

ORIGINAL PAPER

Clinical significance of Her2/neu overexpression in urothelial carcinomas

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Abstract

HER2/neu is a defective transmembrane tyrosine kinase receptor, homologue to the epidermal growth factor receptor, showing overexpression in a large variety of tumor cells. There are no studies published so far regarding HER2/neu overexpression and sensitivity of the urothelial tumors of the urinary bladder to anti-HER2/neu therapy. There are a relatively high number of articles in the literature referring to HER2/neu expression in urothelial tumors of the urinary bladder, but only two of them had investigated HER2/neu expression in patients with urothelial tumors of the upper urinary tract. We have studied HER2/neu overexpression in 59 patients with urothelial carcinomas of the urinary tract by immunohistochemistry. Normal urothelium and the elements of the neighboring renal parenchyma were negative. Out of the 59 cases of urothelial carcinomas, 38 were negative (0 and +1) and 21 were positive: eight were moderately and 13 were intensely positive (+2 and +3). The percentage of positive cases was 35.59%. The negative cases were mostly well-differentiated, G1 tumors, no matter the T-tumor stage. Most of the cases were diagnosed as papillary or, rarely, infiltrative. There is no correlation between HER2/neu overexpression and the tumor stage. The same was true for the lymph node status. The expression intensity, however, was significantly correlated with the differentiation grade. Overexpression was most likely present in tumors with high differentiation grade ($p < 0.05$).

Keywords: urothelial carcinomas, upper urinary tract, HER2/neu, immunohistochemistry, trastuzumab.

Introduction

The c-erbB2 gene encodes the second variant of the epidermal growth factor receptor, Her2/neu. It is a defective transmembrane tyrosine kinase receptor, which forms heterodimers with EGFR. Thus, it is involved in the control of the cellular growth and differentiation of the epithelial cells. The overexpression of this protein has been long time considered as a bad prognostic factor in several carcinomas, particularly in breast and ovarian carcinomas, mostly because it characterizes poorly differentiated cells, with no expression of the estrogen and progesterone receptors. In the same time, Her2/neu overexpression is an indication for trastuzumab (Herceptin) therapy [1, 2]. This monoclonal humanized antibody for HER2/neu (p185) has shown its efficiency in these tumors therapy, both alone and in combination with Cisplatin [3].

In urothelial tumors of the urinary bladder, HER2/neu expression has been reported over 10 years ago, and although the staining pattern is well known, there is no clear correlation with prognosis and recurrence rate [4–7]. There are some studies, which associate HER2/neu overexpression with unfavorable prognosis, but only in the patients with invasive tumors [8, 9]. The correlation between HER2/neu, tumor stage and lymph node status was not established [10, 11].

Moreover, there are no studies published so far regarding HER-2/neu overexpression and sensitivity of the urothelial tumors of the urinary bladder to

trastuzumab therapy. It has been shown that BCG adjuvant therapy of the superficial urinary bladder tumors has reduced the incidence of HER2/neu expression [12]. We believe that the results obtained so far justify the study of HER2/neu expression in the urothelial tumors of the upper urinary tract as well.

The tumors of the upper urinary tract are relatively rare, representing less than 5% of the renal tumors. The areas affected by Balkan nephropathy have an exceptionally high incidence of such tumors, as 9% of the renal tumors are carcinomas of the upper urinary tract. HER2/neu expression in the urothelial tumors of the calyces and the basinet and of the ureters is mentioned in only two studies published so far, without a correlation with the prognosis. There are a relatively high number of articles in the literature referring to HER2/neu expression in urothelial tumors of the urinary bladder, but only two of them had investigated HER2/neu expression in patients with urothelial tumors of the upper urinary tract [13, 14].

Material and Methods

Tumor specimens from 59 patients with tumors of the upper urinary tract were resected by open surgery, fixed in 4% buffered formalin, embedded in paraffin and sectioned in 3–5 μ m thick slices for the morphological diagnosis and immunohistochemistry. Additional sections of normal renal parenchyma and ureters were cut for each patient. Hematoxylin–Eosin staining was

performed for the morphological diagnosis, and it was completed by immunohistochemistry for cytokeratin 7 and cytokeratin 20 expressions in order to select strictly urothelial carcinomas.

Antigen retrieval for HER2/neu immunohistochemistry was performed by heating the slides submerged in citrate buffer at pH 6 at 95–99°C for 40 minutes in the microwave oven. The retrieval buffer was part of the HercepTest kit supplied by DAKO, as well as the rest of the reagents.

Antigen retrieval was followed by incubation with the primary antibody for 30 minutes, and then by incubation with EnVision working system, followed by diaminobenzidine visualization of the final reaction product in brown color.

The interpretation of the staining was done according to the producer specification. Cell culture slides with

known expression of HER2/neu supplied by the producer were used as external control. Only membrane staining was assessed.

Results

HER2/neu expression was negative in the normal urothelium adjacent to the tumor (Figure 1), and so were all of the elements of the neighboring renal parenchyma. The epithelial cells of the urinary tubules, as well as those of the renal corpuscle and inflammatory elements were also negative, even in the cases with areas of necrosis.

In the interpretation of HER2/neu immunostaining, the cases with only cytoplasmic staining were considered negative, no matter the intensity of the staining (Figure 2).

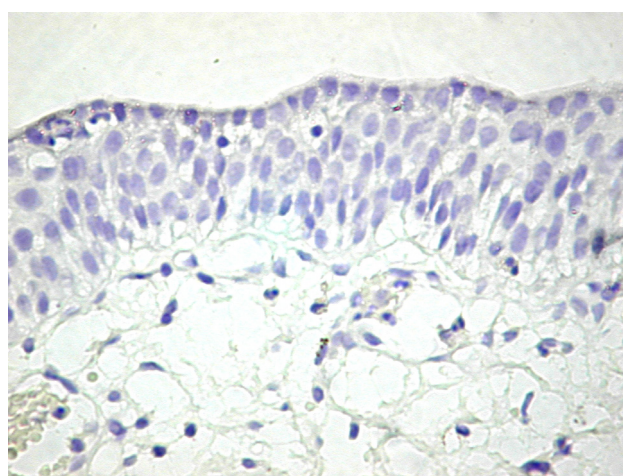


Figure 1 – Normal urothelium from the renal pelvis. Negative immunostaining for HER-2/neu ($\times 400$).

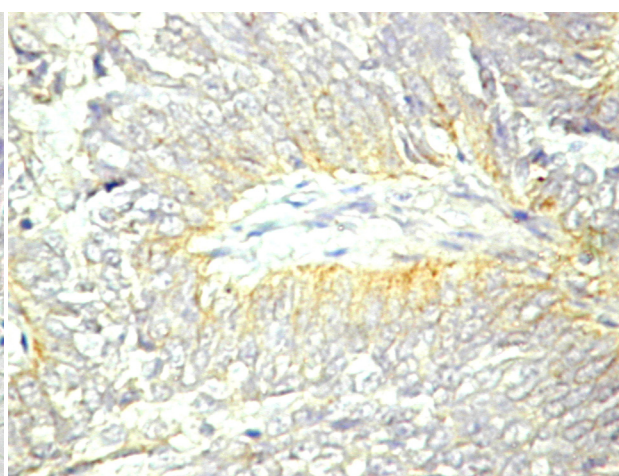


Figure 2 – Cytoplasmic immunostaining of HER-2/neu in the urothelial carcinoma cells, without membrane staining. The staining was considered negative ($\times 400$).

Out of the 59 cases of urothelial carcinomas, 38 were negative (0 and +1) and 21 were positive: eight were moderately and 13 were intensely positive (+2 and +3). The percentage of positive cases was 35.59%. The negative cases were mostly well differentiated, G1 tumors, no matter the T-tumor stage. Most of the cases were diagnosed as papillary or, rarely, infiltrative.

The final staining pattern was membranary, and the intensity of the staining depended upon the differentiation grade and on the tumor area selected. From this point of view, we have distinguished homogenous and heterogeneous reaction patterns. The tumors scored +1 (considered negative) were characterized by the staining of a small number of cells, with discontinuous membrane pattern, and with weak to moderate intensity (Figure 3). For the correct assessment of these cases, serial multiple sections were stained in order to certify the focal reaction pattern.

HER2/neu overexpression, characterized by an intensely positive staining in almost the entire tumor was noticed in the cases with +3 score. In the cases with +2 score, the final intensity of the reaction was weaker, but both the cases labeled +2 had over 30% positive cells (Figure 4, a and b).

The usual pattern of staining in the cases with

HER2/neu overexpression was heterogeneous. Thus, we have noticed areas with intensely stained alternating with areas with weak HER2/neu expression. Even if the intensity of the staining was weak, the cells were stained with continuous membrane pattern (Figure 5).

The heterogeneous immunostaining pattern was noticed also by the presence of weakly positive or negative areas with isolated intensely positive cells, usually in the close vicinity of intensely positive areas (Figure 6).

There is no correlation between HER2/neu overexpression and the tumor stage. The same was true for the lymph node status.

The expression intensity, however, was significantly correlated with the differentiation grade. Overexpression was most likely present in tumors with high differentiation grade ($p < 0.05$). Only two of the well-differentiated (G1) tumors were positive (Figure 7a); the other ones were negative. The intensity of the staining in those two cases was weak to moderate, but the cellular membrane was completely stained in more than 20% of the tumor cells. The majority of the moderate or low differentiated tumors were positive, with +2 and +3 scores, with high intensity and present in the majority of the tumor cells (Figure 7, b–d).

HER2/neu expression is not correlated with tumor stage, but is correlated with the differentiation grade. There is no linear correlation between HER2/neu

overexpression and the differentiation grade, but the existent one is statistically significant ($p=0.034$).

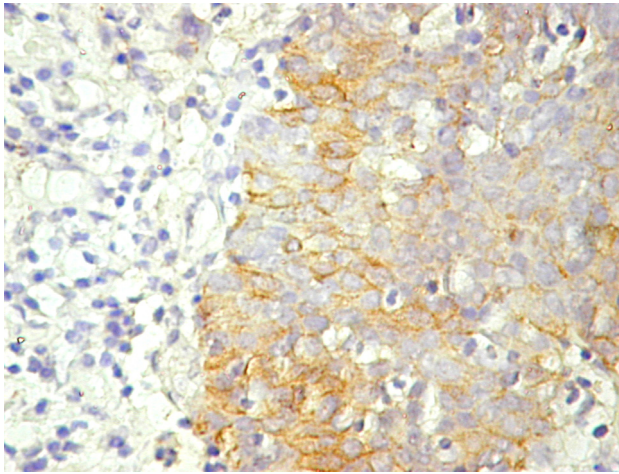


Figure 3 – Focally positive HER-2/neu immunostaining of the cells membranes, with discontinuous pattern, in a small number of tumor cells, +1 score ($\times 400$).

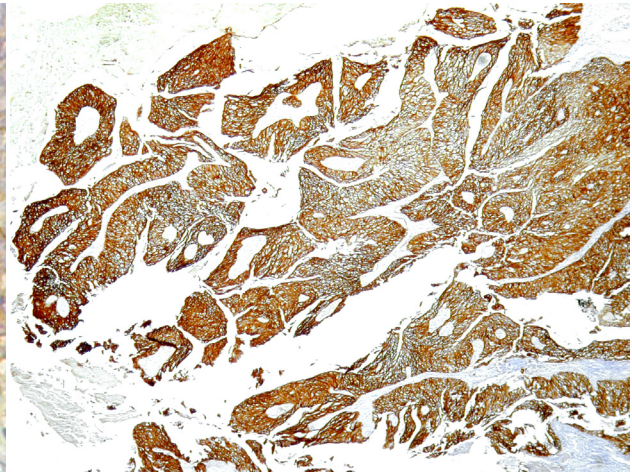


Figure 4a – HER-2/neu overexpression. Almost all of the tumor cells are stained (low magnification).

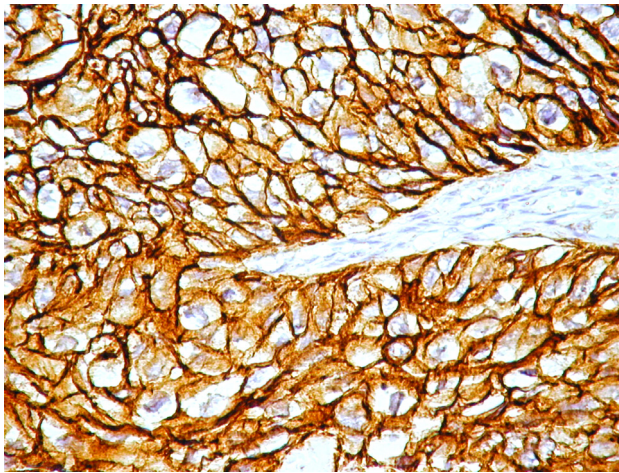


Figure 4b – HER-2/neu overexpression. Intensely positive membrane staining of all the tumor cells ($\times 400$).

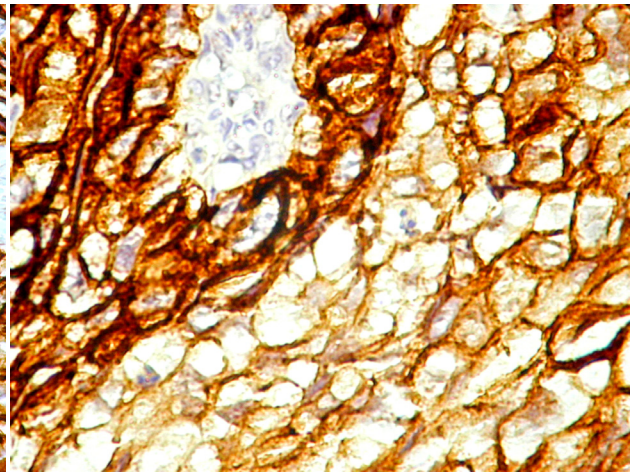


Figure 5 – Heterogeneous HER-2/neu overexpression, +3 score. Even if the intensity of the staining is not homogenous, continue membrane staining was noticed in all of the cells ($\times 400$).

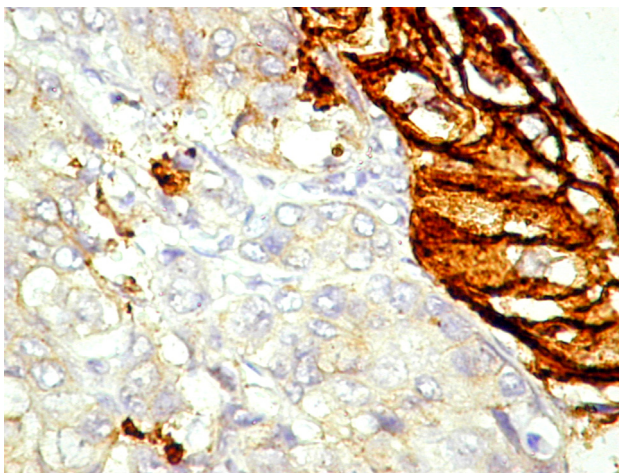


Figure 6 – Urothelial carcinoma with heterogeneous expression. Negative tumor area, with few isolated positive cells in the close vicinity of a tumor area with +3 score. HER-2/neu immunostaining ($\times 400$).

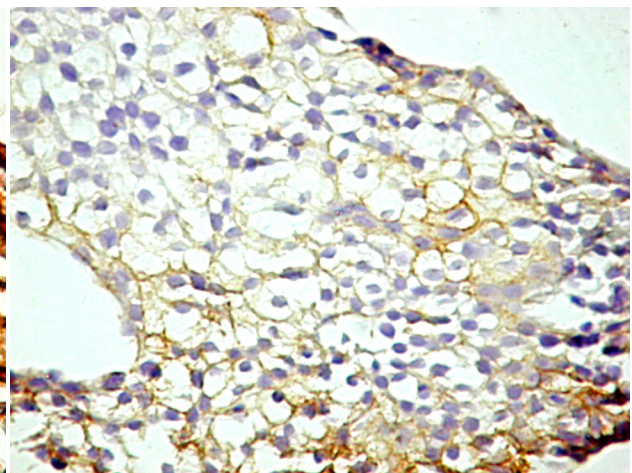


Figure 7a – Well-differentiated G1 urothelial carcinoma, with moderate staining, but present in the majority of the tumor cells ($\times 200$).

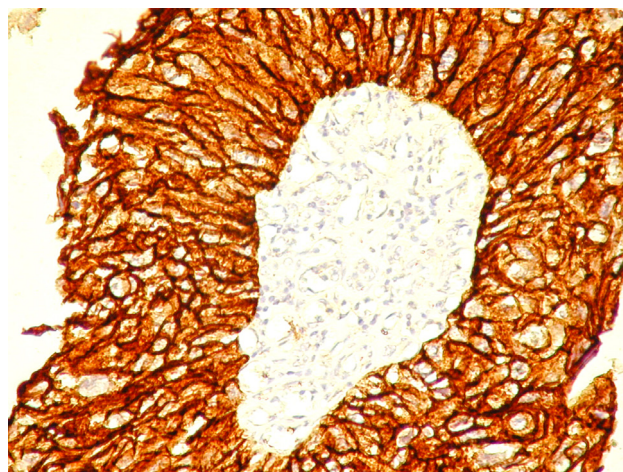


Figure 7b – G2 urothelial carcinoma, +3 score (×200).

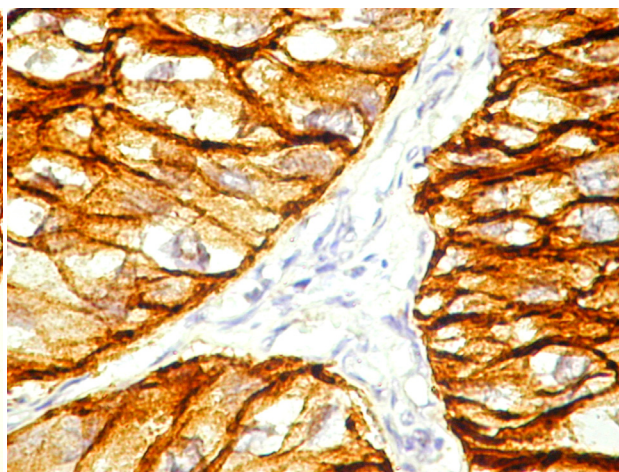


Figure 7c – G3 urothelial carcinoma, +3 score (×400).

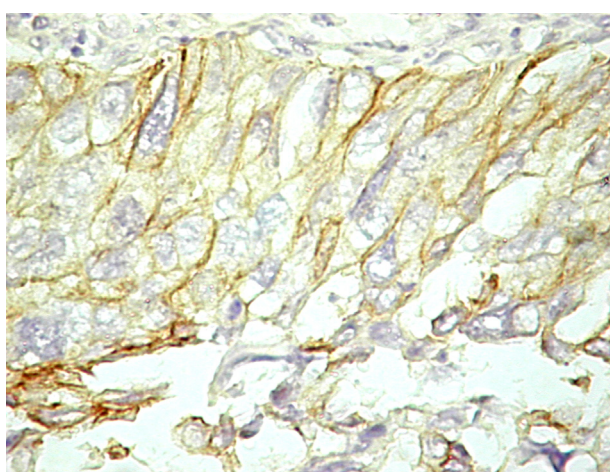


Figure 7d – Infiltrative T3G2 carcinoma, +2 score. HER-2/neu immunostaining (×400).

The overall expression rate was 35.59%. Overexpression was correlated with tumor differentiation grade, reflecting the aggressivity of the tumor cells. Taking into account these results, we consider that approximately one third of the cases with moderately and poorly differentiated invasive urothelial carcinomas could benefit from Herceptin therapy. The major therapeutic indication is for the cases labeled with +3 score. Particularly the cases with +2 score need further investigation of the c-erbB2 gene by *in situ* hybridization in order to assess the status of HER2 overexpression.

Discussion

The assessment of HER2/neu overexpression is not a new idea, the first observations have been published over 10 years ago [15–17].

The assessment of HER2/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 [3, 18].

The gene that encodes the HER2/neu protein, c-erbB2, is localized on chromosome 17, and encodes a defective tyrosine kinase receptor homologue to epidermal growth factor receptor [19]. In malignant

tumors, the protein overexpression is the direct result of gene amplification.

In breast, ovarian, prostate, pancreatic and liver malignant tumors, HER2/neu overexpression is associated with bad prognosis. Moreover, HER2/neu overexpression induced experimentally has provided the malignant cells with chemotherapy resistance. This phenomenon was inhibited by the use of Emodin, a tyrosine kinase inhibitor [20].

HER2/neu overexpression was investigated in a large number of malignant tumors: breast, prostate, urinary bladder carcinomas [2, 7, 8, 21–25]. In the case of the urothelial carcinomas of the urinary bladder, these results have an important degree of certitude only in low differentiated carcinomas. HER2/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 HER2/neu, but the expression is not correlated with the tumor stage and survival [6, 9, 26]. These data are in concordance with our results, particularly in the correlation of HER2/neu overexpression and tumor stage. On the other hand, 45% of the cases with negative primary tumors had lymph nodes metastases with positive HER2/neu expression.

A single recent study that assessed HER2/neu overexpression in metastatic urothelial tumors of the urinary bladder shows 81% of the cases with positive primary tumor and 67% with positive metastases [27]. These results are a good justification for the introduction of anti HER2 therapy in these lesions. As for the tumors of the upper urinary tract, the data from the literature are lacunar, because of the small number of articles published on small series of cases [13, 14]. In 2002, Fontana LO *et al.* [14] did not find any prognostic correlation for these tumors, in a study on 61 cases. There is no study published so far regarding the correlation between HER2/neu overexpression and survival. Our results notice HER2/neu overexpression in 35.59% of the cases, which is similar with most of the

studies regarding the same tumor type of the urinary bladder. We have preferred immunohistochemistry because it has similar sensitivity as *in situ* hybridization, at least for negative and for the cases labeled with +3 score. We have considered as positive only the tumors labeled with +2 and +3 scores, whereas tumors with +1 score were considered negative. Most of the cases that we have considered positive (n=21) were localized in the calyces or the basinet, and were locally advanced (T2 or T3) and poorly differentiated (G2 or G3). All of the tumors of ureters (n=8) were negative (0 and +1), but well differentiated (seven cases were G1 and one case was G2). In these conditions, we may assert that HER2/neu overexpression is correlated with the differentiation grade ($p<0.05$) and not with the localization of the tumor.

The 5-year survival of these patients is to be followed, since the number of the patients (n=59) is significant for this tumor type. Herceptin, either alone or in combination with Cisplatin and Paclitaxel (which are frequently used in the therapy of urothelial carcinomas) might be efficient for the therapy of the tumors with HER2/neu overexpression.

Conclusions

HER2/neu overexpression was present in 21 of the upper urinary tract tumors (35.59%). HER2/neu overexpression was not correlated with the tumor stage or lymph node status. HER2/neu overexpression was significantly correlated with the differentiation grade ($p<0.05$). There is enough evidence that supports the introduction of trastuzumab as an adjuvant therapeutic agent in the cases with HER2/neu overexpression.

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Received: November 11th, 2009

Accepted: March 15th, 2010