Pathology (December 2015) **47(7)**, pp. 641–646

ANATOMICAL PATHOLOGY

Concordance of HER2 expression in paired primary and metastatic sites of gastric and gastro-oesophageal junction cancers

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Summary

HER2 is amplified/overexpressed in a subset of gastric and gastro-oesophageal junction cancers. Addition of anti-HER2 therapy has been shown to provide survival benefit in this setting. However, there are limited data assessing the concordance of HER2 status between primary and metastatic sites. A total of 113 samples from 43 paired primary and metastatic tumours were tested for HER2 status, by immunohistochemistry (IHC) for protein expression and silver *in situ* hybridisation (SISH) for gene amplification. Primary sites tested included endoscopic biopsies ($n=30$) and resections ($n=24$). Metastatic samples included lymph nodes ($n=29$), peritoneal effusions ($n=21$) and miscellaneous sites ($n=9$). The overall HER2+ rate was 11%. Of 41 (95%; 95% CI 88.5–100%) concordant cases, 38 were HER2– and three were HER2+. There were two (5%) discordant cases, one of which showed heterogeneity of HER2 expression. This series confirms a high concordance rate of 95%, supporting that testing of primary tumours and metastases is equally valid and providing clinical rationale for the addition of anti-HER2 therapy in HER2+ disseminated disease.

Key words: Concordance, gastric cancer, HER2, Herceptin, metastases.

Received 28 April, revised 25 June, accepted 29 June 2015

INTRODUCTION

Gastric cancer remains a frequent and lethal disease despite a steady decline in incidence and mortality since the 1930s. Approximately one million new cases occur annually, making it the fourth most common malignancy worldwide.[1] Advances in surgical treatment and conventional multimodality therapy have led to better prognosis over the past two decades, although the improvement has been modest. Gastric cancer is still the second most common cause of cancer-related death in both males and females, with an overall 5-year survival rate of less than 30%.[1,2]

Recently, there has been growing interest in the role of human epidermal growth factor receptor 2 (HER2), an oncogene which codes for a transmembrane tyrosine kinase glycoprotein involved in signal transduction pathways regulating cell growth.[3] HER2 gene amplification and/or protein overexpression in gastric and gastro-oesophageal junction (GOJ) cancers was recognised as early as the 1990s,[4] with an incidence variably reported as between 7 and 42%,[5,6] although most studies since then have primarily focused on breast cancer. The recent renewal of interest in the role of HER2 in gastric cancer has been largely driven by the findings of the widely publicised ToGA (Trastuzumab for Gastric Cancer) clinical trial.[7] In this multicentre, randomised phase III study, clinically and statistically significant benefit was seen in response rates, median progression-free survival and overall survival with the addition of the anti-HER2 biological agent, trastuzumab, to standard chemotherapeutic regimens. The survival advantage was reported in patients with advanced gastric and GOJ carcinoma showing HER2 gene amplification and protein overexpression, with the greatest benefit demonstrated in those with a 2+/3+ reaction on immunohistochemistry (IHC) and gene amplification confirmed on fluorescence *in situ* hybridisation (FISH).

In view of these findings, selection of patients most likely to benefit from trastuzumab and other novel anti-HER2 therapies is critically dependent upon reliable methods of HER2 testing. To date, this assessment has been largely based on the primary tumour, either endoscopic biopsies or surgical resections, and experience with testing of metastases is limited. However, most patients with gastric carcinoma in Western populations present with advanced disease with up to 40% having metastatic spread at initial diagnosis.[8] Although it has been suggested that HER2+ clones within gastric cancers are more likely to metastasise,[9] there are limited data on the concordance of HER2 status between primary and metastatic sites, and hence on whether HER2 amplification/overexpression is truly maintained in the metastatic process. A high concordance would not only provide support for testing of metastatic sites which might be more easily and rapidly accessible, but also offer clinical rationale for the use of anti-HER2 therapies in advanced disease. Therefore, in this study we aimed to compare HER2 status between primary and metastatic sites of gastric and GOJ carcinomas and to assess whether factors such as age, sex, site of origin, histological subtype and heterogeneity of HER2 expression might influence concordance rates.

MATERIALS AND METHODS

Samples from cases with paired primary and metastatic gastric and GOJ carcinomas were identified from the database of a single, tertiary level, hospital-based laboratory. HER2 status was assessed by immunohistochemistry (IHC) for protein overexpression and silver *in situ* hybridisation (SISH) for gene amplification. According to current guidelines, brightfield methods of ISH testing such as SISH are now preferred over fluorescence *in situ* hybridisation (FISH) for their superior interobserver agreement, lower cost, preserved signals

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: 10.1097/PAT.0000000000000323

Table 1 Immunohistochemical scoring of HER2 protein expression in endoscopic biopsy and resection specimens for gastric and gastro-oesophageal adenocarcinomas according to the modified criteria of Hofmann *et al*.[5,8]

Score	Biopsy specimens	Resection specimens
0	No staining or membrane staining in clusters of <5 tumour cells	No staining or membrane staining in <10% of tumour cells
1+	Cluster(s) of at least 5 cohesive tumour cells with barely visible (requires ×400 magnification) complete, basolateral, or lateral membrane staining	Barely visible (requires ×400 magnification) complete, basolateral, or lateral membrane staining in >10% of tumour cells
2+	Cluster(s) of at least 5 cohesive tumour cells with weak to moderate (generally visible at ×100–200 magnification) complete, basolateral, or lateral membrane staining	Weak to moderate (generally visible at ×100–200 magnification) complete, basolateral, or lateral membrane staining in >10% of tumour cells
3+	Cluster(s) of at least 5 cohesive tumour cells with strong (generally visible at ×25–50 magnification) complete, basolateral, or lateral membrane staining	Strong (generally visible at ×25–50 magnification) complete, basolateral, or lateral membrane staining in >10% of tumour cells

over time and, importantly, for their ability to incorporate assessment of heterogeneity of HER2 amplification, which is seen in up to 40% of gastric cancers.[10–12]

HER2 IHC was performed on 4 μm thick sections, freshly cut from formalin fixed, paraffin embedded (FFPE) tissue using the Ventana Benchmark XT autoimmunostainer (Ventana Medical Systems, USA) which employs an indirect polymer method, following heat-induced epitope retrieval. The polyclonal CERB2 antibody (Dako, Denmark) was applied at 1:4000 dilution. IHC was scored for HER2 overexpression according to the modified criteria of Hoffman *et al.*[11,13] (Table 1). Briefly, these criteria assess a minimum cluster of five cohesive malignant cells in biopsy samples and at least 10% of malignant cells in resection specimens. A four tiered scoring system (0–3+) is proposed, based on the intensity of either complete, basolateral or lateral membrane staining.

HER2 SISH was performed on freshly cut sections from FFPE tissue using the automated Ventana INFORM HER2 Genomic Probe platform. Gene amplification was assessed based on HER2 copy number, which has been shown to be more reproducible than assessments based on the HER2/centromeric probe 17 ratio.[12,14] In clinical practice, an average of ≥6 HER2 signals per nucleus in a minimum of 20 malignant cells is considered amplification.[15]

A proportion of specimens from metastatic sites tested in this study were from effusions and fine needle aspirations (FNAs), for which IHC and SISH were performed on sections cut from cell block material. Cell blocks were prepared from fresh or refrigerated aliquots of the fluid samples using the plasma thrombin clot procedure. Specifically, the cells were concentrated by centrifugation and the supernatant discarded. An equal volume of plasma was added to the cell deposit and mixed to ensure even distribution of the cellular material. Clot formation was achieved with the addition of 0.25 mL of human thrombin. The sample was then fixed in 10% neutral buffered formalin and processed as a biopsy specimen.

IHC and SISH were scored by two experienced pathologists (MPK and WBdB) with expertise in gastrointestinal pathology and SISH scoring. For the purpose of the study, HER2 status was considered positive for cases showing both amplification on SISH and an IHC 2+/3+ reaction, or an IHC 3+ reaction alone when SISH could not be performed due to inadequate or unavailable samples. Our experience has previously demonstrated good concordance between IHC3+ and amplification,[10,12] similar to the basis of current therapeutic goods administration (TGA) and European guidelines.[16] All other combinations were considered to be HER2 negative.

Statistical comparisons of proportions and means were performed using Fisher's exact test and Student's independent samples *t*-test, respectively. Statistical analyses were performed using SPSS for Windows Version 17.0 (SPSS, USA) with the two-sided statistical significance level set at 5%.

This study was approved by the institutional human research ethics committee. Informed consent was waived as there was no direct patient involvement and the results of the study were not anticipated to alter the management of individual participants.

RESULTS

A total of 113 samples from 43 cases with paired primary and metastatic gastric and GOJ carcinomas were identified from our database. The cases were from 25 (58%) males and 18 females (42%), with median age of 60 years and mean age of 61 years (range 31–87 years) (Table 2). Thirty-six (84%) cases were gastric adenocarcinomas and the remaining seven (16%) were GOJ in origin.

Primary tumour samples comprised endoscopic biopsies from 26 patients, two of which had multiple biopsies producing a total of 30 (56%) specimens. There were also resections from 24 (44%) patients (Table 3). Seven patients had both endoscopic biopsies and subsequent resections available for comparison. Histological subtyping according to the Lauren and the World Health Organization (WHO) classifications, the degree of histological differentiation and staging information (as assessed on resections) are summarised in Table 2.

A total of 59 samples were available from metastatic sites including lymph nodes ($n = 29$, 49%), peritoneal effusions ($n = 21$, 35%), liver biopsies ($n = 5$, 8%), small bowel ($n = 1$, 2%), large bowel ($n = 1$, 2%), bone ($n = 1$, 2%) and skin ($n = 1$, 2%) (Table 3). Two of the lymph nodes and two of the liver specimens were sampled by FNA.

Table 2 Clinicopathological characteristics of gastric and gastro-oesophageal carcinomas

	No. patients ($n = 43$)	%
Gender		
Male	25	58
Female	18	42
Age, years (median/mean; range)	60; 61.1	31–87
Primary tumour site		
Gastric	36	84%
Gastro-oesophageal	7	16%
Histological classification (Lauren)[a]		
Intestinal	26	60%
Diffuse	8	19%
Mixed	9	21%
Histological classification (WHO)[a]		
Tubulopapillary	25	58%
Poorly cohesive	8	19%
Mixed	9	21%
Mucinous	1	2%
Histological degree of differentiation		
Well-differentiated	0	0%
Moderately-differentiated	14	33%
Poorly-differentiated	29	67%
T staging (depth of invasion)[b]		
pT1 (submucosal invasion)	1	4%
pT2 (muscularis propria invasion)	1	4%
pT3 (subserosal or adventitial fat invasion)	7	28%
pT4 (invasion of gastric serosa or adjacent structures)	16	64%

[a] Performed on resection specimens where available.
[b] Resection specimens only ($n = 25$).