

# EGFR and Her2/neu immunoexpression in papillary urothelial bladder carcinomas

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## Abstract

The study analyzes the immunoexpression of EGFR and Her2/neu in 45 cases of papillary urothelial bladder carcinoma. The cases have been investigated through histopathological and immunohistochemistry techniques, the quantification of results considering the number of marked cells and intensity of the reactions. Immunoexpression of EGFR has been observed in 53.3% of cases, the reaction score having high values in moderate carcinomas and poorly differentiated carcinomas with invasion in the muscularis propria or in the entire wall. Immunostain of Her2/neu has been positive in 42.2% of cases, the poorly differentiated carcinomas presenting high scores regardless of tumor stage. The analyzed markers proved useful for identifying urothelial papillary carcinomas with a poorly differentiated grade, which are in an advanced stage.

**Keywords:** urothelial carcinoma, EGFR, Her2/neu, immunohistochemistry.

## Introduction

Papillary urothelial bladder carcinoma is a highly complex and heterogeneous disease with a large spectrum of histological aspects and deadly potential. Despite the progress of therapeutic means, up to 30% of patients who suffer from noninvasive tumors in the muscularis propria and 50% of those with invasive tumors in the detrusor muscle present recurrences or they die [1].

The last decade has known an exponential growth in studies and information regarding the molecular markers in the papillary urothelial bladder carcinoma. As a result, the molecular mechanisms and complex biological pathways, which lead to the installment of urothelial tumorigenesis, are better understood due to the discovery of new biological markers, which offer information, the improvement of clinical prediction and the personalization of therapeutic approaches on these patients with the purpose of reducing the risk of progression. Lately, many studies are concentrated on the evaluation of certain markers involved in the regulation of the cell cycle, among them being the human epidermal receptors (HER) [2–7], which can represent a promising therapeutical target.

Analysis of the over expression EGFR and Her2/neu has been the subject of numerous studies in an attempt to make correlations with other markers or with clinical-pathological parameters of prognosis, with discrepant results [3, 8–11].

We had as objective of assessing the expression of EGFR and Her2/neu for primary papillary urothelial

carcinomas of the bladder and identification of eventual correlations with clinical and statistic parameters.

## Materials and Methods

Our study included a number of 45 cases of primitive urothelial bladder carcinomas diagnosed in the Pathology Department of the Emergency County Hospital of Craiova. The biologic material was represented by bladder tissue obtained after cystectomies, fixed in 10% formalin and processed through histopathological technique of paraffin embedding and Hematoxylin–Eosin stain. For classification of the lesions, we used the WHO 2004 staging [12].

The working systems for immunohistochemical reactions have been represented by CSA II, Biotin-Free, Catalyzed Amplification System (code K197, Dako) for EGFR and LSAB+ System-HRP (DAKO) for Her2/neu, their visualization being obtained with DAB (3,3'-diaminobenzidine, Dako).

We used monoclonal mouse anti-human EGFR antibody (clone E30) diluted 1/1000, without antigen retrieval, and for external control was used oral mucosa and rabbit polyclonal anti-human HER2/neu antibody, 1:250 dilution, antigen retrieval with pH 6 citrate buffer, for external control using the breast carcinoma.

An adapted system of case sorting was used to quantify the immunostain results, following the pattern of the labeling for EGFR (score 1) [13, 14] and for Her2/neu (score 2) [15] (Table 1).

Statistical analysis was performed using the non-parametric *chi-square* test, to test the dependence of two factors of classification, of the software SPSS 10.

**Table 1 – Quantification of EGFR and Her2/neu immunoreactions**

EGFR	Positive cells	0	<10% (weak stain)	10–50% (moderate stain)	>50% (intense stain)
	Score 1	0	1+	2+	3+
Her2/neu	Absence of the reaction or <10% membrane stain of the cells.	Weak membrane stain / incomplete for >10% of the cells.	Membrane stain completed, weak / moderate for >10% of the cells.	Intense membrane stain completed for >30% of the cells.	Absence of the reaction or <10% membrane stain of the cells.
	0	1+	2+	3+	0

## Results

The study was performed on a group of 45 cases of urothelial carcinomas with primitive location in the bladder. Tumors were diagnosed in patients aged in the VI and VII decade, predominantly males (males/females 6.5/1).

Histopathological analysis of the tumors has shown a papillary growth pattern and corresponded in 21 cases to well differentiated forms, in 19 cases to moderately differentiated forms and in five cases to poorly differentiated forms. Classification of tumors according to the clinico-morphological stage indicated the fact that most cases were diagnosed in I stage of disease, that was 48.8% of the cases, followed by the II stage with 33.3% and III stage with 17.9% of cases.

The immunohistochemical study aimed the EGFR and Her2/neu immunoexpression according to the stage and the tumoral degree (Table 2). The stain was present in the tumor cells membrane.

**Table 2 – Immunoexpression of EGFR and Her2/neu depending on tumoral stage and grade**

No. of cases / score	EGFR immunostain 24 (53.3%)			Her2/neu immunostain 18 (40%)		
	WD*	MD*	PD*	WD	MD	PD
Stage I	0	5 (1+, 2+)	1 (3+)	2 (1+)	2 (1+)	1 (2+)
Stage II	2 (1+)	7 (2+, 3+)	2 (3+)	3 (1+)	4 (1+)	2 (2+, 3+)
Stage III	1 (2+)	4 (2+, 3+)	2 (3+)	1 (2+)	2 (2+)	2 (3+)

\*WD – Well-differentiated; MD – Moderately differentiated; PD – Poorly differentiated.

Analysis of EGFR expression indicated a positive reaction in 24 cases (53.3%). Negative cases corresponded to well and moderately differentiated tumors, most of them in stage I. We observed positive reaction for every poorly differentiated tumors regardless of tumor stage (score 3+), for 16 of 19 moderately differentiated tumors (score 1+, 2+, 3+) and only three of well-differentiated tumors (score 1+, 2+) (Figure 1, a–c). Expression analysis by tumoral stage indicated positivity for almost all stage III tumors (score 2+, 3+), for 11 of stage II tumors (score 1+, 2+, 3+) and six of stage I tumors (1+, 2+, 3+). All stage I well-differentiated tumors were negative for this marker.

The study of Her2/neu immunoexpression indicated positivity for 19 cases, representing 42.2% of analyzed cases. Expression Her2/neu analysis indicated positivity for six cases of well differentiated tumors, which five cases 1+ corresponding to stage I and II disease and one case 2+ classified as stage III. Concerning moderately differentiated carcinomas, number of positive cases was eight, which corresponded to score 1+ for six cases classified as stage I and II, and two cases score 2+,

which were classified as stage III of disease. Poorly differentiated tumors were positive for all five cases, of which three cases maxim score 3+ corresponding stage II and III disease and two cases 2+ score found in stage I and II disease (Figure 1, d–f).

Statistical analysis indicated significant differences of EGFR score by tumoral grade [ $\chi^2(6, N=45)=43.6, p=0.000$ ] and stage [ $\chi^2(6, N=45)=17.5, p=0.008$ ]. Also, there were significant differences between Her2/neu score by tumoral grade [ $\chi^2(6, N=45)=33.7, p=0.000$ ] and tumoral stage [ $\chi^2(6, N=45)=19.8, p=0.003$ ]. We have not identified statistical associations between analyzed markers scores ( $p=0.116$ ).

## Discussion

Lately immunohistochemical studies on urothelial carcinomas of the bladder are focused on the evaluation of several markers involved in the cell cycle control, with the main purpose of developing nomograms that allow classification of patients with the aim of assessing the predictive factors of the disease.

The cell cycle is regulated by a number of proteins that act in various biomolecular ways. Human epidermal receptors (Her) are a family of four tyrosine kinase receptors: Her1 (EGFR, Erb-B1), Her2 (Neu, Erb-B2), Her3 (erb-B3), Her4 (erb-B4), involved in several cellular processes such as proliferation, growth and survival. They seem to be involved in cell transformation and may be overexpressed in a variety of solid tumors.

EGFR mutations could lead to its constant activation, which causes uncontrolled division of cells and predispose to cancer development [17]. They have been identified in several cancers, being the target of anti-cancer therapies [18].

Her2/neu apart from its intervention in regulating normal cell proliferation also plays a role in cancer cell growth [19]. Her2 alteration by gene amplification or protein overexpression, have been characterized in several types of tumors associated with HER2 gene amplification, including urothelial carcinomas with poor outcome [20, 21].

In the study, EGFR expression was present in 53.3% of papillary urothelial bladder analyzed carcinomas, regardless of stage or tumor grade. Literature indicates various percentages for bladder urothelial carcinoma, with values between 23–75% [9, 22–24].

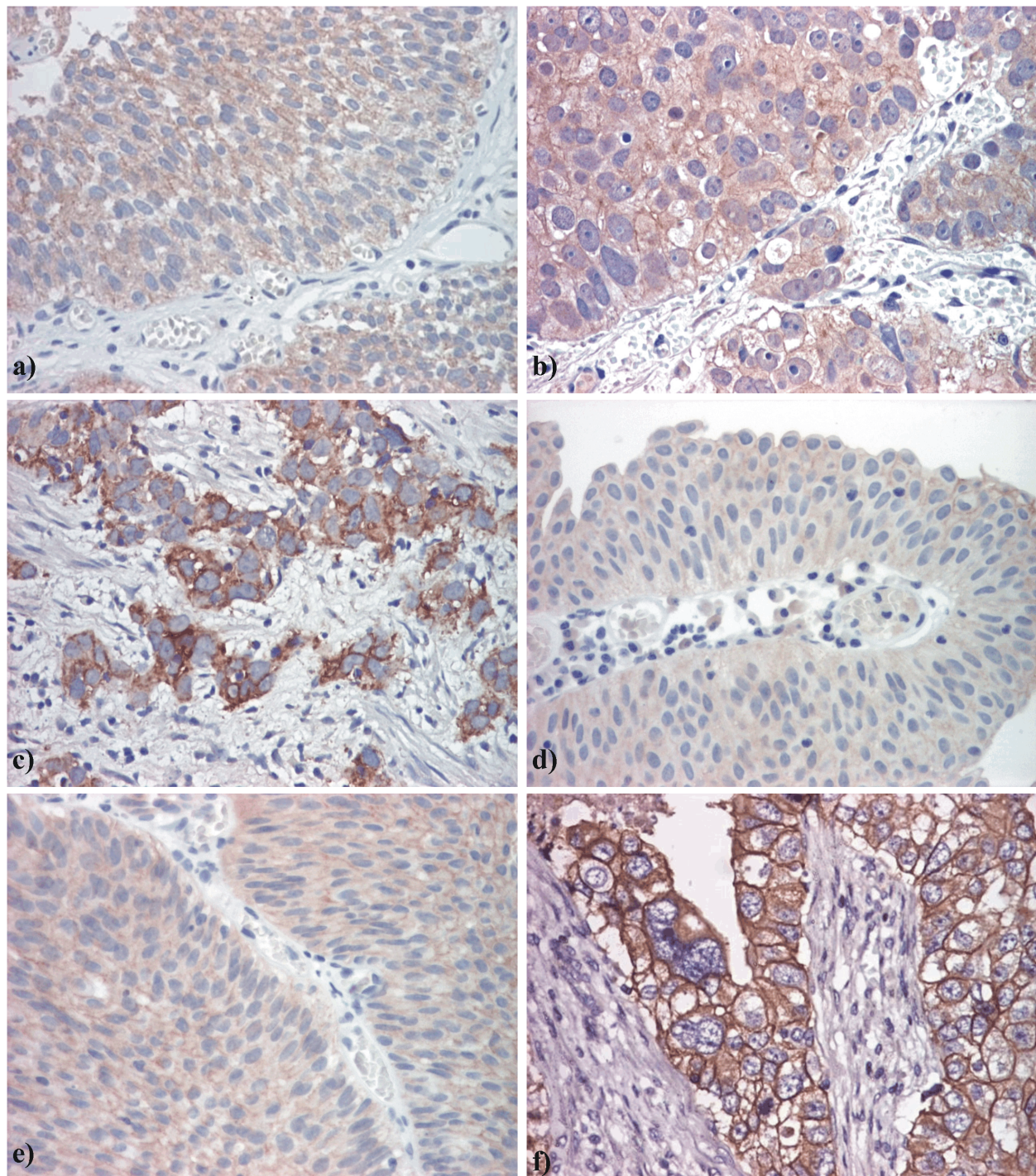
## EGFR expression

EGFR expression analysis indicated positivity for all poorly differentiated tumors, regardless of tumor stage, for 16 of the moderately differentiated tumors (84.2%) and only three of well-differentiated tumors (14.2%). Analysis of expression according to tumor stage



revealed positivity for almost all tumors of stage III (15.5%), for 11 of the tumors in stage II (24.4%) and six

of stage I tumors (13.3%). All well-differentiated stage I tumors were negative for this marker.



**Figure 1 – EGFR stain,  $\times 200$ : (a) Moderate differentiated carcinoma, 2+ score; (b) Moderate differentiated carcinoma, 3+ score; (c) Poorly differentiated carcinoma, 3+ score. Her2/neu stain,  $\times 200$ : (d) Well-differentiated carcinoma, 1+ score; (e) Moderate differentiated carcinoma, 2+ score; (f) Poorly differentiated carcinoma, score 3+.**

Similar studies in the literature indicate the EGFR immunostain with 1+ intensity in 49% of cases, with 2+ in 31% of cases and with 3+ in 20% of cases, 3+ score in univariate analysis being associated with development of metastatic disease ( $p=0.016$ ) [3]. Also, EGFR immunoreactivity was associated with advanced tumor stage (47% vs. 66% in tumors pTa/pT1 vs. pT2–pT4,  $p=0.003$ ) and high tumor grade (45% low-grade carcinomas compared with 67% of high-grade carcinomas,  $p<0.001$ ) [3].

Other recent studies reported EGFR expression in over 70% of invasive bladder tumors in the muscularis propria and it is associated with reduced overall survival [25]. In contrast with these studies, other authors have communicated the lack of any correlation with the clinico-pathological examined parameters [9]. On the other hand, there are also studies indicating positivity for EGFR in univariate analysis significantly associated with improved overall survival, with no disease specific survival (DSS) and intact bladder [21]. Estimation of

specific survival at five years for 121 patients who underwent radical cystectomy with curative intent was 60% in 47 patients with low or moderate EGFR expression, compared with 41% for 45 patients with strong EGFR expression [4].

### Her2/neu immunoexpression

Immunoexpression of Her2/neu was present in 42.2% of analyzed cases, the highest scores being recorded in poorly differentiated and advanced forms of disease. Thus, well and moderately differentiated carcinomas were Her2/neu positive in 13.3% and 17.7%, the maximum score (2+) being observed in lesions with deep invasion, in the entire bladder wall. Poorly differentiated carcinomas were positive in 11.1% of cases, with high scores (2+, 3+), regardless of tumor stage.

Literature data suggests that the incidence of the Her2/neu overexpression in bladder cancer is one of the highest in all human cancers ranging from 9% to 34% [26, 27].

The real incidence rate of the Her2/neu overexpression and/or of the amplification remains uncertain varying between 23–80% for overexpression [7] and between 0–32% for amplification [7, 8]. In a complex study on 1005 patients, the overexpression of the Her2/neu protein has been present in 9.2% of cases, while the amplification of the Her2 gene has been present in 5.1% of tumors [28]. Another recent study shows overexpression of Her2/neu in 59% of bladder carcinomas, more frequent in the transitional ones or in adenocarcinomas.

Recent similar studies indicate an elevated expression of Her2 protein (2+ or 3+) present in 36% of high-grade urothelial invasive carcinomas and 26% of these cases have presented a 3+ reaction. Correspondingly, ElMoneim HMA *et al.* (2011) reports Her2/neu overexpression statistically correlated with high-grade tumors but did not find any correlation with the tumor stage [29]. Wülfing C *et al.* (2005) communicated the Her2/neu expression in 74.8% of cases and in 32.2% of cases the score has been 2+ and in 17.3% of cases the score has been 3+ [30].

The authors found correlation between the Her2/neu expression and lymph node metastasis ( $p=0.06$ ), the invasion of lymph vessels ( $p=0.07$ ) and distant metastasis ( $p=0.002$ ) but not with global survival ( $p=0.73$ ) or with disease free survival ( $p=0.63$ ).

Another study reports the Her2/neu overexpression in 41% of T2 invasive bladder carcinoma cases, more frequent in the high-grade tumors than in the low-grade ones ( $p=0.036$ ) [11]. The authors show the lack of correlation between the overexpression of Her2/neu and the tumor stages or the lymph nodes status. In the multivariate analysis, the results suggest that the Her2 expression could offer supplementary information for prognosis in patients with muscularis propria invasive bladder carcinoma.

In a similar study concerning upper urinary tract tumors, HER2/neu overexpression was present in 21 of the studied 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 ( $p<0.05$ ) [31].

### Conclusions

We have noticed positivity of EGFR and Her2/neu in a considerable amount of cases, respectively in 53.3% and 42.2% of studied lesions. The two members of the growth factor receptors family have been mostly expressed in high-grade tumors and in advanced stages of disease.

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