# Analysis of HER2 expression and gene amplification in adenocarcinoma of the stomach and the gastro-oesophageal junction : rationale for the Belgian way of working

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## Abstract

**The Human Epidermal growth factor Receptor 2 (HER2) has been established as a key player in the development of certain human tumors. ToGA trial has demonstrated that the addition of the monoclonal antibody blocking HER2 receptor, trastuzumab (Herceptin®), to chemotherapy significantly improves overall survival of patients with HER2-positive advanced or metastatic adenocarcinoma of the stomach or gastro-oesophageal junction. Therefore, it is essential that pathologists guarantee an accurate testing of HER2 status in these tumours. Following the international recommendations and the Belgian criteria for reimbursement of trastuzumab, a consortium of expert pathologists (Belgian Working Group Molecular Pathology) proposes an adaptation of the international guidelines in order to develop strategies for optimal performance, interpretation and reporting assays.** (Acta gastroenterol. belg., **2012**, 75, **9-13**).

**Key words** : HER2 testing, gastric cancer, gastro-oesophageal junction cancer, immunohistochemistry, in situ hybridisation, trastuzumab.

## Introduction

Human epidermal growth factor receptor 2 (HER2, ErbB2, Neu) is a 185-kDa transmembrane tyrosine kinase receptor. HER2 overexpression/amplification is implicated in the development of various solid tumours and plays a pivotal role in oncogenic transformation and tumorogenesis (1). The HER2 signalling pathway has been recently proposed as a major therapeutic target.

In breast cancers, HER2 overexpression/amplification is associated with aggressive tumour growth, poor prognosis and an increased risk of disease recurrence (2,3). Furthermore, HER2 overexpression/amplification in breast tumors is a predictive marker for targeted therapy with the monoclonal antibody trastuzumab, a fully humanised anti-HER2 monoclonal antibody (4,5). In gastric adenocarcinomas, overexpression/amplification of HER2 was first described in 1986 (6). Most data reported in the literature for HER2 positivity rates in gastric cancer vary from about 15 to 25% (6-9), but HER2-positive status appears to be associated with poorer prognosis, more aggressive disease and shorter survival (8-10). Most information concerning the effect of trastuzumab in gastric cancer treatment came from the ToGA (trastuzumab for gastric cancer) trial, an open-label randomised multicenter phase III study conducted in 24 countries. The combination of chemotherapy plus trastuzumab was shown to be statistically superior to chemotherapy alone, with an increased median overall survival of nearly 3 months (OS 13.8 vs. 11.1 months without trastuzumab). Moreover, increased benefit from trastuzumab was seen in patients who had higher levels of HER2 protein expression, including subgroups with immunohistochemistry (IHC) score 2+/FISH + and IHC score 3+. In these patients, median overall survival increased from 11.8 months for the chemotherapy treatment arm to 16.0 months for the chemotherapy with trastuzumab arm (11). In addition, the ToGA trial confirmed the previous observations that HER2 overexpression is found in approximately 15-25% of cases of advanced gastric and gastro-oesophageal junction adenocarcinoma and that there is a strong correlation with tumour type and location in the stomach (6,7,11-15).

According to the data of the literature, the breast scoring system for HER2 IHC is not applicable to gastric cancer and should be modified taking into account the differences between HER2 overexpression in gastric and breast cancers, i.e. the incomplete membrane positivity (lateral or basolateral membrane staining) and the heterogeneity of the overexpression/amplification of HER2 in gastric cancer (7,8). Indeed, in gastric cancer, lateral membranous staining with linear staining at contact sites between two cells or basolateral membranous staining creating a U-shaped staining pattern is observed (Fig. 1). This is due to the higher frequency of glandular formations with lumen in gastric cancer (intestinal type), in which basolateral (not luminal) membranes are stained. The basolateral staining pattern is probably secondary to the absence of growth factor receptors at the luminal part of the cell. This incomplete membrane positivity should be considered “positive” in gastric cancer, in contrast to breast cancers in which the completeness of membrane staining is a “conditio sine qua non”.

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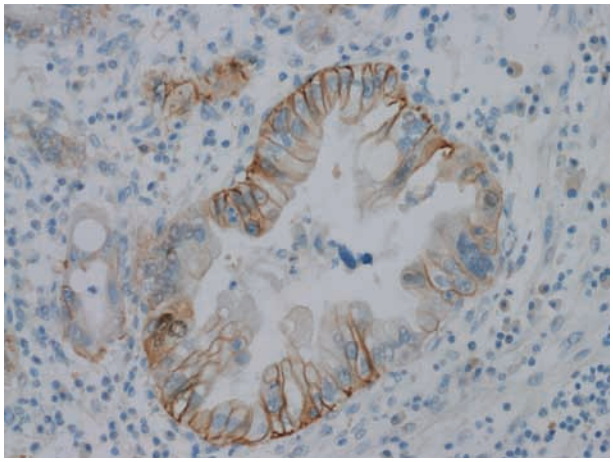


Fig. 1. — Lateral membrane staining or basolateral membranous staining creating a U shaped staining pattern (40×).

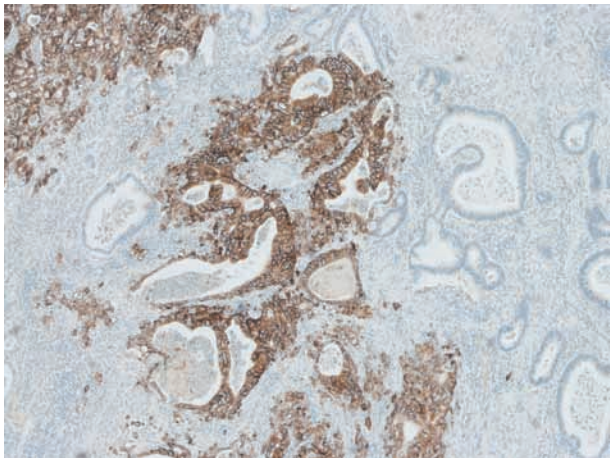


Fig. 2. — Tumour heterogeneity (5×)

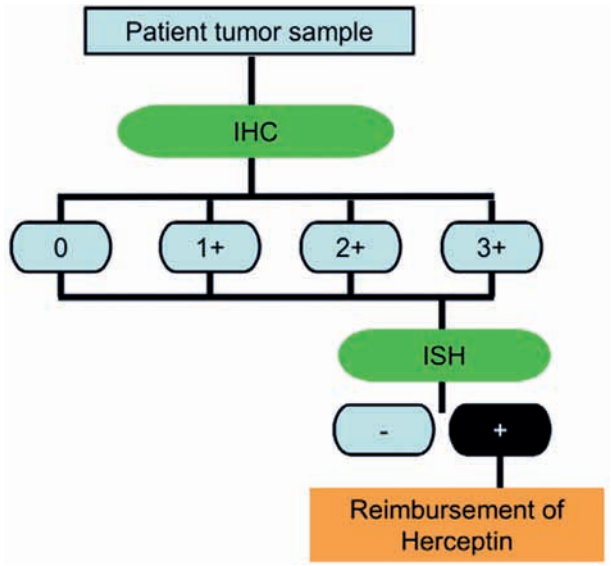
Tumour heterogeneity is more common in gastric cancer (Fig. 2) (7,8,15,16). A recent study evaluated the percentage of heterogeneity as 52% of cases (17). Heterogeneous staining can sometimes be seen within one gland. The main explanation to such heterogeneous staining is that up to one third of gastric cancers are of mixed intestinal/diffuse type. Strong staining is often seen in areas of intestinal type of differentiation, while areas of diffuse type of differentiation are negative. Signet-ring-cell carcinomas are almost always negative. Because of heterogeneity, the 10% cut-off level for positivity, which is required in breast cancer, is omitted in gastric cancer for biopsy specimens. Positivity in gastric cancer specimens is thus independent of the percentage of stained cells, and a cohesive group of cells displaying HER2 positivity represents a sufficient criterion. The first paper on HER2 scoring in gastric cancer stated that a least 5 cohesive HER2-positive cells were required (8).

Following the results of the ToGA trial, EMEA (European Medicines Agency) reported on December 17th, 2009, the approval of trastuzumab (Herceptin®) for the treatment of advanced/metastatic adenocarcinoma of the stomach or gastro-oesophageal junction, in combination with capecitabine or 5-fluorouracil (5-FU) and cisplatin, for patients who have not received prior anticancer treatment for their metastatic disease. The EMEA limited the approval to patients whose tumours have HER2 overexpression as defined by HER2 IHC score 2+, confirmed by a positive FISH, or by HER2 IHC score 3+, as shown by an accurate and validated assay (18).

## Belgian recommendations

The Belgian Working Group Molecular Pathology supports the decision to require HER2 ISH as the sole criterion to determine which patients are eligible for trastuzumab treatment and thus for reimbursement of therapy and not to rely solely on IHC in case of IHC score 3+ (Table 1). Indeed, it has been shown that reproducibility between laboratories is worse for IHC than for ISH, predominantly due to non-standardized fixation methods and times of tissue samples. ISH is relatively independent of tissue fixation, compared to IHC, where staining intensity is highly dependent on fixation time (17,20). Moreover, ISH requires counting the number of signals, while IHC includes an interpretation step. IHC scoring of the intensity of the staining relies on human eyes, which cannot adequately distinguish small differences in staining intensity as described by Rüschoff *et al*. in 2010 (8). For reproducible intensity scoring it is advised to apply the algorithm, so called magnification rule, proposed by J. Rüschoff (Table 2).

Table 1. — **Belgian algorithm for HER2 testing in gastric and oeso-gastric adenocarcinoma**




Since overexpression/amplification of HER2 in gastric cancer is often heterogeneous, a precise screening of

Table 2. — **Algorithm for reproducible intensity scoring in HER2 IHC in oeso-gastric and gastric adenocarcinoma (magnification rule)**

| | |
|---|---|
| Score 3+ : Tumour cell clones with a strong basolateral or lateral membranous reactivity irrespective of percentage of tumour stained | Positivity directly visible by eye or at low magnification (5×) |
| Score 2+ : Tumour cell clones with a weak to moderate basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained | Requires a more detailed magnification with a 10× or 20× objective for demonstration of membranous staining |
| Score 1+ : Tumour cell clones with a faint/barely perceptible membranous reactivity irrespective of percentage of tumour cells stained | If high magnification, a 40× objective, is required for demonstration of membranous staining |

Table 3. — **Results for HER2 testing in three Belgian academic centers**

| | Hospital A | Hospital B | Hospital C |
|---|---|---|---|
| N | 54 | 17 | 125 |
| Location | | | |
| Gastric | 42 | 7 | 72 |
| Oeso-gastric | 12 | 10 | 53 |
| Type of cancer | | | |
| Intestinal | 43 | 14 | 61 |
| Diffuse | 11 | 1 | 38 |
| Mixed | | 2 | 26 |
| HER2 status (%:+) | 25.9% | 23.5% | 16.8% |
| IHC 0-1+/ISH - | 26 | 11 | 76 |
| IHC 0-1+/ISH+ | 0 | 2 | 3 |
| IHC 2+/ISH - | 12 | 2 | 28 |
| IHC2+/ISH+ | 4 | 1 | 6 |
| IHC 3+/ISH- | 2 | 0 | 0 |
| IHC 3+/ISH+ | 10 | 1 | 12 |

the whole tumour area is very important. The Belgian working group for HER2 testing in gastric cancer recommends performing first IHC for HER2, in order not to miss a small focus of HER2 overexpression/amplification and to facilitate identification of an amplified region. This IHC-positive focus can subsequently be selected for further ISH analysis. When HER2 IHC is performed in a primary pathology laboratory and HER2 ISH in a reference laboratory, the Belgian Group recommend to send the HER2 IHC slide to the reference laboratory together with the tissue block (19).

In most studies, a high concordance between IHC and ISH for IHC score 3+ is observed, but this seems quite different for IHC score 2+ and score 1+. In the study of Rüschoff *et al*., all IHC 3+ showed HER2 gene amplification, whereas only 32% of cases with IHC score 2+ and 5% of IHC score 1+ were amplified (8). By contrast, Bilous *et al*. showed a significant number of gastric cancers with HER2 IHC score 0/1+ with gene amplification (16). In our Belgian Group, we also observed a significant number of oesogastric cancers with HER2 IHC score 1+ and gene amplification and, conversely, a significant number of patients with HER2 IHC score 3+ without gene amplification (Table 3). These patients with amplification but low level HER2 expression might respond less to trastuzumab therapy, as revealed by the ToGA trial (11). These observations, however, are based on a small number of patients and it has not yet been unequivocally proven that these patients with IHC score 0/1+, but with amplification of HER2, do not respond to trastuzumab treatment.

Thus, like Barros-Silva *et al*., we supports the decision to analyze all tumours by ISH, including those with IHC score 0/1+, to avoid false negatives (13,19).

The Belgian Working Group recommends using both IHC and ISH in clinical testing. HER2 IHC and ISH procedures should be standardised using written procedures, and regularly validated using the quality control and quality assurance measures. Reliable HER2 testing is of key importance. Specificity and sensitivity of the currently available anti-HER2 antibodies (e.g. HercepTest – Dako, 4B5 clone – Ventana, CB11 clone – Novocastra, SP3-Labvision) used in IHC testing differ. IHC positivity for HER2 should be determined by an accurate and validated assay. A recent study comparing the HER2 status in gastro-oesophageal adenocarcinomas

Table 4. — **Standardisation of the pathological report for HER2 testing in oeso-gastric and gastric cancer**

| |
|---|
| Patient identification : |
| Requesting physician identification : |
| Date specimen received : Date results available : |
| Tissue block number (including name of primary pathology department) : |
| Specimen type : Specimen site : |
| Histological type : |
| Fixation method and time of fixation (if available) : |
| HER2 IHC |
| Antibody clone used : |
| IHC score : |
| Estimated number of tumour cells on which the scoring was performed and percentage of positive cells : |
| HER2 ISH |
| Technique used : HER2/CEP17 ratio : |
| Interpretation of the results |

comparing SP3 and 4B5 immunohistochemistry with dual probe HER2 (fluoresence in situ hybridisation-FISH and silver in situ hybridisation-SISH) concluded that sensitivity for amplification is higher with 4B5 IHC than SP3 (21). Therefore, it is advised to mention the antibody clone used in the pathological report.

Evaluation of HER2 in situ hybridisation can be made by fluorescence (FISH) or by a dual colour silver enhanced in situ hybridisation (SISH). Recent studies have compared FISH versus SISH. Practically, FISH and SISH yield similar results, but the assessment seems much easier with SISH (15,17,21). It would be worth mentioning the type of ISH used in the pathological report but the Working Group does not recommend to preferentially use any of both.

Because of tumour heterogeneity, the 10% cut-off level for positivity was in first instance only omitted for biopsy specimens, while for surgical resection specimens a 10% cut-off level was required instead of the cut-off for breast cancer resection specimens (7,8). However, the 10% cut-off level is now also considered inappropriate for testing in surgical resection specimens, as often these cases with less than 10% positive IHC score 2+ or IHC score 3+ cells as determined by IHC can contain a cluster of 20 cells with amplification of HER2 as detected by ISH (J. Rüschoff, personal communication). Positivity in gastric cancer specimens is thus independent of the percentage of stained cells and it is sufficient to have a cohesive group of cells displaying HER2 positivity. The first papers on HER2 scoring in gastric cancer stated that a least 5 cohesive HER2-positive cells were required (8). However, it is more appropriate to require at least 20 cohesive cells for positivity, as a number of 20 cells showing amplification for HER2 is required to conclude for positivity in amplification studies with ISH (J. Rüschoff, personal communication).

For this reason, we advise to apply the same rules to resection specimens as to biopsies, and to require in both cases for positive IHC at least 5 or better 20 cohesive HER-2 positive cells (Table 2).

According to Russhoff *et al*., biopsies as well as surgical specimens can be used for HER2 testing with similar success rates. At least 6 biopsies are required to increase sensitivity. On resection specimens, because of the strong correlation between HER2 overexpression/amplification and tumour type, blocks with areas of gland formation (intestinal type) should be selected if present.

In addition, there is no statistical difference between the overexpression/amplification of HER2 in metastastic tissue vs. primary tumours in ToGA trial as well as in a recent paper of Bozetti *et al*. which showed a high concordance between HER2 results obtained by both IHC and FISH on primary tumours and corresponding metastases (22).

In accordance with Rüsshoff publication, the evaluation of HER2 ISH in gastric cancer is similar to that in breast cancer except for HER2/CEP17 ratio ≥ 2.0 indicating HER2 gene amplification. In case of a ratio between 1.8 and 2.2 an additional count of 40 cells in a different area is required. The final cut-off is then a ratio of ≥ 2.0 (8). Interpretation must include the counting of at least 20 non-overlapping, adjacent tumour cells in the chosen cancer area, only leaving out those cells that do not meet the quality criteria (overlapping nuclei). Counting can be done by a trained technologist, but must be confirmed by a pathologist.

The pathological report should be standardised as described in Table 4. The primary pathologist should include the results into the original or a complementary pathology report and transmit the results to the requesting oncologist. The report should include interpretation of the results.

## Conclusion

The Belgian Working Group for HER2 testing in gastric cancer recommends to perform ISH preceded by IHC in all cases in clinical testing in gastric cancer, in order not to miss the amplified region in cases of heterogeneity. Amplification as demonstrated by HER2 ISH (HER2/CEP17 ratio ≥ 2) is, however, the sole criterion for decision to treat with trastuzumab. Moreover, all patients that are considered for trastuzumab treatment, as well as all patients with IHC score 0/1+, should be analysed by ISH, since they are also eligible for

trastuzumab therapy if they show amplification. Concerning surgical samples, the pathologist should select the tissue blocks with the largest area of intestinal differentiation (glandular structures). The pathologist should clearly identify the strongest IHC-positive area in order to perform ISH confirmation on that particular area. We advise to apply the same rules for resection specimens as for biopsies, i.e. to require in both cases for positive IHC at least 5 or better 20 cohesive HER-2 positive cells.

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