

## HER2-Neu Gene Testing in Gastric Cancer by Immunohistochemistry in Tunisian Patient's Samples

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### Abstract

Immunohistochemical (IHC) testing for HER2/neu is becoming the standard of care for guiding the adjuvant treatment of gastric carcinoma with trastuzumab. Up to now, gastric cancer has been considered the most commonly diagnosed tumors leading to death.

In this study, we evaluate the detection of HER2 expression by IHC in Tunisian patients with gastric cancer according to the international consensus. A total of 84 tumor specimens was assessed for HER2 expression by immunohistochemistry (IHC) using the antibodies HercepTest™. Doubtful IHC results (IHC 2+) were resolved by HER2 Chromogenic in situ hybridization (CISH). Thus, 10.5% of samples were HER2-positive (3+), 8.3% having a negative score IHC, Her2-Neu (+1) and 3.6% to be a dubious immunostaining Her2-Neu (2+).

**Keywords:** HER2-Neu Gene; Gastric cancer; Trastuzumab; Immunohistochemistry; In Situ Hybridization

### Introduction

Gastric cancer (GC) is the fourth most commonly diagnosed tumor and the second leading cause of cancer-related death in the world [1-4]. Conventional cancer therapies are surgery, radiation, and chemotherapy [1]. Surgery is the main treatment for early stage GC, while patients with advanced GC require chemotherapy to improve their chances of survival [2]. Nevertheless, chemotherapy can cause damage or toxicity to both the adjacent and distant normal tissues, which in turn limits the effectiveness of these therapy approaches [1,2].

The ToGA study showed that trastuzumab plus chemotherapy prolonged median survival in patients with human epidermal growth factor receptor 2 (HER2)-positive advanced gastric cancer [5-7].

Trastuzumab, is a monoclonal antibody used to target Human Epidermal Growth Factor receptor 2 protein and is used worldwide with standard chemotherapy [4,6].

The HER2 protein is a member of the epidermal growth factor receptor family and is coded by HER2/neu gene located on the long arm of chromosome 17 [8]. The product is a 185 kD transmembrane glycoprotein [9]. It is a transmembrane tyrosine kinase receptor [3], involved in tumor cell proliferation, apoptosis, adhesion, migration, and differentiation [8].

It was confirmed that tumor cells release circulating HER2 peptides originated from the plasmic membrane, and thus it could be used as a diagnostic marker for tissue HER2 status [10]. Meanwhile tissue HER2 assessment by immunohistochemistry (IHC) and in situ hybridization

has become a routine practice in the analysis of advanced gastric cancer [4,10].

In this study of 84 samples, we carried out our assessment by using the approach of immunohistochemistry and we confirmed doubtful samples by in Situ hybridization.

### Material and Methods

Our study is retrospective, it was performed on 84 cases of gastric adenocarcinoma involving 42 gastric biopsies, 37 operating samples of gastrectomy and 5 biopsies of metastasis.

All tissue samples were processed according to standard protocols, with formalin fixation. Clinicopathological parameters, including age, gender, histological classification, and pathological TNM stage, were retrieved from the medical folder. Histological classification was determined according to the Lauren's classification.

All tissues were fixed with 10% buffered formalin and then paraffin-embedded.

Sections (4 µm thick) were de-paraffinized in xylene and hydrated through a graded ethanol series.

IHC staining of HER2 was manually performed with the HercepTest II™ (DAKO, Glostrup, Denmark).

Out of the 84 specimens, 3 expressing HER2 were defined as either or IHC 2+ and subsequently were evaluated by CISH.

CISH analysis was carried-out using the ZytoDot SPEC Her2-Neu.

**Statistical analysis**

Statistical analysis was performed using the chi-square test to analyze associations between HER2 status and clinicopathological parameters.

A value less than 0.05 was considered significant. Data were analyzed using the SPSS statistical software program for Microsoft Windows.

		Her2-Neu 0	Her2-Neu 1	Her2-Neu 2	Her2-Neu 3	Total
<b>Age</b>	≤ 60	36	3	2	2	43
	>60	30	4	1	6	41
	Total	66	7	3	8	84
<b>Gender</b>	Male	44	4	2	6	56
	Female	22	3	1	2	28
	Total	66	7	3	8	84
<b>Digestive history</b>	Cardia	8	0	1	1	10
	Fundus	8	0	1	0	9
	Body	5	0	0	2	6
	Den	20	6	1	3	30
	Pylorus	1	0	0	0	1
	Location broadcasts	23	1	0	2	26
	Total	65	7	3	8	83
<b>Macroscopic</b>	Budding	8	0	0	2	10
	Infiltrating	7	0	0	1	8
	Rankled	9	0	1	0	10
	CD-budding	24	4	2	5	35
	CDinfiltrating	18	3	0	0	21
	Total	66	7	3	8	84

**Table 1:** Correlations between epidemiological, paraclinical data and the score of the Her2-Neu status.

		Her2-Neu 0	Her2-Neu 1	Her2-Neu 2	Her2-Neu 3	Total
<b>Size of the tumor</b>	≤ 5 cm	26	4	0	3	33
	>5 cm	40	3	3	5	51
	Total	66	7	3	8	84
<b>Histological type</b>	Intestinal type	40	7	2	6	55
	Diffuse type	26	0	1	2	29
	Total	66	7	3	8	84
<b>Histological differentiation</b>	Well-differentiated	3	0	0	0	3
	Medium Differentiated	22	5	1	4	32
	Undifferentiated	15	2	1	2	20
	Isolated cells in ring kitten	26	0	1	2	29
	Total	66	7	3	8	84

<b>Mucosecretion</b>	Tumor mucosecreteur	32	1	1	2	36
	Tumor not mucosecreteur	34	6	2	6	48
	Total	66	7	3	8	84
<b>Associated precancerous lesions</b>	Chronic gastritis	36	3	1	5	45
	Intestinal metaplasia	18	4	0	2	24
	Helicobacter pylori	13	2	1	2	18
	Operating parts	30	3	0	4	37
<b>Histopathological sampling type</b>	Gastric biopsy	32	4	3	3	42
	Biopsy of metastasis	4	0	0	1	5

**Table 2:** Correlations between histopathological data and the score of the Her2-Neu status.

		Her2-Neu 0	Her2-Neu 1	Her2-Neu 2	Her2-Neu 3	Total
<b>T-stage</b>	T1+T2	16	3	0	2	21
	T3+T4	34	4	3	6	47
	Total	50	7	3	8	68
<b>N-stage</b>	N (+)	42	6	3	7	51
	N (-)	14	1	0	1	16
	Total	56	7	3	8	74
<b>M-Stage</b>	M (+)	28	3	2	5	37
	M (-)	10	4	1	3	14
	Total	38	7	3	8	56

**Table 3:** Correlations between TNM Stage and the score of the Her2-Neu status.

## Results

### Clinical investigation

Out of 84 patients, 67% were male. The mean age of our patients at diagnosis was 59 years with extremes ranging from 27 to 86 years. The maximum frequency is between 40 and 79 years. Patients' clinicopathological data are summarized in Tables 1-3.

### Immunohistochemistry

An immunostaining was revealed in 18 samples, 21.4% of cases. Eight patients, (10.5%) of cases have an intense and full stained membrane in 'U', covering 40% to 95% of the cells, and therefore having a score IHC 3+ (Figures 1e and 1f).

Three patients, (3.6%) of cases have a moderate stained membrane, full for two of them and incomplete 'L' for the third, covering respectively 20%, 30% and 70% of the cells, and therefore a score IHC 2+ (Figures 1c and 1d).

Seven patients, (8.3%) of cases have an incomplete low stained membrane, detected in 20% to 60% of the cells, and thus having a score IHC 1+ (Figure 1b).

The rest of cases (77.6%) showed no marking and therefore an IHC score 0 (Figure 1a).

### Chromogenic in Situ Hybridization CISH

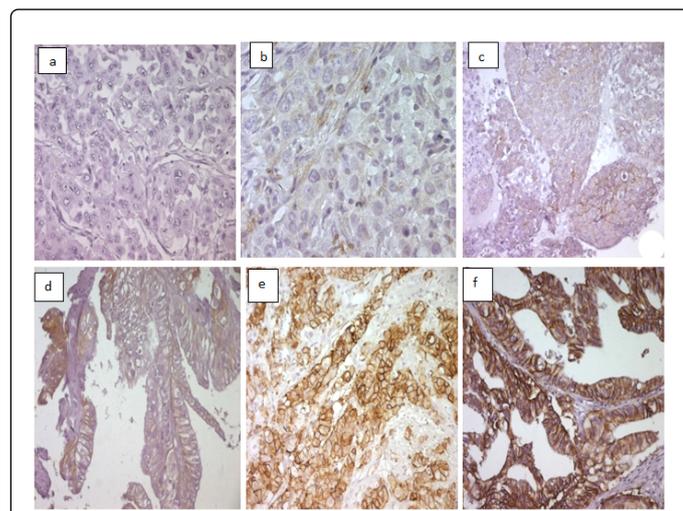
The CISH technique on three ambiguous cases Her2-Neu (2+) looking for a possible amplification of Her2-Neu gene was used. The result was negative and there was no CISH signal (Figure 2).

## Discussion

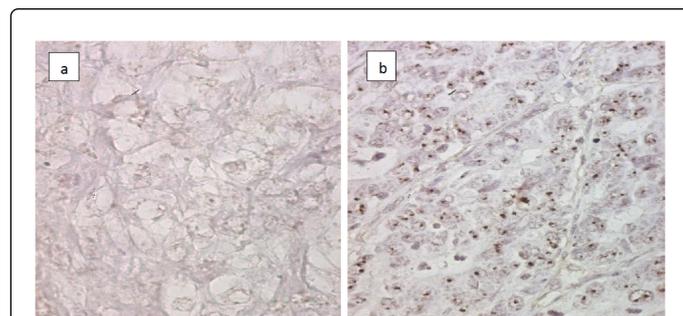
In our study a total of 84 tumor samples (42 gastric biopsies, 37 gastrectomy operating parts and 5 biopsies of metastasis) were tested. 10.5% of samples were Her2Neu (3+), 3.6% were 2+ and 8.3% were 1+. The proportion of stained cells was more than 10% except for cells with score 1+, it was less than 10%.

Recent studies have reported that overexpressing Her2 samples in gastric and gastroesophageal junction carcinomas is ranging between 10% and 20% [7]. But Saito et al. [10], in a series of 224 patients, they found 21% of tissues were HER2-positive. Furthermore, Huang et al. determined only a proportion of 7.8% of HER2-positive in fourteen biopsy and surgical specimens [8]. However, in the study of Rajagopal et al., in 60 cases, positive HER2 expression was observed only in

26.7% of tumors, predominantly in males and intestinal type [3]. Besides, in the study of Koopman et al. [11], HER2 positivity was found in 50 samples out of 323 (15.5%). Whereas, Van et al. determined the status of HER2 in 3803 tumor samples, they found 22.1% of positivity rate. Rates were similar between European and Asian patients (23.6% vs. 23.9%), but higher in intestinal- vs. diffuse-type (31.8% vs. 6.1%), and gastroesophageal junction cancer versus gastric tumors (32.2% vs. 21.4%) [12,13]. Moreover, Yoshida et al. evaluated the expression of Her2 Neu gene by fluorescence in situ hybridization and HER2 overexpression was observed in 17% of both surgically resected tumors and biopsy specimens [14].



**Figure 1:** Different scores of marking Immunohistochemistry of the Her2-Neu. Photo a: score 0, absence of membrane marking, Photo b: score 1, low and incomplete membrane marking in less than 10% of the cells, Photo c and d: score 2, medium and full membrane marking in more of 10% of the cells, Photo e and f: score 3, intense and complete membrane marking in more than 10% of the cells.



**Figure 2:** Search for amplification of Her2-Neu by CISH gene. a-lack of overexpression of the gene Her2-Neu (amplification ++), b-amplification of the gene on one cup witness.

Rakhshani et al. examined the overexpression of HER2/neu in 101 of gastric tissue samples by IHC. In their study, they demonstrated a significant difference in the overexpression of HER2/neu in gastric tumors. They found that the overexpression of HER2/neu was significantly higher in intestinal type, poorly differentiated grade, large size ( $\leq 5$  cm) and positive nodal involvement tumors [15]. In our study, we found an increase Intestinal type expression of Her2-Neu

comparing with diffuse type. Additionally, we found that Her2 was overexpressed in medium differentiated type.

Rabahoo et al. used several antibodies to study the expression of Her2. HER2-positive expression (3+) in the whole-tissue sections was observed in 23 cases (11.6%) using the 4B5 antibody, in 18 cases (9.1%) using the SP3 antibody and in 10 cases (5.1%) using the HercepTest antibody [16].

In a different study, HER2/neu overexpression (IHC 3+) was detected in 19% (10 of 54) of gastric tumors. HER2-positive expression (3+) in the whole-tissue sections was observed in 23 cases (11.6%) using the 4B5 antibody, in 18 cases (9.1%) using the SP3 antibody and in 10 cases (5.1%) using the Hercep Test antibody [17].

HER2 overexpression (2+ or 3+) was observed in 40 (13.8%) cases by HercepTest, 46 (15.9%) by A0485, 40 (13.8%) by 4B5 and 27 (9.3%) by CB11 [18].

In our study, we used HercepTest antibody and we found Her2 expressed (3+) in 8 cases (10.5%).

In another study Hunag et al. reported that HER2-positive tumors were identified in 12.0% (88/734) of the GC and GJC cases. No significant difference in HER2 positivity was identified between resection and biopsy samples, or between early and advanced disease stages [19].

In the study of Gasljevic et al. HER2 over-expression was found in 25.2%; HER2 3+6.6%, HER2 2+ 18.7% of tumors [20].

In the study of Shan et al., HER2 overexpression (3+) was detected in 9.8% of carcinomas and more frequently observed in GEJ cancer cases, in the intestinal type, and in the well or moderately differentiated type [21].

In a series of the 775 gastric cancer samples examined by IHC, a total of 88 (11%) cases were positive for HER-2/neu overexpression at a score of 3+; 44 (6%) cases were equivocal with a score of 2+; and the rest 643 (83%) cases were negative scored as 0/1+. Intestinal-type and early-stage cancers exhibited higher rates of HER-2/neu overexpression than those of diffuse/mixed-type and advanced cancers [22]. Similarly, Sekaran et al. confirmed the overexpression of HER2 in 23 of 52 (44.2%) patients. Two patients had equivocal result by IHC (2+), one of whom was positive on analysis by FISH [23]. Conversely, in another study, HER2 amplification was found prevailed in intestinal-type and low grade tumors, showing no correlation with patients' age/sex, tumor location, stage, and Ming histotype.

The expression of the Her2-Neu gene increases with age (16.2% for patients older than 60 years compared to 26.8% for very elderly patients (over 60 years)), but there was no significant correlation between the overexpression of Her2-Neu and age ( $P=0, 23$ ). Similarly, very elderly patients overexpress more this bothers Her2-Neu (3+) (14.6% vs. 4.6%).

Yan et al. have reported that HER2-neu over-expression significantly predicts poor outcome in pN0 EGC, and exact HER2-neu testing would be done before endoscopic therapy. For HER2/neu-positive patients, radical surgery should be performed [24].

In the study of Madani SH et al., patients with advanced cancer of GC and GEJ, HER2-neu overexpression was more associated with the intestinal cancer subtype. This could be a guide to new complementary therapy for affected patients [25].

In his report, Liu X argued that the value of HER2/neu for a potential role as a negative prognostic factor in the equivocal gastric cancer cases is limited. Indeed, FISH (Fluorescent In Situ Hybridization) is necessary for further classification when IHC (Immunohistochemistry) gives a score of 2+ [9].

Beside FISH assessment, HER2 DISH (Dual In Situ Hybridization) assay, utilizing 10% buffered formalin-fixed CB, would be a reliable and the ideal method to assess the HER2 gene status of breast cancer cytological specimens [26].

More new techniques as next generation sequencing enable Reliable Detection of HER2 (ERBB2) Status in Breast Cancer and Provides Ancillary Information of Clinical Relevance [27].

With the help of NGS, we have now been able to identify actionable mutations such as in the isocitrate dehydrogenase 1 (IDH1), FGFR2, BRAF and HER2/neu genes for targeted therapeutics and correlate the genetic variations with distinct clinical prognoses. This recent genetic information has the potential to make precision medicine a part of routine clinical practice for the management of BTC patients [28].

In our study the expression of Her2 (2+, 3+) was 14.1%. Three cases (2+) were negative on CISH.

There was no difference in HER2 overexpression (positivity) or negativity in relation to age, gender, tumor site, histological subtype, tumor differentiation, serosal involvement or lymph nodal status. HER2 overexpression rates were similar to intestinal type as compared to diffuse histologically [23].

In the study of Sin et al., HER2 gene amplification was detected in 10/85 (11.8%) cases of gastric carcinoma. In the 10 cases with HER2 amplification, HER2 immunoreaction scorings of 3+, 2+ and 0/1+ were present in 7, 2 and 1 cases, respectively [29].

An accurate assessment of HER2 expression in gastric cancer patients is of importance and utility in the optimal selection of patients for Trastuzumab (Herceptin) therapy. Our study found an HER2 overexpression of 10.5% in gastric cancers similar to many studies in the world. Additional studies are needed to explore the role of HER2 as an independent prognostic factor. Though Herceptin is approved for advanced gastric and GEJ cancers, role of herceptin in adjuvant/neo-adjuvant setting in early stages needs to be evaluated with newer agents like Pertuzumab, Bevacizumab, especially in young patients.

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