ORIGINAL PAPER

Immunohistochemical determinations in evaluating the prognostic in patient with urinary bladder tumors

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Abstract

A set of 50 cases of urinary bladder tumors in patients predominantly resident in BEN area was histologically investigated again, in order to establish new prognostic factors. Histological type, histologic stage and invasive pattern were correlated to immunohistochemical determinations. Determinations of C-erb B2, pRb, p53 and Ki67 were performed and aggressivity degree and proliferation and prognostic indice were also established.

***Keywords*:** urinary bladder tumors, prognostic, imunohistochemistry.

Background

The prognostic of the urinary bladder tumors is generally difficult to be establishes by only a speciality. Therefore, a multidisciplinarity analysis is necessary where the urologist's observations should take into account the histologic, cytologic exams and the oncologic monitorization.

Prognostic factors most frequently used have been represented by the stage of the tumoral invasion, the tumoral anaplasya degree and the locations at the level of the urinary bladder [1–3].

To those prognostic elements, the histologic tumoral type, peritumoral histologic structure and also *in situ* carcinoma lesions have been taken into consideration [4].

Cytokeratinic profile changes could provide information concerning the positive differential diagnosis especially upon the prognostic.

Material and methods

A number of 50 cases of urinary bladder were available to be re-examined; they all came from the collection of Drobeta Turnu-Severin Hospital during the years 2005–2006.

All the cases studied by us were males aged between 38–75 years, with an average of 56 years.

The cases were grouped by using theyr residence, as belonging to BEN area, joining all the types of histologic degrees.

The set for studying was made both of tumors with invasive and superficial papillary zones and tumors with exclusively infiltrative pattern. Gradation was made by nuclear and cytoplasmatic differentiation histologic criteria ranging from well-differentiated (G1) to poor-differentiated (G3) [5].

Retrospective study was performed for same IHC markers and surviving was evaluated on each case followed along a period up to five years. Histopathologic re-examination of the cases could provide other morphologic criteria, too, aiming the prognostic value assessment [6].

As to make up the monoclonal antibody set applied in IHC studying, a number of four immunohisto-chemical antibodies have been tested in the present stage. Staining were performed for monoclonal antibodies C-erb B2, pRb, p53, and Ki67 and we followed the agressivity degree, proliferation and prognostic indices [7–13].

Results

Within the histologic exam (HE stain) there have been established such as: 29 cases (58%) of urothelial carcinomas (Figures 1 and 2), 11 cases of squamous carcinomas (22%), six cases of mixed carcinomas – urothelial and squamous (Figure 3) (12.2%), and four undifferentiated carcinomas (8%). Architecture was alveolar in nine cases (18%) (Figure 1), papillary in 24 cases (48.8%), micro-trabecullary in three cases (6%), and a solide one in 14 cases (28%).

Invasive pattern was expanded in 14 cases (28%), infiltrative in 14 cases (28%), noninvasive in 19 cases (28%), and mixed in three cases (6%); invasion was up to the external muscle or extravesical fat tissue.

Thus, in just nine cases invasion was limited to the internal own muscle, seven of which having been urothelial carcinomas and two cases of urothelial and squamous mixed carcinomas.

As concerning the histologic degree, the tumors were G2 and G3 (Figures 1–3).

In tumors with invasion in the external muscle or in perivesical tissue (25 cases), mixed and nondifferentiated carcinomas were predominant and the differentiation degree was especially G3.

C-erb B2 (Figure 4) was positive in 19 cases (four with +++, seven with ++, eight with +), and negative in 31 cases. Positivity was semiquantitative appreciated as +, ++, +++; positivity +++ was appreciated by a positive, intense and continuous membrana reaction which could be found all over the section; a limitted surface reaction, if it is intense is only + marked.

pRb gene protein was zonal cytoplasmatic positive in 28 cases, diffuse cytoplasmatic in 19 cases, nuclear in 10 cases and negative in 22 cases. Positivity for pRb was lost in most of the cases if only the nuclear reaction is appreciated. Practically it can be considered as positive just the nuclear localization of the reaction; as considering our set of study it appeared in only seven cases (Figure 5).

IHC reaction for p53 mutant (Figure 6) in our cases did not give any conclusive indices upon the tumoral aggresivity or prognostic; therefore, 14 cases were negative, 18 cases were undetermined, two cases unconcludent and 15 cases were positive with nuclear reaction of 10 and 60% intensity.

We noted that, in cases with external muscle invasion, p53 appeared as positive without correlation to other factors, excepting the proliferation ones, as it had already been established (20).

In a case of urothelial carcinoma with squamous differentiation with G2-G3 degree with extensive invasion in muscle, p53 was relatively well expressed in association to the poor expression of C-erbB2 and a moderate cell proliferation in Ki67 of 20%.

Ki67 (Figures 7 and 8) is a proliferation and prognostic index with a higher accuracy than p53, IHC reaction being positive in 46 cases (reaction intensity was between 5 and 70%) a negative one in four cases (further mentioning that five cases were not histological graduated therefore; a right correlation could not be made between the tumoral grading and those indices).

Discussions

It can be noted that, though the urothelial histologic type is predominant, with various differentiation degrees, a significant number of vesical tumors presented both a squamous or mixed proliferation types, a higher rate than reported in the literature (~10%), representing a parameter in addition to establish the degree of differentiation, as it is known that the presence of keratinization represents by itself a sign for differentiation [5].

Therefore, we took into account not only the forming of keratosic perls but also the presence of isolated cell keratinizations or the cell arrangement of squamous type, as concerning the squamous differentiation.

As for the tumors without forms of papillary structures, the urothelial origin is harder to be established and also the limit between the various histopathologic degrees.

Usually, infiltrative tumors are histopathologic graduated as well-, moderate- or poor differentiated, in fact, without existing morphologic criteria prescribed for those differentiations [5].

We especially followed the cell morphologic character but not an architectural one, just like into the superficial papillary tumors five cases were not graduated and 1 case was graduated Gx (undetermined and undifferentiated degree). Correlation between the clinical staging and the histopathological one showed a relatively good concordance but it proved that a precise prognostic appreciation was not allowed. That was due to the difficulty of establishing some morphologic criteria enough exactly for a histopathologic graduating, when the urothelial character of the tumor was absent [5].

For many times we tried to use the graduating criteria for the papillary superficial tumors but they were based on deviations of cytology and architecture from the normal urothelial type, thus supposing to extend morphologic characters of some noninvasive tumors to the infiltrative ones [5].

We could note both on the clinical set and for the histopathologically examined one, that, the patients surviving could not be exactly correlated neither to TNM or G cases, just like in the cases of superficial papillary tumors, as it was reported in literature [3]. As a consequence it is neecssary for some criteria with more precise value prognostic to be introduced.

In the se studied by us up to date, we didn't find any correlation between the C-erb B2 positivity and the tumoral grading or other IHC markers (i.e. one case was C-erb B2 +++ with G1 and in other cases with G3, C-erb B2 was negative; this is matched to the most of the reports in literature [3], though there are authors sustaining the contrary [13].

Also, cases with C-erb B2 +++ presented p53 negative and decrease Ki67 < 10%, and in cases with C-erb B3 negative, they were intense positive at Ki67 about 60–70%.

Recent studies of molecular biology concerning the increase of gena with Her-2 neu/C-erb B2 appeared but they are just at the beginning in the urinary bladder tumors [3, 7, 13].

Positive cells were rare and difficult to be identified due to the overlapped cytoplasmatic reaction. Correlation to the deep muscle invasivity is less obvious than it was specified in literature [3, 6].

The observation of that association is limited to the isolated cases. Surviving in that case was of 25.7 months that is understandable if taken into account all these factors. The question is asked why this type of antigenic configuration appeared just like the G2 urothelial papillary cracinomas, with infiltration limited to the chorion and having the same surviving period. It can be said that what is firstly needed to be improved is for the beginning, the morphologic characterization of the tumor.

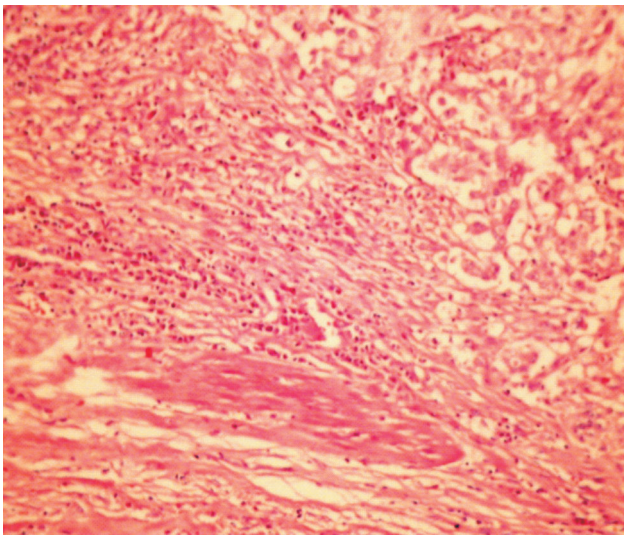

Figure 1 – *Urothelial bladder carcinoma G2; invasive alveolar pattern (HE stain, ob. ×20)*

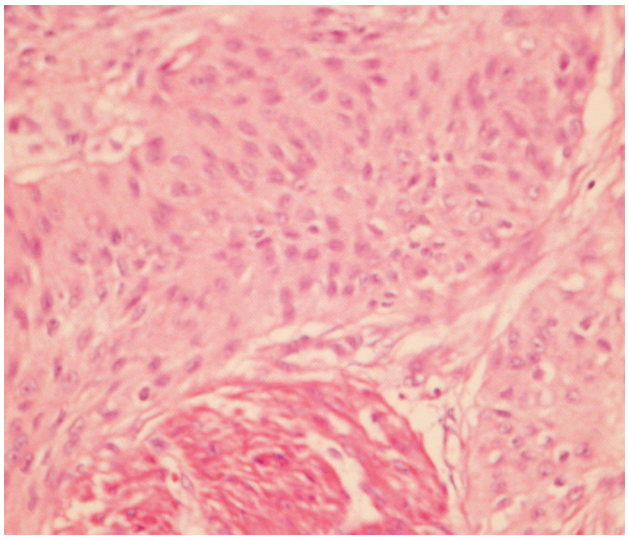

Figure 2 – *Urothelial bladder carcinoma G2; invasive (HE stain, ob. ×20)*

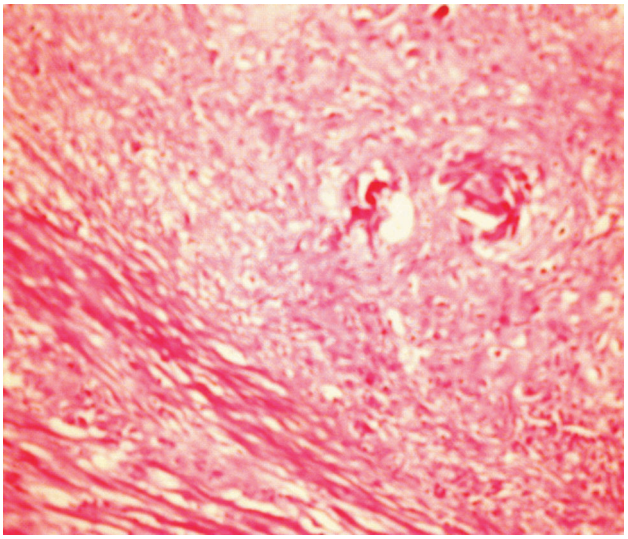

Figure 3 – *Urothelial bladder carcinoma with squamous differentiation G3; invasive (HE stain, ob. ×20)*

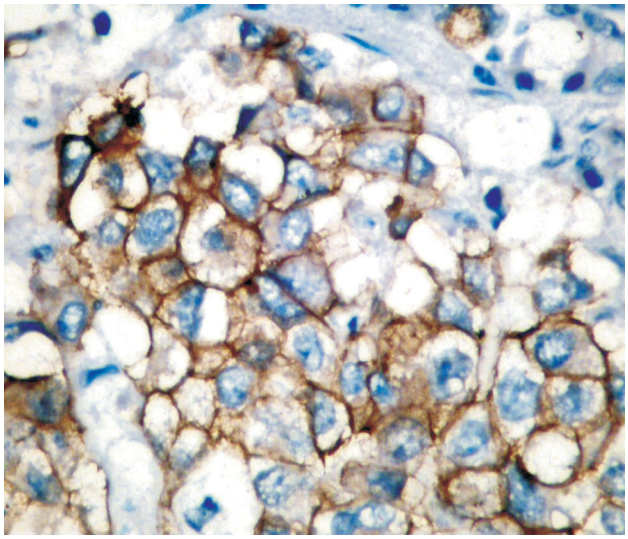

Figure 4 – *Urothelial carcinoma. Positive reaction +++ (IHC stain C-erb B2, ob. ×40)*

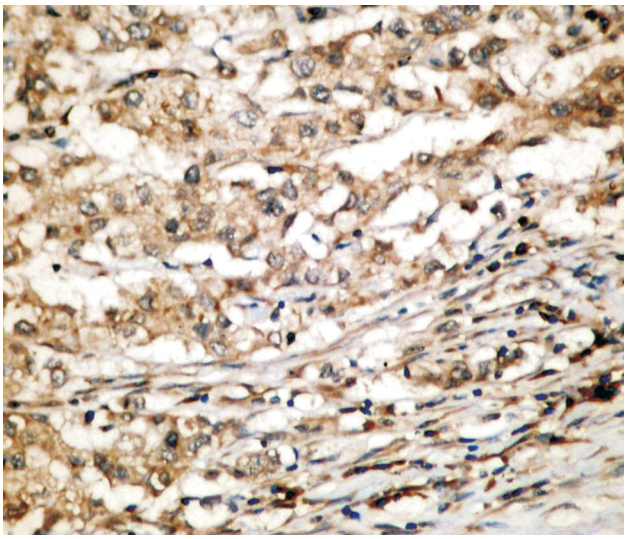

Figure 5 – *Urothelial carcinoma. Positive zonal reaction (IHC stain pRb, ob. ×20)*

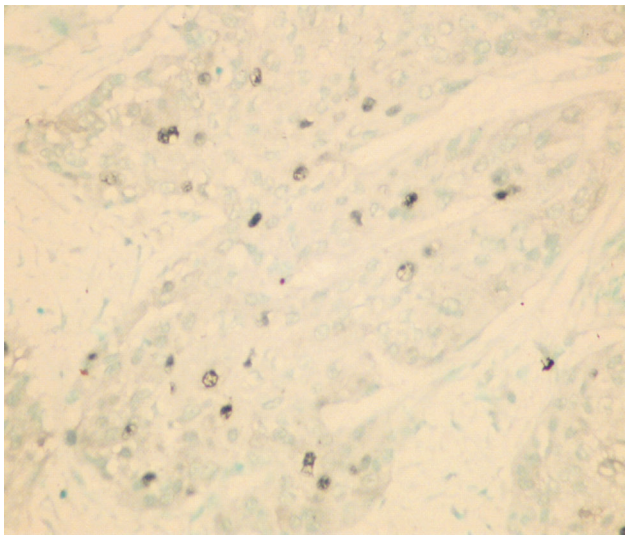

Figure 6 – *Urothelial carcinoma. Positive reaction in 5% (IHC stain p53, ob. ×20)*

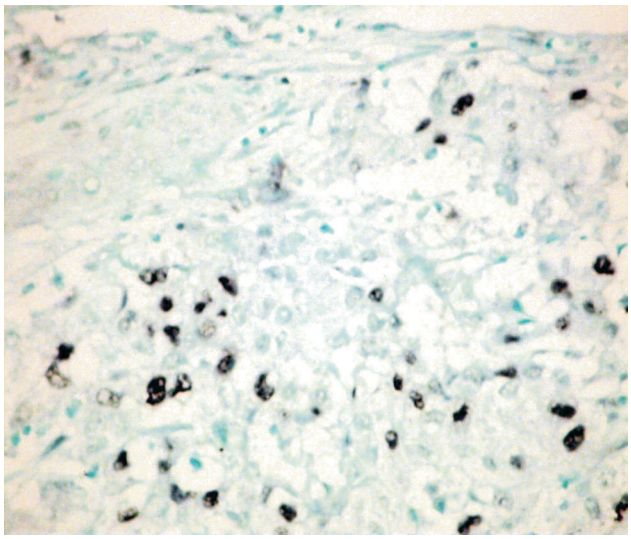

Figure 7 – *Urothelial carcinoma. Positive reaction in 20% (IHC stain for Ki67, ob. ×20)*

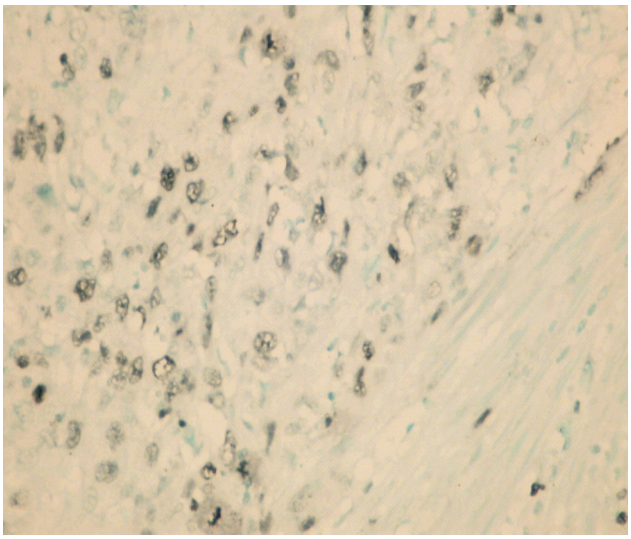

Figure 8 – *Urothelial carcinoma. Positive reaction in 40% (IHC stain for Ki67, ob. ×20)*

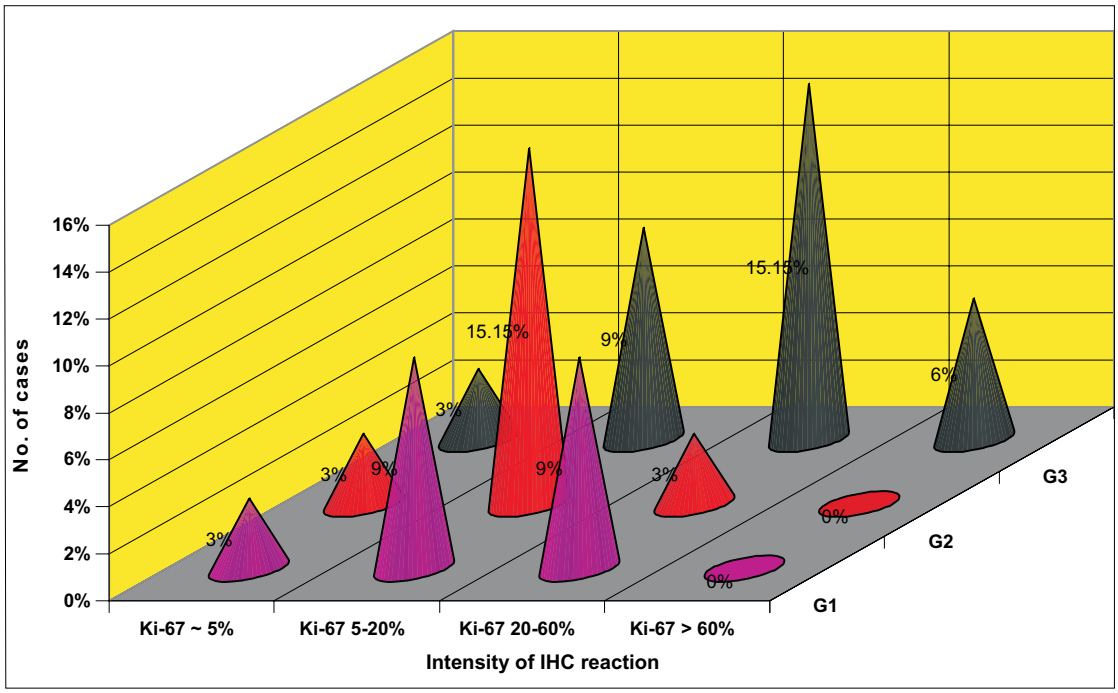

Figure 9 – *Correlation between Ki67 index and tumoral grading*

Relationship between Ki67 and tumoral grading is presented in Table 1 and Figure 9.

Table 1 – *Correlation between Ki67 and tumoral grading*

	Proliferation Ki67			
grading	*</= 5%*	*5–20%*	*20–60%*	*>60%*
G1	one case (3%)	three cases (9%)	three cases (9%)	0
G2	one case (3%)	five cases (15.5%)	one case (3%)	0
G3	one case (3%)	three cases (9%)	five cases (15.5 %)	two cases (6%)

Conclusions

The stage and the degree of the tumor are recognized as morphologic and prognostic factor.

Immunohistochemical determinations represent the ascendent of the tumoral morphologic criteria.

Ki67 and p57 are prognostic factors of great accuracy.

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