ORIGINAL PAPER

HER-2 immunohistochemical expression as prognostic marker in high-grade T1 bladder cancer (T1G3)

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Summary

Objectives: *To evaluate if the Human epidermal growth factor receptor 2 (HER-2) expression levels may be used as potential prognostic marker in high grade T1 bladder cancer (T1G3)*

Methods: *Specimens from transurethral resection of bladder tumour (TURBT) of 103 patients with high-grade T1 bladder cancer were collected. This pathologic database was reviewed. Four-year follow-up data were matched with pathologic data. Eighty-three patients entered the study. HER-2 staining was performed. Patients were grouped for HER-2 status. Statistical analysis included Kaplan Meier survival analysis and Log-rank test.*

Results: *Pathological review of TURBT specimens confirmed high-grade T1 transitional cell bladder cancer in all patients. Median follow-up was 12 months (mean 23,5; range 3-48). Twenty-one patients (25.4%) present strong HER-2 expression (3+), 28 (33.7%) moderate expression (2+), 26 (33.7%) weak staining (1+) and 8 (9.6%) negative expression (0). Thirty-one patients of 83 (37.4%) had not evidence of disease, 41 (49.4%) recurred, 11 (13.2%) had a progression of disease. Forty-one patients had high grade T1 recurrence. Patients with HER-2 status 0 did not showed progression of disease. Patients with HER-2 status 3+, undergoing cystectomy because progression of disease, had a pathological stage > pT2 and a nodal involvement. Median Disease-Free Survival (DFS) for all patients was 12 months (DFS probability (pDFS) = 49.3%; 95% CI, -11.1/+10.1). Median DFS in HER-2 groups was 8 (pDFS 37.5%; 95% CI,-28.8/+29.9), 24 (pDFS 46.1%; 95% CI,-19.5/+17.5), 20 (pDFS 46.4%; 95% CI,-18.8/+16.9) and 10 months (pDFS 47.6%; 95% CI,-21.9/+19.1) respectively in HER-2 status 0,1+,2+,3+. Log-Rank test is not statistically significant (p = 0,39).*

Conclusions: *This study showed that HER-2 expression does not represent a prognostic marker of recurrence/progression of disease in high-grade T1 bladder cancer.*

KEY WORDS: *HER-2 expression; Prognostic marker; Bladder cancer; T1G3.*

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INTRODUCTION

Bladder cancer is the second most common tumour of the genito-urinary tract. In 2010, approximately 70,000 new cases of bladder cancer with almost 15,000 deaths were estimated in the USA alone (1). When the disease is first diagnosed it is non-muscle-invasive (NMIBC) in 75-80% of cases while the remaining cases are muscle-invasive (MIBC) (2). Over 50% of NMIBC recur, while 15-20% advance towards a muscle-invasive form. Early diagnosis of bladder tumour improves the patient's prognosis and reduces the number of cases where cystectomy is needed. High-grade T1 lesions of the bladder (T1G3) have a high propensity to recur and progress to muscle invasion and are associated with a significant risk of metastasis and death. Long-term progression and death rates as high as 53% and 34%, respectively, have been reported (3).

These bladder tumours are heterogeneous in nature and

thus difficult to treat. Nevertheless, many of these tumours can be treated successfully with bladder preservation approaches. The dilemma facing the urologist is how best to treat these tumours in a timely manner so that the chances of bladder preservation and cancer control are maximised, while the risks of overtreatment with radical therapy are minimised (4). Useful prognostic variables and various biological makers have been proposed to assess the prognosis of bladder cancer, but the efficacy of these variables is still inadequate to accurately predict its heterogeneous behaviour. New reliable molecular indicators are required yet.

Also, during the past few decades, numerous trials have been conducted to develop new treatment regimens for both NMIBC and MIBC, because there is an urgent need to identify new agents to prevent bladder cancer recurrence and progression.

Human epidermal growth factor receptor 2 (HER-2) is a transmembrane tyrosine kinase receptor in the *Epidermal Growth Factor Receptor* family and it plays a fundamental role in cell growth, survival and migration. Abnormal activation of HER-2 has been proposed to lead to oncogenic transformation (5, 6).

Human epidermal growth factors are involved in oncogenesis through its action on several pathways leading to proliferation, angiogenesis, cell survival and metastatic potential. The role of HER-2 has been most studied in breast cancer, in which constitutively active HER-2 is overexpressed in 18-22% of cases, correlating with poor prognosis (5, 6). But the prognostic significance of HER-2 expression status in transitional cell carcinoma (TCC) of the bladder remains uncertain. Numerous studies showed that higher HER-2 expression levels are associated with poor prognosis (7-10).

Recently HER-2 positivity was identified as an independent predictor of disease recurrence and disease specific survival in patients with TCC of the bladder after radical cystectomy (11). In contrast, other analysis showed only limited or no prognostic value of HER-2 expression (12-17).

Using Tissue MicroArray (TMA) data of 184 patients with primary TCC of the bladder, *Kassouf et al.* reported no significant correlation between HER-2 expression status and clinical outcomes (16). Similarly, another study reported no statistically significant difference in survival rates of 80 consecutive patients with MIBC between cases positive and normal HER-2 status (12). However, a positive HER-2 protein expression status could represents a potential prognostic factor in patients affected by TCC of the bladder, particularly in high-grade T1 lesions, and it could be used as a novel target for adjuvant therapy (18, 19).

Thus, the purpose of our work was to evaluate HER-2 immunohistochemical (IHC) expression as prognostic marker of disease recurrence and/or progression in high-grade T1 bladder tumour (T1G3).

Methods

Patients selection, inclusion and exclusion criteria.

From June 2005 to October 2006, specimens of high-grade T1 transitional cell bladder cancer were collected from 103 subjects (74 males and 29 females; average age 67,8 years, range 41-90) undergoing complete transurethral resection of the bladder tumour (TURBT) at *Department of Urology, Catholic University of Sacred Hearth, Rome-Italy*.

In January 2010 we have performed a review on this pathologic database. Our uropathologist (F.P.) reviewed bladder tumour resection specimens in order to confirm stage/grade of the bladder tumour.

The 2002 TNM classification (updated to 2009 TNM Classification) was used for pathological staging. The 2004 WHO/ISUP classification was used for pathological grading.

Then, 4-year follow-up data (clinic database) of all patients were matched with pathologic data. Inclusion criteria of the study encompassed, namely: presence of high-grade T1 transitional cell bladder cancer (established by pathological examination of complete TURBT specimens in whom muscularis propria was present and negative), all patients were first-diagnosed bladder cancer, all patients had complete 4-year follow-up clinical data.

Exclusion criteria encompassed Bacillus-Calmette-Guèrin(BCG)-treated patients following TURBT; incomplete follow-up data.

Ten patients were excluded because not first-diagnosed, 8 because lost at first follow-up, 2 because incomplete follow-up data. Eighty-three patients (67 males and 16 females, average age 69,2 years, range 45-88) of 103 entered the study.

Thus, HER-2 IHC analysis was performed. This study was carried out in accordance with the guidelines set out by the Ethics Committee and all subjects prior to participation were required to sign an informed consent form.

Follow-up data

The follow-up assessment adopted for these patients includes 3-months cystoscopy and urinary cytology for the first 2 years, then every 6 months for the following 2 years. No second TUR was done. Approximately after 2 to 4 weeks following TURBT, BCG intravesical therapy induction course was performed, followed by BCG maintenance therapy if there was not evidence of disease recurrence/progression.

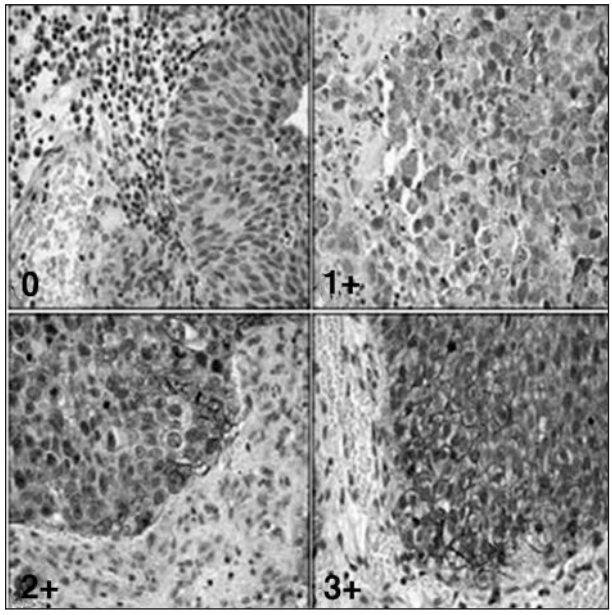
IHC analysis of HER-2

Four-micron-tissue sections, prepared from a formalin-fixed and paraffin-embedded representative of the tumor sample, were used (one to two conventional slides of tumor when available). After deparaffinization, rehydration and antigen retrieval in citrate buffer (10 mMol, pH 6,1), tissue sections were stained for HER2 (*A0485 policlonal antibody; 1/1500, Dako, Glostrup, Denmark*). HER2 positivity was assessed using the ASCO scoring system, evaluating only membranous staining (20).

Specimen of normal breast tissue were used for negative control and invasive ductal breast carcinoma served as positive controls. The level of HER2 protein expression was assessed semiquantitatively by the intensity and percentage of staining and score on a scale of 0 to 3+. Score of 0 and 1+ are categorized negative, 2+ as weakly positive, and 3+ as strongly positive. Score 0 was defined as negative membrane staining in all neoplastic cells or

Figure 1.

Membrane HER-2 stain intensity.



when membrane staining was observed in <10% the tumor cells. Score 1+ was defined as faint/ barely perceptible membrane staining in > 10% of the cells and the cells exhibit incomplete membrane staining. Score 2+ was defined weak-to-moderate complete membrane staining detected in > 10% of tumor cells. Score 3+ was defined a strong complete membrane staining in > 10% of tumor cells (Figure 1).
A cytoplasmic staining was considered non specific.

Outcome measures and statistical analysis

After HER-2 staining, patients were grouped for HER-2 status in 4 groups.
Kaplan-Meier survival analysis was performed to obtain survival values as *Disease-Free Survival* (DFS) for all patients and DFS between 4 patient groups of HER-2 status.
The difference in survival rates was determined by Log-rank test. Statistical significance (p) was set at 0.05.
Statistical tests were carried out using *MedCalc Statistical Software* (*MedCalc Software bvba, Mariakerke - Belgium*).

Results

Pathological review of bladder tumour specimens confirmed high-grade T1 transitional cell bladder cancer in all patients (average diameter of lesions 2 cm, range 1-3,5 cm). Median follow-up was 12 months (mean 23,5; range 3-48)
Regarding the expression of HER-2 protein, 21 patients (25.4%) present strong expression (HER-2 score 3+), 28 (33.7%) moderate expression (HER-2 score 2+), 26 (33.7%) weak staining (HER-2 score 1+) and 8 (9.6%) negative expression (HER-2 score 0).
Table 1 shows HER-2 status of patients (grouped in 4 groups) and its matching with follow-up data (no evidence of disease (NED), recurrence (REC) and progression (PROG) data).

Table 1.

HER-2 status of patients (grouped in 4 groups) and its matching with follow-up data (no evidence of disease (NED), recurrence (REC) and progression (PROG) data).

HER-2 status	N (%)	NED (%)	REC (%)	PROG (%)
(0)	8 (9.6)	1 (3.2)	7 (17.2)	0 (0)
(1+)	26 (31.3)	11 (35.5)	11 (26.8)	4 (36.4)
(2+)	28 (33.7)	12 (38.7)	13 (31.7)	3 (27.2)
(3+)	21 (25.4)	7 (22.6)	10 (24.3)	4 (36.4)
Total	**83 (100)**	**31 (100) (37.4)**	**41 (100) (49.4)**	**11 (100) (13.2)**

Table 2.

HER-2 status and its association with pathological examination of cystectomy specimens.

HER-2 status	PROG (%)	Pathological examination of cystectomy specimens
(0)	0 (0)	No cystectomy specimens
(1+)	4 (36.4)	3 (pT2a pN0 pMx G3) 1 (pT3a pN1 pMx G3)
(2+)	3 (27.2)	2 (pT2b pN0 pMx G3) 1 (pT3a pN1 pMx G3)
(3+)	4 (36.4)	2 (pT3a pN1 pMx G3) 1 (pT3a pN2 pMx G3) 1 (pT4a pN2 pMx G3)
Total	**11 (100)**	

Please note that 31 patients of 83 (37.4%) had not evidence of disease, 41 (49.4%) recurred, 11 (13.2%) had a progression of disease. Thus, 41 patients had high-grade T1 recurrence, 8 patients of whom with an association of carcinoma in situ of the bladder (CIS).
Eleven patients of 83 showed progression from NMIBC to MIBC, requiring cystectomy. HER-2 status and its relative association with pathological examination of cystectomy specimens is reported in Table 2.
High-grade T1 lesions with HER-2 status 0 did not showed progression of disease. Interestingly, all patients with HER-2 status 3+, undergoing cystectomy because progression of disease, had a pathological stage > pT2 and a nodal involvement.
Figure 2 and 3 shows the Kaplan Meier plots of DFS for all patients and DFS between the 4 patient groups of HER-2 status.
Median DFS for all patients was 12 months (DFS probability (pDFS) = 49.3%; 95% CI, -11.1/+10.1). Median DFS in HER-2 groups was respectively:

- HER-2 status 0 = 8 months (pDFS 37.5%; 95% CI, -28.8/+29.9);
- HER-2 status 1+ = 24 months (pDFS 46.1%; 95% CI, -19.5/+17.5);

- HER-2 status 2+ = 20 months (pFS 46.4%; 95% CI, -18.8/+16.9);
- HER-2 status 3+ = 10 months (pDFS 47.6%; 95% CI, -21.9/+19.1).

Log-Rank test was not statistically significant (p = 0,39).

Figure 2.

Kaplan Meier plot of disease-free survival (%).

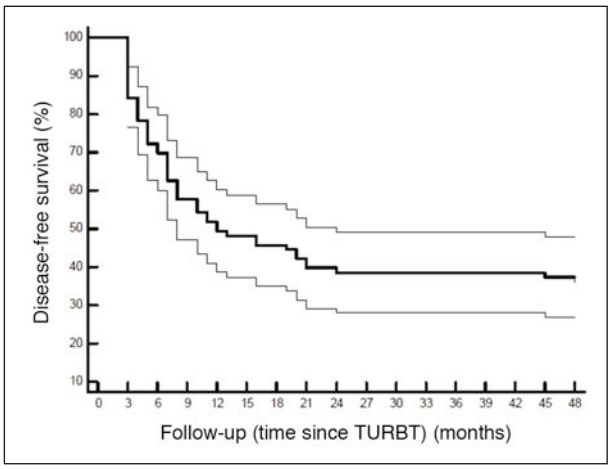
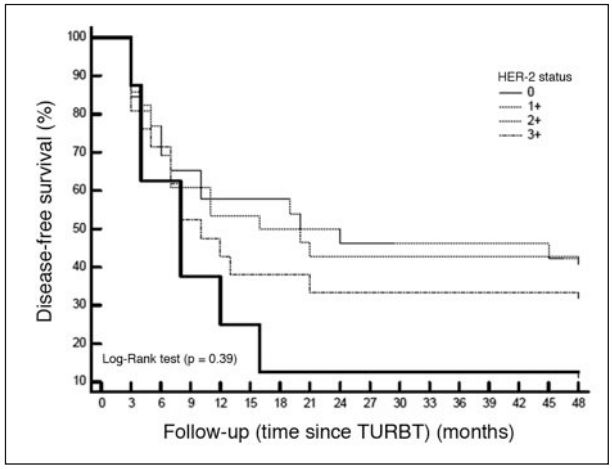


Figure 3.

Kaplan Meier plot of disease-free survival (%) for groups of HER-2 status.



Discussion

At each stage of bladder cancer, clinical management strategies are aimed at preventing disease recurrence/progression and the use of unnecessary and potentially life-altering procedures.

Once the disease becomes muscle-invasive, the main goal of treatment is threefold: to maximize long-term survival, to prevent pelvic recurrence or metastases, and to provide a good quality of life (21).

General guidelines exist for treatment of high-risk TCC of the bladder (22, 23).

However, to predict exactly which patients will progress, and who could, therefore, require more aggressive therapy, needs an individualized approach, although assessment remains more an art than science (24).

Zhau et al. reported HER2 amplification and overexpression in bladder cancer for the first time in 1990 (25).

In contrast with its known importance in breast cancer, the significance of HER2 expression and/or HER-2 gene amplification in bladder cancer is controversial. It was found that HER2 is overexpressed with a greater frequency in higher grades (40%) and stages (38%) than in lower grades (0%) and stages (8%)[8] and several studies confirmed that HER-2 could have a role as prognostic factor in bladder cancer, correlating its overexpression with poor prognosis for patients (shorter median survival time, reduced complete response to chemoradiation therapy) (8, 10, 13, 26-29).

Recently *Bolenz et al.* identify HER-2 positivity as an independent predictor of disease recurrence and specific survival in patients TCC of the bladder after radical cystectomy (11).

In literature other data seem to indicate limited or no prognostic value of HER-2 expression (12, 14-17).

In a large series of patients with primary TCC of the bladder, no significant correlation between HER-2 expression status and clinical outcome it has been reported (15).

Moreover, no statistically significant difference in the survival rates of 80 consecutive patients with MIBC has been observed (14).

A recent study showed that, in a large series of transurethral resection and cystectomy (1005 cases), 5,1% of MIBC had a HER-2 gene amplification with complete concordance (100%) between IHC and Fluorescence in situ Hybridization (FISH) analyses (30).

These variations in results are due to the heterogeneity of studies with respect to kits and type of antibodies used for IHC analysis, protocols, stage of the disease studied (non-muscle-invasive vs muscle-invasive), definition of HER-2 positivity and the material studied (fresh/formalin fixed).

Thus, discordant results reported in the literature highlight a need for standardized laboratory methods.

In our work we evaluate HER-2 IHC expression as prognostic marker of disease recurrence and/or progression in high-grade T1 bladder tumour (T1G3)

High grade T1 lesions with HER-2 status 0 did not showed progression of disease. Interestingly, all patients with HER-2 status 3+, undergoing cystectomy because progression of disease, had a pathological stage > pT2 and a nodal involvement.

No statistically significant association between HER-2 IHC expression and recurrence/progression of disease it has been found.

Conclusions

This study showed that HER-2 expression does not represent a prognostic marker of recurrence/progression of disease in high-grade T1 bladder cancer.

The numbers of this cohort are actually quite small and they could affect the significance of statistical analysis. Further studies analyzing a large group of disease progression are needed.

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