Original Article

Status of Her2 over expression in muscle invasive urothelial bladder carcinoma: Report of 21 cases

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Abstract

Background: Invasive urothelial bladder carcinomas have a poor prognosis even with cystectomy and chemotherapy. A high number of these patients have Her2 overexpression. The goal of this study is to assess the Her2 status in muscle invasive urothelial bladder carcinoma, to evaluation heterogeneity and discordance with metastases.

Patients and Methods: We retrospectively analyzed 21 specimens of transurethral resection or cystectomy in patients with invasive urothelial bladder carcinoma. We selected one representative section from primary tumors and metastases for immunohistochemistry analysis. Staining was evaluated according to the same criteria of breast cancer. A chromogenic *in situ* hybridization (CISH) was performed in case of 2+ score or in heterogeneous samples.

Results: Median age of our patients was 62 years. Intratumoral heterogeneity was observed in 2 cases (less than 1%). One case showed a Her2 3+ score (high grade, pT2 stage) and 3 cases showed a 2+ score (all low grades, stage T2, T4, M1, respectively). Two metastatic lymph nodes scored 1+ for the first (primary 1+) and 2+ for the second (primary 1+). Two cases showed CISH gene amplification. The first one scored 2+ and had area of 3+ score. The second one scored 1+ and had area with 2+ score.

Four patients died from disease, one of them had Her2 3+ score.

Conclusion: Her2 overexpression can be observed in muscle invasive urothelial bladder carcinoma in an important number of patients. Evaluation criteria must be standardized, especially with heterogeneous cases. Metastases tests can also readdress the expression of Her2, which gives the patient a supplementary therapeutic tool.

Key Words: Her2, immunohistochemistry, prognosis, targeted therapy, urothelial invasive bladder carcinoma

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INTRODUCTION

Urinary bladder cancer is the fourth most common malignancy in men and the ninth most common in women. Eighty percent of urothelial bladder carcinoma are superficial at the time of diagnosis and have a high survival rate.[1] The invasive form is however associated with a lower survival rate decreasing from 80% to 50%. Metastases occur most of the time in lymph nodes but also in lung, liver, and skeleton. In fact, 30% of patients are invasive or metastatic at the time of diagnosis.

Approximately 10 to 15% of superficial tumors progress to invasive stage. This risk is dependent on stage and grade, which are subjects of inter-and intra-observer variation.[1] More accurate prognostic factors have been developed such as polysomy 17 and Her2 overexpression.

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The human epidermal growth factor receptor (Her2) encodes for a tyrosine kinase receptor of the epidermal growth factor. Its activation increases the mitotic activity and metastatic potential of the cell leading to oncogenic transformation. Several studies on transitional bladder carcinoma examined the overexpression of Her2 protein, with a range reported between 17% and 76% of invasive carcinoma.[2] This overexpression seems to be correlated with earlier time recurrence, higher grade, and worse prognosis. Her2 can be assessed with IHC staining or with Fluorescent *in situ* hybridization (FISH). Variant results of correlation between those techniques have been reported. Her2 overexpression can be observed in the primary tumor and in the metastatic lesions but correlation is still a controversy.

A reliable evaluation is needed to introduce targeted therapy in the management of invasive urothelial bladder carcinoma.

The goal of this study is to evaluate the status and pathological heterogeneity of Her2 overexpression in urothelial muscle invasive bladder carcinoma. We also studied Her2 expression in primary and metastatic samples.

PATIENTS AND METHODS

Patients

We selected 31 patients with muscle invasive urothelial carcinoma (pT2 and more) from the department of pathology, Salah Azaiez institute in 18 years period from 1993 to 2011. Patients who did not have complete follow-up or representative sections were excluded. Data of 21 patients were collected from surgical records. A total of 21 specimens from primary tumors were included and two additional metastatic lymph nodes were added.

Tumors were staged and graded according to the World Health Organization (WHO) 2004.

All patients had clinical follow up (age, sex, stage, grade, treatment, survival). Samples for histological examination were obtained after endoscopic resection (10 cases) and/or cystectomy (18 cases).

All samples have been reviewed and pathologically staged by two pathologists with a double blind examination. One representative block was selected to immunohistochemistry (IHC) analysis. In cases with metastatic lymph nodes, one block was chosen to IHC analysis. Heterogeneity was defined by at least one Her2 negative field in a Her2-positive tumor.

Immunohistochemistry

In each case, sections cut containing representative area were stained immunohistochemically. We used a Her2 antibody type Leica clone NCL-N-CD11.

Only membrane staining was scored according to the same standard criteria used in breast cancer. Her2 positivity was assessed using the following scoring system:

0 : No membrane staining or less than 10% of cells.
1+: Partial membrane staining in more than 10% of cells.
2+: Weak, circumferential membrane staining in more than 10% of cells, or intense membrane staining in less than 30% of cases.
3+: Intense membrane staining in more than 30% of cells.

Protein overexpression was considered present if IHC score was 3+. Specimens with 2+ score were selected of chromogenic *in situ* hybridization (CISH) analysis.

Metastatic tumors and lymph nodes were stained and scored with the same criteria.

Chromogenic *in situ* hybridization

Only cases scored 2 + or/and had an intratumoral heterogeneity were analyzed by CISH to evaluate Her2 gene copy number. All samples were carried out on a single block according to the instruction from the test kits.

RESULTS

Patients

The average age of our patients was 62 years (range, 50-78 years). There were 19 males and 2 females with sex ratio M/F of 8.

There were 15 cases (75%) with stage T2, 3 cases (15%) with stage T3, and 3 cases with stage T4. Two cases of lymph node involvement and four cases of metastasis were observed (lung, liver).

Four deaths related directly to disease progression were observed; one case was associated with Her2 positive (3+) and had metastatic disease.

The time of follow up was of 3 months in Her3+ patient, 25 months in Her2+ patients, and 11 months in Her2-negative patients.

The relationship between the distribution of age, sex, and tumor stage and grade is summarized in Table 1.

Microscopic aspects

We observed two cases with glandular differentiation, two cases with micro papillary features, one case with sarcomatoide differentiation, and four cases with squamous features. Most of our patients were high grade (17 cases, 70%).

Immunohistochemistry

One sample scored 3+, three scored 2+, and 17 scored 0/1+ [Figure 1].

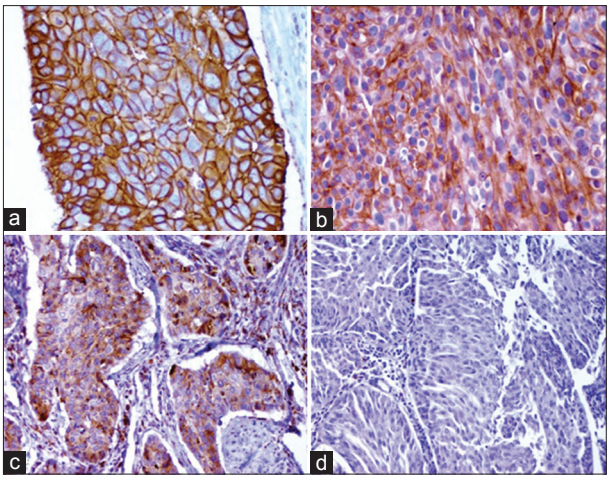

Figure 1: (a) Her2 (3+) score, (b) Her2 (2+) score, (c) Her2 (1+) score, (d) Her2 (0) score

Heterogeneous staining was observed in two cases; in one case, we found 10% of Her2 3+ area in a Her2+ tumor and in the other case more than 10% of Her2+ area in a Her1+ tumor.

Her2-positive staining was more frequent in higher stage (2/4 cases were stage pT4 or M+) or higher grade (2 cases with high grade 3). However, among the four cases of death, only one patient had Her2-positive staining.

In Her2-negative tumors, 63% (12/19) were high grade and 57% (11/16) were stage T2, two cases with lymph node involvement and one case with metastases (lung not histologically examined).

IHC analysis of two added metastatic lymph nodes showed Her2 1+ staining in 1+ primary tumor and 2+ staining in 1+ primary tumor.

The distribution of Her2-positive and negative cases among stages and grades is summarized in Table 2.

Chromogenic *in situ* hybridization

Four samples were analyzed. Only two cases showed gene amplification. The first one scored 2+ and had area of 3+ score [Figure 2a]. The second one scored 1+ and had area with 2+ score [Figure 2b]. The two other samples scored 2+.

DISCUSSION

Since the important prognostic and therapeutic impact that Her2 status had in breast carcinoma, more interest has been given to its expression in other cancers. In 1990, Zhau *et al.* reported first an increased amplification and an overexpression of the Her2 in bladder carcinoma.[1] Since then, several studies tried to evaluate its status and prognostic role.

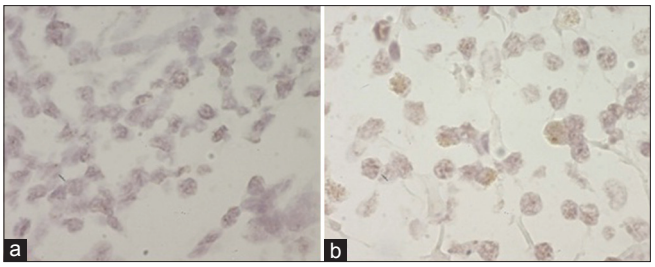

Figure 2: (a) CISH amplification in a sample scored 2+ with 3+ score area. (b) CISH amplification in a sample scored 1+ with 2+ area

Table 1: Relationship between the distribution of age, sex, and tumor stage and grade

Variable	No. of patients
Age	
Median	62
Range	50-78
Sex	
Male	19
Female	2
Grade	
Low grade	6
High grade	15
Stage	
T2	15
T3	3
T4	3
N0	19
N+	2
M0	17
M+	4
Follow up	
Median	15,37 months
Died of disease	4

Table 2: Distribution of HER2-positive and negative cases among stages and grades

	Her2+	Her2–
T2	3	11
T3	0	3
T4	1	2
M+	2	2
Low grade	2	4
High grade	2	13

Incidence of Her2 overexpression in muscle-invasive urothelial bladder carcinoma is variable in the literature. It occurs in a median of 45%, and ranges from 4 to 32% in invasive stages and from 2 to 50% in high-grade forms.[1,2]

The largest series (1005 cases) reported by Laé *et al.* suggested an incidence reaching 9.2%, which was lower than that reported in the literature (23-80%).[3,4]

Some authors consider both 2+ and 3+ score as a positive Her2 status and other consider only 3+ score. In our study, Her2 2+/3+ staining was observed in 19% (4 cases: Only one cases 3+ score) of tumor samples. This rate is similar to the rates described previously.

Heterogeneous IHC staining, found in only 5% of invasive breast carcinoma, was less frequent (1%) in our study. M Laé *et al.* found 35% of intratumoral heterogeneity, which made analysis more difficult.[3-5] However, the study of Edwards *et al.* found a uniform IHC staining of Her2 in all 39 cases tested.[4] These discordant results are explained by the diversity of kits, antibodies, and interpretations, and highlight the necessity to standardize kits and assessment criteria. That is why many authors recommend to carry out a FISH in these cases, in order to avoid ambiguity.

Gene amplification in invasive urothelial bladder carcinoma reported in the literature ranges from 0% to 30% in urothelial muscle-invasive bladder carcinoma.[6,7] Laé *et al.* reported a 5.1% of gene amplification in their series.

Several studies compared IHC to FISH results.[3-12] Sauter *et al.* reported in 141 cases an overexpression in 43%, but only 7% of gene amplification (all of these cases showed Her2 overexpression); similar results have been reported by Zhau *et al.* This discordance seems to be more frequent than in breast cancer where it has been reported in 36% of cases.[8] It can be explained by the several mechanisms leading to protein expression (translocation, mutation), the bias in sampling, cross-reactivity of antibodies, or by interobserver variation. Thus, they suggest that the decision algorithm used in breast cancer can be used in bladder carcinoma; selection of patients with invasive urothelial carcinoma might rely on strong IHC staining and when it is difficult to assess, a FISH or CISH analysis become necessary. In our series, we performed a CISH analysis, in Her2 2+ specimen and in heterogeneous cases, in order to confirm the diagnosis. Two cases had CISH amplification.

Her2 status in primary tumor and in distant metastases has been also compared in several studies.

100% concordance for Her2/neu amplification and 88% concordance in overexpression have been reported.[3] Differences between primary and metastatic tumor can be explained by the heterogeneity of overexpression as reported by Jimenez *et al.* Other theories have been advanced, such as growth-stimulating signals from Her2 are different in primary and in metastatic lesions.[9]

In our series, Her2 overexpression in metastatic lymph node lesions have been assessed in two cases and showed a concordant result in a case (1+ score) and discordant result in the other one; a 2+ score in metastases but 1+ score in primary tumor.

The prognostic impact of Her2 overexpression is still a controversy.

Several studies (Jamez, Undewood, and Kringer) found that Her2 overexpression is predictive of bladder cancer-related death in patient with invasive tumors.[10] B. Kolla *et al.* observed a significantly high disease-free survival in Her2-negative patients compared to Her2-positive patients; this difference was more profound in patients with locally advanced disease (T2b, T4, N+). But high controversial results have been published; Rafeael *et al.* reported no significant difference in survival between Her2-positive and negative patients.[9]

Recently, an anti-Her2 antibody (Trastuzumab) was proposed as therapeutic tool in invasive urothelial carcinoma of the bladder. Response rates range from 3% to 63%.[13]

All these results suggest that IHC assessment of Her2 overexpression might represent an additional tool to evaluate individual prognosis of bladder patients.

CONCLUSION

HER2 overexpression, expressed in a median of 45% of invasive urothelial bladder carcinoma, became an interesting tool to assess the patient prognosis and to select those who can be candidates for targeted therapy. Evaluation criteria must be standardized, especially with heterogeneous cases. Metastases tests can also readdress the expression of Her2, which gives the patient a supplementary therapeutic tool. Trastuzumab (targeted therapy for Her2) can improve the overcome of patients with Her2 overexpression. More investigation and clinical trials are needed in order to standardize diagnostic criteria and tools and the optimal use of Her2 expression in targeted therapy.

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