IMMUNOHISTOCHEMICAL EXPRESSION OF HER-2 IN BLADDER CANCER DOES NOT ALWAYS MATCH WITH HER2 GENE AMPLIFICATION BY IN SITU HYBRIDIZATION

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Background

Infiltrating bladder cancer (BC) is the most common malignant neoplasm of the urinary tract, characterized by the propensity of divergent differentiation. It can be distinguished in usual type, and special types. The two most common special types of differentiation are squamous and glandular. Other less common differentiation patterns include trophoblastic, nested, microcystic, micropapillary, lymphoepithelioma-like, plasmacytoid, sarcomatoid and clear cell, all bearing more aggressive behavior. The micropapillary type is very similar to the counterpart found in breast with nests of cells lacking true fibrovascular core and with lacunar spaces.

Her2 is a membrane receptor of EGFR family, with tyrosin kinase function that is important for transduction of signal and activate intracellular signaling pathway. Her2 overexpression is mainly relevant in breast cancer but it is described in BC, and in particular in the micropapillary type.

Overexpression, amplification and mutations of HER2 protein have been assessed so far by immunohistochemistry (IHC), chromogenic in situ hybridization (ISH) and sequencing but mainly in bladder cancer with micropapillary histology.

Here we evaluated the IHC overexpression as well as the amplification of HER2 in a cohort of BC patients covering the whole spectrum of histotypes, using a tissue microarray (TMA) strategy.

Methods

We selected 61 patients (50 males, 11 females) with a diagnosis of muscle-invasive BC with usual and special types who underwent radical cystectomy. A TMA was built containing 3 tumor cores for each case and serial sections were submitted to IHC and ISH to detect HER2 overexpression.

IHC was performed using the 4B5 anti-HER-2 antibody while ISH was performed using the INFORM HER2 Dual ISH DNA Probe Cocktail Assay both on a Ventana Benchmark Ultra automatic instrument.

HER2 scoring was performed according to the following revised ASCO/CAP guidelines for HER2 amplification in breast cancer:

- **IHC: HER2 3+: complete and intense circumferential membrane staining. HER2 2+: complete, weak to moderate, circumferential membrane staining in >10% of tumor cells. HER2 1+: incomplete membrane staining >10% of tumor cell. HER2 0: no staining observed or incomplete membrane straining <10% of tumor cell.**
- **ISH: AMPLIFIED with HER2/CER17 ratio ≥ 2 or HER2 copy number ≥6; NOT AMPLIFIED with HER2/CEP17 ratio <2.**

Results

The series incorporated the whole spectrum of BC histotypes: 41 cases with usual type (41/61), 20 cases with special type (20/61).

Among the special types 8 cases (13%) showed squamous differentiation, 4 (6%) micropapillary histology, 4 (6%) sarcomatoid, 1 (1.6%) chordoid, 1 giant cell (1.6%), 1 plasmacytoid (1.6%) and 1 neuroendocrine (1.6%).

IHC: HER2 3+ in 2/61 cases (3%), HER2 2+ in 2/61 case (3%) and HER2 1+ 2/61 cases (3%).

ISH: The ISH analysis was performed in all cases, but it was valuable only in 37 cases.

HER2 amplification was found in 13/37 cases (35%). In particular, all cases with any IHC HER2 positivity (3+, 2+, 1+) showed HER2 amplification. In addition, the amplification of HER2 was found in 7 cases with IHC score 0.

HER2 CORRELATIONS:

- **The 2 cases with IHC HER2 3+ were either special type (1 micropapillary, 1 giant cell); the 2 cases with IHC HER2 2+ were 1 chordoid and 1 usual type; the 2 cases IHC HER2 1+ were 1 micropapillary and 1 usual type.**
- **The 7 cases HER2 IHC negative were 1 neuroendocrine, 1 micropapillary and 5 usual type.**
- **The 13 amplified cases at ISH were 7 usual type and 6 special types: 2 micropapillary, 1 giant cell, 1 chordoid and 1 neuroendocrine.**
- **Among the micropapillary tumors, 2 cases (50%) were IHC positive (1 case 3+, and 1 case 1+) and 3 (75%) were amplified in ISH.**

Figure 1

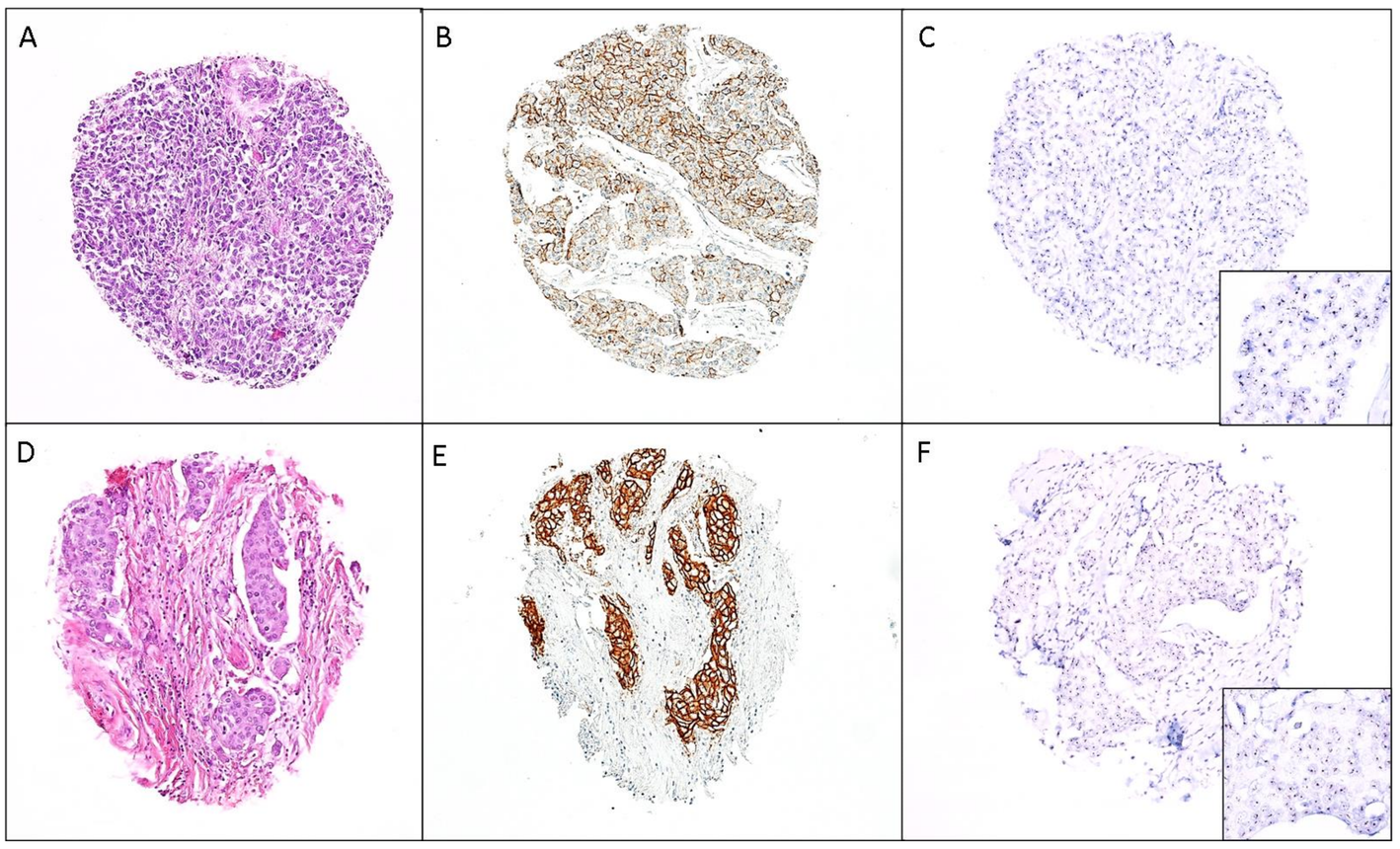

Figure 2

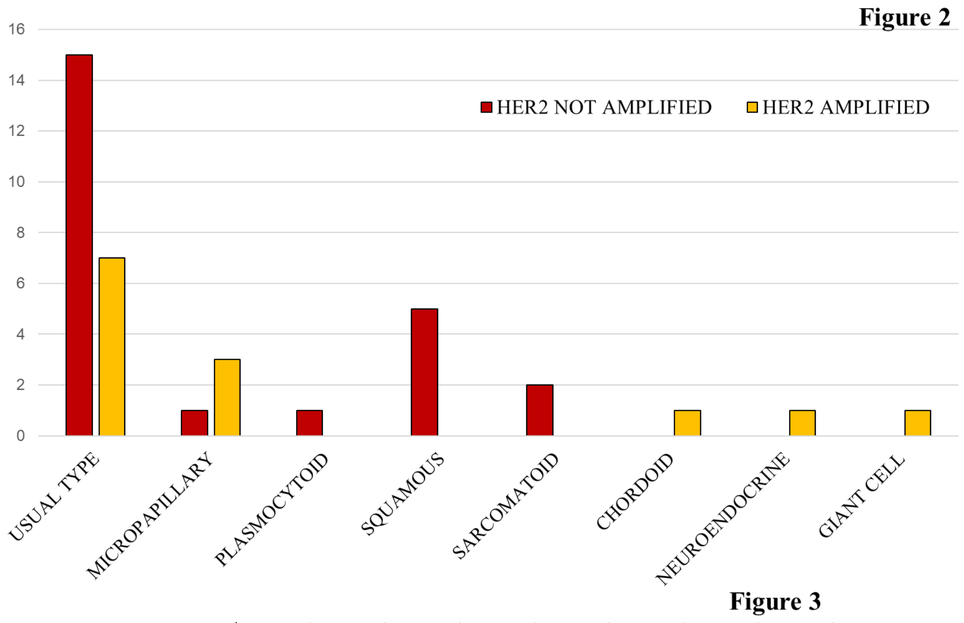

Figure 3

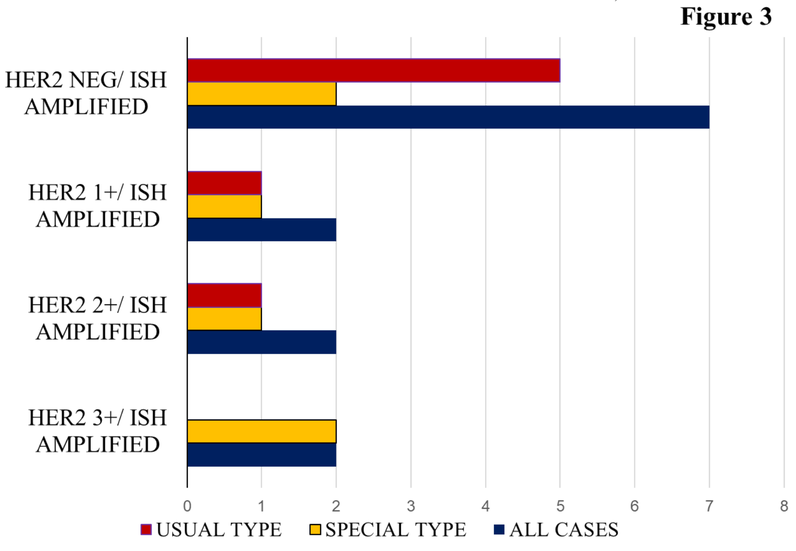

Figure 1 - A. BC usual type (H&E, x10), B. IHC HER2 3+ (x10), C. ISH HER2 AMPLIFIED, cluster (x10, square x40), D. BC micropapillary (x10), E. IHC HER2 3+ (x10), F: ISH HER2 AMPLIFIED, cluster (x10, square x40)

Figure 2 - Number of cases HER2 AMPLIFIED (yellow column) and HER2 NOT AMPLIFIED (blue column) for each histotype

Figure 3 - Distribution of cases HER2 AMPLIFIED for any results at IHC HER2, all cases blue bar, BC special type orange bar, BC usual type yellow bar

Conclusions

- **Unlike breast cancer we found in BC a strong correlation between any extent of IHC immunoreativity for HER2 and ISH amplification.**
- **We have also found HER2 amplification at ISH in 7 cases that were completely negative at IHC.**
- **HER2 was amplified not only in micropapillary type (23%) but mostly (54%) in usual type BC.**
- **In view of the possible therapy of BC with HER2 targeting agents, the candidate cases must be investigated not only by IHC but ISH is mandatory.**