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# HER2 status in advanced gastric cancer: the dark side of the moon

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## Abstract

The present study evaluated HER2 status between primary gastric and paired metastatic disease to lymph nodes, collecting 62 formalin-fixed paraffin-embedded representative tissue blocks as well as synchronous metastatic lymph nodes by immunohistochemistry and FISH. The discordant HER2 pooled rate, regardless either negative or positive conversion, was 9.26% in primary gastric carcinoma and corresponding nodal metastasis. Moreover, a high level concordance in HER2 expression between primary carcinoma and synchronous metastatic lymph nodes was achieved in 90.74% of cases. In our opinion, the observed event of discordant HER2 status should be ascribed to intra-tumor heterogeneity. In any case, the shift from positive to negative HER2 expression suggests that Trastuzumab could be the targeted treatment choice, while the opposite shift should be evaluated by a simultaneous HER2 determination in both primary and metastatic lymph nodes.

**Key words**: HER2, advanced gastric cancer, lymph node, metastasis, prognosis

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## Introduction

Trastuzumab is a humanized monoclonal antibody targeting HER2, able to increase the disease-free and overall survival of women with tumors HER2 positive expression in breast cancer (1,2); moreover, a significant percentage of gastrointestinal adenocarcinomas shows an HER2 gene amplification with a range from 6% to 23% (1,3). HER2 amplification or overexpression has reported to be variable rates in advanced gastric carcinoma (AGC), in relation to the primary tumoral site, being more frequent in cancers located in gastroesophageal junction (GEJ) (24–35%) compared with those from others gastric localizations (9.5–21%) (4,5). The histological assessment and classification of neoplastic histotypes revealed that the intestinal is more likely to be HER2 positive (16–34%) compared with diffuse (2–7%) or mixed (5–20%) types (6). Rarer histotypes of gastric carcinomas were also studied, including hepatoid carcinomas (HAS) that have been shown the highest HER2 immunoreactivity (7). In the present paper we analyzed the

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discordance in HER2 status encountered in primary AGC and corresponding synchronous lymph node metastases.

## Materials and Methods

In a cohort of 62 AGC (37 male and 25 female patients; mean age of the patients: 66.06 years; range 39–90 years), together with the corresponding regional synchronous metastatic lymph nodes, we have utilized the two recommended methods in HER2 testing, such as immunohistochemistry (IHC) for protein expression and in situ hybridization (ISH) for gene amplification, although there is controversy regarding the validity of HER2 testing in gastrointestinal malignancies (8). In contrast to breast cancer, the distribution of HER2-positive gastric cancer cells in tissue is heterogeneous, frequently multi-focal and incomplete membrane staining. Consequently, the ASCO/CAP guidelines for HER2 IHC testing used in breast cancer were not appropriate for AGC (9); therefore, a specific gastric cancer HER2 testing protocol has been developed (9). In biopsy specimens, it has been recommended that a cluster of at least 5 positive tumour cells was the minimum required to qualify a result as positive, replacing the surgical specimens cut-off criteria of $\geq$ 10% (9). In case of equivocal 2+ results, an ISH test is strongly recommended to clarify the HER2 status in represented by ISH techniques, mainly fluorescence in situ hybridization (FISH) (10). Fleiss-Cohen weighted k statistics were used to assess the concordance rate between HER2 status of the primary AGC and metastatic synchronous lesions. *k* values between 0 and 0.2 were regarded as no agreement, between 0.21 and 0.4 as fair agreement, between 0.41 and 0.6 as moderate agreement, between 0.61 and 0.8 as substantial agreement, and between 0.81 and 1 as almost perfect agreement. The statistical correlations between HER2 status and the other histopathological parameters were investigated by using Chi-squared test. This test was also carried out to assess whether any variation in the clinico-pathological parameters was present between discordant and concordant AGC. A *p*-value lower than 0.05 was considered to be statistically significant. Data were analyzed by using the SPSS package version 6.1.3 (SPSS, Chicago, IL, USA).

## Results

HER2 immunohistochemical staining showed that 11 primary AGCs (17,74%) were scored for 3+ HER2 expression (Fig.1), while four cases were 2+ (6.42%), five cases 1+ (8.10%), and 42 cases (67.74%) were not expressed HER2 at all. FISH analysis revealed no amplification in all of these cases with HER2 scores of 2+ or more. The overall concordance rate with corresponding nodal metastases was 90.74%; by contrast, changes in HER2 status between primary AGC and

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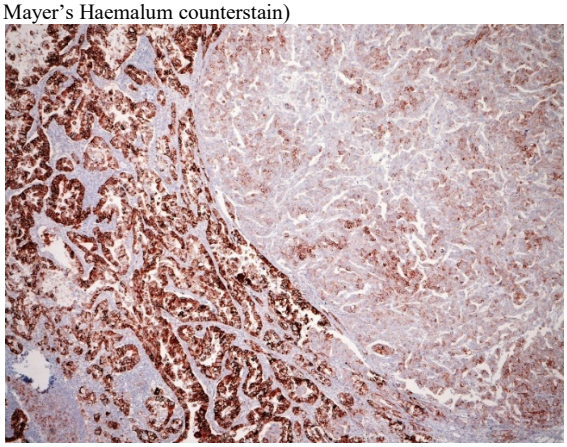
matched synchronous metastases were evidenced in 10 (9.26%) cases.

Of these, 6 cases were HER2 amplified in the primary AGC and not amplified in the metastases, while 4 were HER2 not amplified in the primary tumour and amplified in the lymph node metastases. No significant differences in the clinico-pathological parameters were encountered between discordant and concordant AGC.

Fig. 1: Intratumoral heterogeneous 3+ HER2 expression in a surgical specimen of intestinal type gastric carcinoma (120x, Mayer's Haemalum counterstain)



**Discussion and Conclusions**

HER2 testing is often done on neoplastic biopsy samples of primary lesions, the overall concordance range of HER2 status between biopsy and surgical specimens advanced gastric and GEJ adenocarcinomas ranged from 74.1% to 96.1 %, with a predictive positive value of 71.4 % and a negative predictive value of 94.4 % (11) indicating that the considerable heterogeneity of HER2 expression raises issues with respect to tumour sampling and block selection. It has been described, that the IHC HER2 analysis with a single block of the primary tumor could not be adequately confident to compensate for the heterogeneity of HER2 expression in a gastric cancer (12); in fact, additional tissue blocks improved the rate to 20%, a value slightly higher than that obtained using 1 block (17%) (12).

In AGC many papers showed that HER2 status varied in the metastatic lesions compared to the primary tumour and this discrepancy was more frequently encountered in distant metachronous metastases (87.5%–94.9%) than in locoregional ones (13,14). In detail, in gastric cancer the discordance rate ranged from 2% to 24%, with a mean pooled estimated about 7%, with a pooled proportion of negative conversion of 17% and the positive conversion of 4% (14).

In order to explain this discordant phenomenon, a variety of reasons has been hypothesized including variability in technical assessment and/or pre-analytical factors, such as use of different fixation intervals and different staining procedures. To avoid the possibility that variability in HER2 status might depend on external factors, both neoplastic primary and metastatic gastric specimens should be collected during the same surgical procedure, without any influence of therapy and with a common tissue fixation methodology (14-15). In conclusion, the results suggest to reassess HER2 status not only in metachronous metastases, but also in synchronous nodal metastases since it may have a relevant clinical impact for the therapeutic choice.

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