

# Human epidermal growth factor receptor 2/neu overexpression in urothelial carcinoma of the bladder and its prognostic significance: Is it worth hype?

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## Abstract

**Aims:** In urothelial tumors of the urinary bladder, human epidermal growth factor receptor 2 (HER-2)/neu expression has been reported over 10 years, but there is no clear correlation between prognosis and recurrence rate. The present study evaluates prognostic implication of HER-2/neu expression. **Subjects and Methods:** In this study, 100 formalin-fixed paraffin-embedded specimens of primary transitional cell carcinoma of the bladder were processed. HER-2/neu monoclonal antibody immunohistochemistry staining procedure used for the study. **Results:** A total of 70 (70%) patients were positive for overexpression of HER-2/neu. HER-2/neu was positive in patients with 42 (70%) superficial tumor, 28 (70%) muscle invasive tumor, 41 (75.9%) high-grade tumor, 29 (63%) low grade tumor, 31 (68.9%) recurrent tumor, and 6 (66.6%) had positive lymph nodes. **Conclusions:** Human epidermal growth factor receptor 2/neu over expression was not correlated with the tumor stage, lymphnode metastasis or recurrence of the disease. HER-2/neu overexpression was statistically insignificantly correlated with the differentiation grade ( $P < 0.161$ ) as compared to previous studies. Future studies on HER-2 expression with chemo-sensitivity and efficacy of HER-2-targeted therapies in urothelial carcinomas is needed.

**Key words:** C-erbB2 gene, human epidermal growth factor receptor 2/neu, immunohistochemistry, urothelial cancer

## Introduction

The bladder is a common site for cancer development in the urinary tract. Urinary bladder cancer ranks ninth in worldwide cancer incidence. It is the seventh most common malignancy in men and 17<sup>th</sup> in women. In the United States and Western Europe, the lifetime risk is about 1 in 25 and 1 in 80 for white males and females, respectively.<sup>[1]</sup> The mortality from transitional cell carcinoma (TCC) of the urinary bladder increases significantly with the progression of superficial or locally invasive disease (pTa/pT1) to detrusor muscle-invasive disease (pT2+). The most common prognostic markers in clinical use are tumor stage and grade, which are subject to considerable intra and inter-observer variation. Polysomy 17 and HER-2/neu gene amplification and protein overexpression have been associated with the more advanced disease.<sup>[2]</sup> The c-erbB2 gene encodes the second variant of the epidermal growth factor receptor (EGFR), Her-2/neu. It is a defective transmembrane tyrosine kinase receptor which is involved in the control of epithelial cells growth and differentiation. The overexpression of this protein is considered as a bad prognostic factor in many carcinomas (breast, ovary), and amplification is more common in patients with lymph-node metastases.<sup>[3]</sup> In urothelial tumors of the urinary bladder, HER-2/neu expression has been reported over 10 years, but there is no clear correlation between prognosis and recurrence rate. The incidence of over expression of HER-2/neu in bladder cancer has been reported to have a wide variation ranging from 2% to 71% of cancers tested.<sup>[4]</sup> In TCC of the bladder it was found that HER-2/neu is overexpressed with a greater frequency in higher grades (40%) and stages (38%) than in lower grades (0%) and stages (8%).<sup>[5]</sup> HER-2/neu protein overexpression and gene amplification occur more commonly in pT2 tumors compared with pTa/Ti tumors and are associated with a poorer prognosis. There are some studies in the patients with invasive tumors which associate HER-2/neu overexpression with an unfavorable prognosis. However, correlation between HER-2/neu, tumor stage and

lymphnode status was not established.<sup>[6]</sup> Based on the limited effectiveness of systemic chemotherapy there is significant interest in alternative therapies for metastatic urinary bladder carcinoma. Development of targeted therapies and their effectiveness in other malignancies has resulted in an interest in applying these therapies in patients with urinary bladder carcinoma.<sup>[2]</sup>

The present study evaluates prognostic implication of HER-2/neu expression in primary urothelial carcinomas of the urinary bladder and its correlation with clinical parameters, tumor stage, grade, and recurrence.

## Subjects and Methods

Tumor specimens from 100 patients with new or recurrent TCC of the urinary bladder were obtained by transurethral resection and open surgery in case of radical cystectomy.

Tumor tissues were fixed in 4% buffered formalin embedded in paraffin and sectioned in 2–5  $\mu$ m thick slices. Hematoxylin–Eosin (H and E) staining was performed for 5  $\mu$ m thick slices for the morphological diagnosis and it was completed by immunohistochemistry (IHC) of 2  $\mu$ m thick paraffin sections on albumin coated slides by peroxidase and anti-peroxidase staining for cytokeratin 7 and cytokeratin 20 expressions in order to select strictly urothelial carcinomas.

HER-2/neu monoclonal antibody (Novocastra, UK, clone CB11) and detection system was used for the study. Following IHC staining procedure used for the study;

- 2  $\mu$ m thick paraffin sections were encircled on the glass slide with a diamond knife. Then de-paraffinization of section achieved by keeping at 60°C in over for 15 min and transfer to xylene: (Xylene I for 10 min followed by Xylene II for 5 min and in Xylene III for 5 min)
- Slides were rehydrated by putting into absolute alcohol for 5 min followed by 50% ethanol for 5 min and then into distilled water for another 5 min

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- To block the endogenous peroxidase, the section was treated with an endogenous block solution, that is, methanol +3% hydrogen peroxidase in 9:1 ratio for 20 min. Then slides were rinsed in distilled water and then washed in tris buffered solution (TBS) 5 min
- Target antigen retrieval was done by pressure cooker method by putting the slide into a coupling jar containing 0.01 mm/L citric acid at 6.0pH, to be operated under 15 psi on a domestic hot plate
- After antigen retrieval slides were allowed to cool for 10 min or placed in cool (TBS, 0.05 mol/L, Tris0.15 mol/L NaCl with pH of 7.2–7.4)
- The section was covered with the primary antibody by wiping the excess liquid around the tissue section (primary antibody diluted in 1% bovine serum albumin)
- Then slides were washed with TBS and triton solution in two changes of 5 min each. Then sections were covered with secondary antibody or detection system. Slides were stained with diaminobenzidine tetrahydrochloride 1–5 min and then washed with distilled water
- Counter staining with haematoxylin performed for 1–2 min. Then slides were washed with water and placed in 1% acid alcohol. After washing slide with water, they were placed in scosch's tap water for 2 min. Then slides were dried and cleaned and mount with DPX.

A negative control without the primary antibody and a positive control of known breast carcinoma antigen was put up side by side.

#### Interpretation of human epidermal growth factor receptor 2/neu protein immunostaining

It was done subjectively by counting 1000 cells selecting the maximum positive area with strongest positive intensity and expressing it in percentage.

#### Immunohistochemistry staining score

Representative areas were identified from the H and E sections and corresponding areas were scored using a conventional light microscope. Only membrane staining was scored, with cytoplasmic staining being ignored. A 4-point scale was used: '0' if there was no membrane staining, '1' if there was weak membrane staining in at least 10% of cells, '2' if there was moderate membrane staining in at least 10% of cells, '3' if there was strong membrane staining in at least 10% of cells. Sections with grade 2 and grade 3 staining were considered positive for Her-2/neu.

#### Results

The mean age of patients was 59.30 years (range of 28–94) and there were 84 male and 16 female patients. Five patients (all male patients) were age below 40 years. Our data reconfirming the fact, that cancer of the bladder is more common in male patients compared to female patients.

Forty-six male patients out of 84 male patients were smokers and none of the 16 female patients were smokers. Out of 100 patients, 98 patients had history of hematuria, one had pain in abdomen and one asymptomatic at the time of enrolment in the study. History of smoking is significantly associated with HER-2/neu expression (78.30%), but no statistical significance difference in smokers and nonsmokers ( $P = 0.096$ ). In present study 54 patients had

high grade TCC (44 male and 8 female), while 46 had low grade TCC (35 male and 8 female). In which 41 (75%) patients in high grade and 29 (63%) patients in the low grade TCC group expressed HER-2/neu. 31 (68.90%) patients with recurrent TCC were positive for HER-2/neu which was not statistically significant as compared to newly detected urothelial carcinomas. Only 9 patients with metastasis were there in the study group in which 6 patients were positive and in nonmetastatic group 64 (70.30%) were positive for HER-2/neu. Metastasis is also not correlated with HER-2/neu expression ( $P = 0.819$ ) [Table 1].

#### Discussion

The HER-2/neu oncogene is located on chromosome 17 q11–21 and encodes for a tyrosine kinase trans-membrane growth factor receptor. Activation of the HER-2/neu receptor following autophosphorylation of the tyrosine kinase residues results in the activation of a cascade of intracellular proteins. Ultimately the mitotic activity and metastatic potential of the cell increases. The assessment of HER-2/neu overexpression in urothelial carcinomas is studied by many authors, because it has been shown that this protein is involved in the pathogenesis of these tumors, to an extent nearly as important as in breast cancer.<sup>[7,8]</sup> In malignant tumors, the protein overexpression is the direct result of gene amplification. In breast, ovarian, prostate, pancreatic and liver malignant tumors, HER-2/neu overexpression is associated with bad prognosis. In the case of the urothelial carcinomas of the urinary bladder, these results have an important degree of certitude only in low differentiated carcinomas. HER-2/neu expression is variable, with an incidence between 2% and 74%, and still has a controversial prognostic significance. The differences between the results reported may be due to different techniques and methods of assessment. Some observations are worthy to be mentioned here. First of all, in large series studies, 28% of the T2–T4 urothelial tumors overexpress HER-2/neu, but the expression is not correlated with the tumor stage and survival.<sup>[9,10]</sup> These data are in concordance with our results, particularly in the correlation of HER-2/neu overexpression and tumor stage. In present study HER-2/neu expression was seen in 70%

**Table 1: HER 2/neu and urinary bladder tumor co relation**

Tumor characteristics	HER-2/neu negative (%)	HER-2/neu positive (%)	Total	P
Type of tumor				
Superficial	18 (30)	42 (70)	60	1.00
Muscle invasive	12 (30)	28 (70)	40	
Grade of tumor				
High grade TCC	13 (24.1)	41 (75.9)	54	0.161
Low grade TCC	17 (37.0)	29 (63.0)	46	
Diagnosed first time/recurrent				
Diagnosed first time TCC	16 (29.10)	39 (70.90)	55	0.826
Recurrent TCC	14 (31.10)	31 (68.90)	45	
Lymph node status				
Negative	09 (24.33)	28 (75.87)	37	0.723
Positive	03 (33.33)	06 (66.66)	09	
Metastases				
M0	27 (29.70)	64 (70.30)	91	0.819
M1	03 (33.30)	06 (66.70)	09	

of superficial and muscle invasive urothelial bladder tumor each. But no statistical significance difference of HER-2/neu expression in superficial or invasive bladder tumor ( $P = 1.00$ ) was noticed. HER-2/neu negativity was correlated with well-differentiated low grade papillary or rarely infiltrative tumors. HER-2/neu over-expression did not correlate with stage or lymph node status; however, high staining intensity was associated with high grade. Similar results are shown by other researchers.<sup>[11,12]</sup> HER-2/neu expression was noticed 63.00% in low grade and 75.90% in high grade urothelial tumor. We noticed a trend of increase HER-2/neu expression as grade increases. Chakravarti *et al.* in their study suggested that HER-2/neu could be more related with resistance to chemo radiation. Therefore may be more implied in local control of the disease as well as EGFR expression seems to be a prognostic factor for distant relapse but less related than HER-2/neu with response to chemoradiation.<sup>[13]</sup> In contrast Vollmer *et al.* showed that the role of an excess of HER-2/neu and EGFR was of minor importance particularly in high-grade tumor in these cases presence of HER-2/neu and EGFR was related with a lesser probability of tumor invasion into the stroma of the bladder.<sup>[10]</sup> Her-2/neu expression was not reported in any case of Ta, while there was a no statistical significant difference between T1 and T2/T3/T4 urothelial tumor ( $P = 0.985$ ). These findings are corresponding to Matsubara *et al.*<sup>[14]</sup>

In present study the HER-2/neu expression was reported in 68.90% of recurrent cancer and 70.90% of those presented for the 1<sup>st</sup> time. But there was no significant difference between HER-2/neu expression with the pattern of tumor ( $P = 0.826$ ). Similarly by Tetu *et al.* noted no association between HER-2/neu over-expression and early tumor recurrence.<sup>[9]</sup>

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#### Conflicts of interest

There are no conflicts of interest.

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