 ratio < 2.0 (amplification of ERBB2 gene copy number is negative); ***F***, HER2: CEP17 ratio >/= 2.0 (amplification of ERBB2 gene copy number is positive).

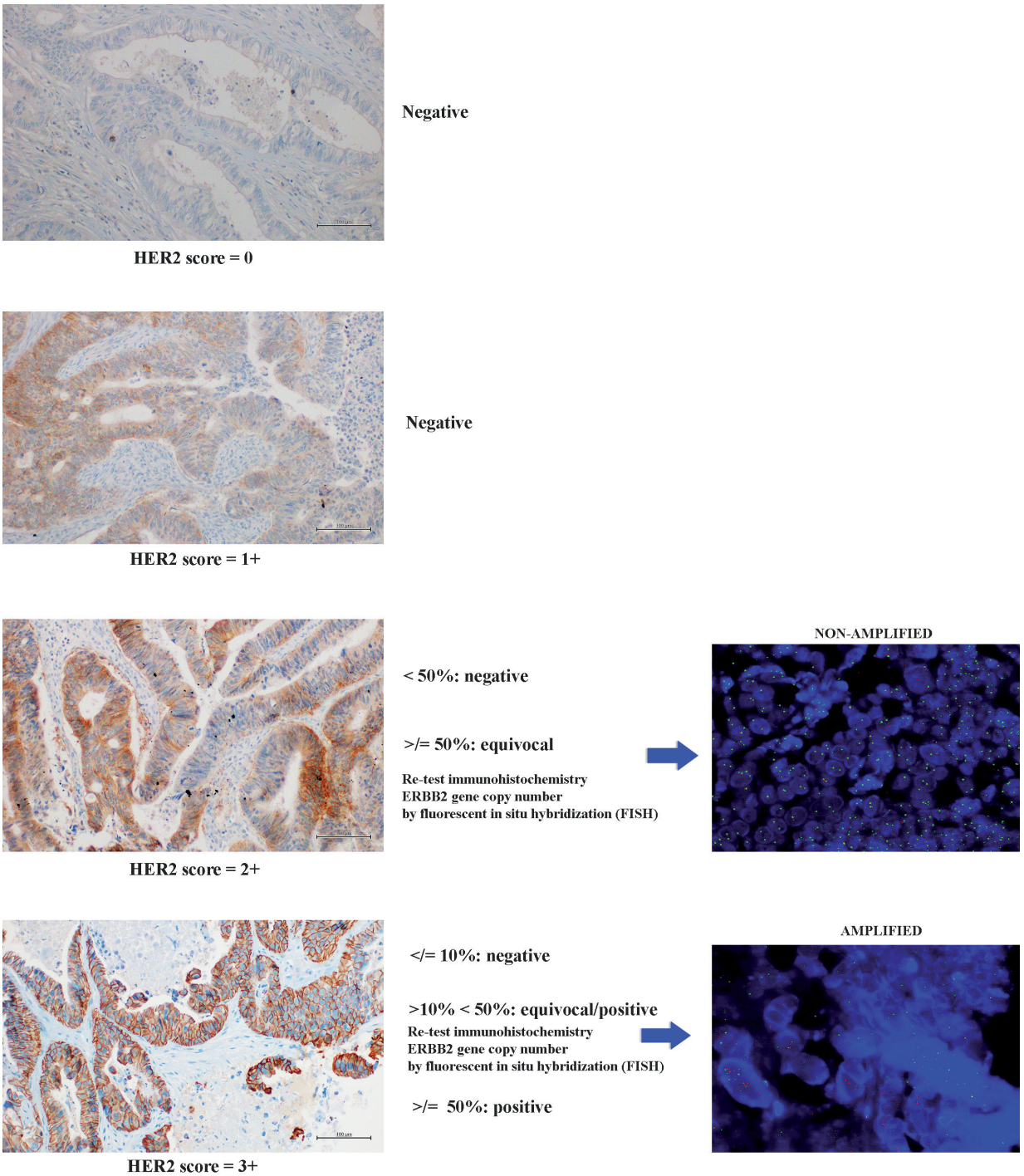


Fig. 2. Immunohistochemistry and fluorescent in situ hybridization for HER2 receptors in colorectal cancer. ***A,*** no membranous staining of tumor cells (score = 0, HER2 overexpression is negative); ***B,*** faint, segmental or granular membranous staining of any cellularity (score = 1+, HER2 overexpression is negative); ***C,*** moderate complete, basolateral or lateral membranous staining in <50% of tumor cells (score = 2+, HER2 overexpression is negative) or moderate complete, basolateral or lateral membranous staining in >/= 50% of tumor cells (score = 2+, HER2 overexpression is equivocal and immunohistochemistry re-test and FISH confirmatory test are mandatory); ***D,*** intense complete, basolateral or lateral membranous staining in </= 10% of tumor cells (score = 3+, HER2 overexpression is negative) or intense complete, basolateral or lateral membranous staining in >10% <50% of tumor cells (score = 3+, HER2 overexpression is equivocal and immunohistochemistry re-test and FISH confirmatory test are mandatory) or intense complete, basolateral or lateral membranous staining in >/=50% of tumor cells (score = 3+, HER2 overexpression is positive); ***E,*** HER2:CEP17 ratio < 2.0 (amplification of ERBB2 gene copy number is negative); ***F,*** HER2:CEP17 ratio >/= 2.0 in >/=50% (amplification of ERBB2 gene copy number is positive).

HER2 IN COLORECTAL CANCER

Recently in colorectal cancer (CRC) HER2 has been described as a potential therapy target, because of the demonstration of its overexpression subsequently to HER2 gene amplification and sequence point mutations[27-29]. Moreover, HER2 genetic deregulation plays a role in the resistance to epidermal growth factor receptor (EGFR)-targeted therapies[30,31]. Thus 30% and 38% of objective response rates were observed in CRC patients with Her2 overexpression respectively in HERACLES and MyPathway studies[31,32]. Although Her2 overexpression does not predict CRC patients' prognosis, its incidence has been reported in 6% of patients in a cohort of 365 consecutive CRC and in a cohort of 174 advanced CRC. In the same cohort HER2 gene amplification was documented in 5.8% of the patients from cohort 1 and 6.3% of the patients from cohort 2. In addition *HER2* gene amplification documented through SISH was more frequently revealed in rectal site[33]. Although a substantial concordance rate of 95.5% between HER overexpression documented through immunohistochemistry and HER2 gene amplification obtained through SISH, some biological condition, such as erroneous post-translation processes leading to decreased HER2 protein expression in a status of *HER2* gene amplification, also the interpretation of HER2 status in CRC could impact on discordant cases. Indeed the interpretation should be based on specific criteria derived from the specific clinical setting, not from experience in breast or gastric cancer[2]. Thus, the clinical trial HERACLES (*HER2* Amplification for Colo-Rectal Cancer Enhanced Stratification), based on the use of trastuzumab and lapatinib in metastatic CRC kRas wild type and Her2 amplification, represented the right base for a correct proposal of a specific Her2 scoring system. The trial led to an objective response in 8/27 patients and stable disease in 12/27 patients[31]. The trial was preceded by an accurate definition of inclusion criteria, based on Her2 status derived for immunohistochemistry and in situ hybridization[2]. Thus the samples collected in the HERACLES study were studied using HercepTest antibody (Dako A/S Glostrup, Denmark) and automatically on the automated Bench Mark Ultra system using the VENTANA 4B5 antibody and then for in situ hybridization through Her2 amplification analysis by FISH was performed with a PathVysion HER-2 DNA Probe Kit (Abbott Laboratories, Des Plaines, IL, USA) and SISH with a VENTANA 4B5 Inform HER2 dual-color on the BenchMark Ultra system (Inform HER2 DNA dual-color assay—Roche Tissue Diagnostics, VENTANA Medical Systems, SA) [2] (Figure 2). The conclusion of this pre-clinical study adds important information to the interpretation of HER2 status as predictive biomarker in CRC. Particularly the consensus of pathologist selected 4B5 clone for immunohistochemical evaluation of HER2 because of the lower incidence of false negatives respect to HercepTest. In addition, being HER2 amplifications supporting the trials, the Consensus of pathologist define a diagnostic algorithm, in order to minimize the false positive cases. Thus, on the base of Her2 amplification data they define two order of 2+ and three order of 3+ immunohistochemical scores. Indeed 2+ score with any membrane positivity in less of 50% of neoplastic cells and 3+ score with intense circumpherential, basolateral and lateral positivity in less than 10% of neoplastic cells are not considered as eligible patients; 2+ score with moderate circumpherential, basolateral and lateral positivity in more than 50% of neoplastic cells and score 3+ with intense circumpherential, basolateral and lateral positivity in more than 10% of neoplastic cells but not more than 50% of cells require further immunohistochemical confirmation and then ISH documentation of HER amplification to candidate the patients to the treatment. Finally, 3+ score with intense circumpherential, basolateral and lateral positivity in more than 50% of neoplastic cells, after immunohistochemical confirmation, could be candidate to combined therapy without ISH documentation of HER2 amplification, although suggested[2] (Figure 2).

CONCLUSIONS

Multiple reports demonstrate a deregulation of HER2 expression, mainly due to gene amplification, in many cancer types. But the applicability of such information to the specific HER2 target therapy should be revised in a clinical trial, being interpretation of immunohistochemistry and ISH methodologies quite different from a clinical neoplastic setting to another.

Conflict of Interest:

The authors declare no conflict of interest.

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