Prognostic and diagnostic implications of histological differentiation in invasive urothelial cell carcinoma of the bladder: variant or non-classic differentiation number

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KEY WORDS

bladder cancer ◗ divergent differentiation ◗ non-classic differentiation number ◗ histological type

ABSTRACT

Introduction. A tumor's histological organization is of prognostic importance. We evaluated the prognostic value of tumor uniformity (classic, or mixed = classic plus one non-classic type of differentiation as a histological variant) and non-classic differentiation number greater than 1 (NDN >1) in invasive urothelial cell carcinoma of the bladder.

Material and methods. One hundred thirty-three bladders obtained after cystectomy for primary urothelial bladder cancer and classified as T1-4 were examined. Three elements in every tumor were histopathologically evaluated: non-classic differentiation type (NDT), extent of NDT (NDT%), and NDN. Ten ranges of non-classic differentiation were distinguished: from 0% (100% classic pattern) to over 50%. Based on accepted criteria every instance of NDT was diagnostically essential, even trace instances.

Results. The percentage of tumors with pure classic (NDN = 0) differentiation was 16% (n = 21) and with non-classic (NDN >0) was 84% (n = 112). A tumor NDN of ≤2 was a factor that favorably influenced the prognosis (P = 0.024). No significant (P = 0.357) difference was observed between the lengths of survival of the groups with NDN = 0 and NDN = 1.

Conclusions. The NDN of a urothelial tumor is of prognostic importance. The presence of uniform differentiation, i.e., NDN ≤1, is associated with a more favorable prognosis. The repeatability of the results of diagnostic and scientific examinations requires the complete consideration of every instance of non-classic differentiation in invasive urothelial cell carcinoma of the bladder, finding that a classic and/or one non-classic tissue (NDN <2) does not indicate the risk of neoplastic progression.

INTRODUCTION

The histological phenotype of a tumor might be an indicator of the biological potential of neoplastic tissue. Tumor histological organization is of prognostic importance [1, 2] and thorough observation of a tumor's morphology might define its malignancy very well. Bladder cancer (BC) is a good observational subject because it displays the biological property of divergent differentiation [3-6]. Numerous chromosomal aberrations are known to occur in urothelial cancer cells and are also associated with the type of histological differentiation observed in the tumor [3, 5]. However, the presence of specific genetic damage, which is usually observed in cases of cancer and occurs within the normal urothelium of patients with BC, might indicate that the process of tumor development does not depend on the presence of chromosomal aberrations. Tumor phenotype is probably the result of the genetically defined potential of the neoplastic cell and its realization is induced by environment signals [7]. According to Dozmorov et al., the phenotype of bladder cancer cells depends on the activity of the extracellular matrix. They suggest that '... understanding how the extracellular matrix modulates the phenotype of bladder cancer cells is highly relevant to understanding the processes of recurrence and progression' [8]. The biological behavior of the tumor, as well as its morphology, depends on the expression of genes in the neoplastic cells, which might be subjected to changes under the influence of the extracellular matrix [9-11]. The accurate evaluation of the phenotype of urothelial cell cancers of the bladder might be a way to evaluate the cancer cells' reactivity to environmental signals. The outcome of this 'dialogue' may be the histological differentiation of the tumor. It is known that the mixed non-classic (NC) component of urothelial tumors can be associated with a worse prognosis [2, 5]. We have already described the prognostic value of the non-classic differentiation number (NDN) in urothelial cancer and have proposed that the presence of more than two non-classic types of differentiation in one tumor (NDN >2) is associated with a worse prognosis [1, 12]. In this study we evaluated whether the presence of a single type of non-classic differentiation (histological variant), regardless of its extent within the tumor, is of prognostic importance in urothelial cancer of the bladder.

MATERIAL AND METHODS

One hundred thirty-three bladders obtained from patients of Jurasz University Hospital and Jan Biziel Hospital in Bydgoszcz, undergoing cystectomy for primary cancer of the bladder in the years 2000-2005, were examined. This study was approved by the

Committee of Ethics of Scientific Research of Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland. The criteria used to select the tumors for examination were a classification of T1-T4 and the presence of a urothelial classic or mixed (classic plus non-classic) pattern of differentiation. In our previous work we had described the time spent to transport the surgical specimens from the operating room, the processes of preparing and preserving them, the method of cutting the tissue samples from the tumors, and the technology of embedding them in paraffin as well as the processes of cutting and staining the tissue fragments [12]. Three elements were histopathologically evaluated in every tumor: 1) non-classic differentiation type (NDT) according to the histological classification of urinary tumors of the World Health Organization of 2004; 2) the extent of the non-classic differentiation pattern (NDT%), determined separately for each non-classic type present in a tumor, and defined as the average percentage of the surface of the whole neoplastic tissue displaying that pattern, present in all the examined sections from a tumor; and 3) the non-classic differentiation number (NDN). In the histopathological evaluation, every instance of NDT observed was considered. Depending on the value for NDT%, the following ranges of the extent of non-classic differentiation were distinguished: 0% (that is 100% classic tissue), 0-1%, 1-3%, 3-6.5%, 6.5-10%, 10-20%, 20-30%, 30-40%, 40-50%, and 50-100% [3]. The evaluation of NDT% within the range of 6.5-100% was made by subjective approximation, as described in our previous publication [1]. Instances of NDT smaller than 6.5% were divided into two categories: 'trace' and 'greater than trace', to which arbitrary threshold values of 1% and 3% were given, respectively. An NDT% of 10% was defined subjectively as a little more than 6.5%. The NDT% evaluation within the range of 10-100% was also revised by means of the method of multiplicity of defined 10% representation supported with the rule for estimating non-classic tissues in relation to the whole (100%) neoplastic tissue. The NDT% values for each section were summarized and averaged to obtain the 'tumor' proportion for every non-classic differentiation type present. As a consequence of the accepted criteria, every instance of NDT was diagnostically essential. The recognition of a pure conventional pattern was only possible after any occurrence of NDT, even a trace, was excluded.

The dates and causes of death of the remaining patients were determined according to clinical data, follow-up observations, and Registry Office data. Data sorting and initial calculations were performed using Office 2003 (Microsoft Corp, Seattle, WA). The mean values, correlations (r), t-test findings, and survival analysis were assessed, and figures were drawn using the STATISTICA data analysis software system (version 7.1, StatSoft, Inc; www.statsoft.com). A P value lower than 0.05 was considered to be statistically significant.

RESULTS

The average age of the patients was 62 years (range, 27-78.7 years); the ratio of women to men was 4:15; the average ages of the women and men were 59 and 62.7 years, respectively. The average survival of the whole group (n = 133) was 2.07 years, while in the T1 classification group (n = 10) it was 3.91 years, T2 group (n = 20) – 2.91 years, T3 group (n = 87) – 1.81 years, and T4 group (n = 16) – 1.29 years. The correlation between the length of survival and T classification or NDN in the whole group was –0.35 or –0.25, respectively (P <0.05). The percentage of tumors with the pure classic pattern (NDN = 0) was 16% (n = 21) and with the non-classic pattern (NDN >0) was 84 % (n = 112).

In the whole group, it was clear that survival was essentially dependent on NDN. An NDN value for a tumor of ≤2 was the factor that favorably affected prognoses (Fig. 1A).

Thirty-one patients had NDN = 1 (6 women and 25 men, with average ages of 58.1 and 64.7 years, respectively). The presence of non-classic differentiation in the tumors of this group was observed within the scope of all the ranges of NDT%, except two with NDT% of 30–40% and 40-50%. The average age of patients in the whole group with NDN = 1 was 63.4 years. The types of non-classic differentiation present in the tumors are shown in Table 1A.

Twenty-one patients had pure classic differentiation (NDN = 0): 3 women and 18 men. No statistically significant difference (P = 0.357) was observed in the lengths of survival in the groups with NDN = 0 and NDN = 1 (Table 1B).

Because of their prognostic similarity, the patients in both groups (NDN = 0 and NDN = 1) are listed in one table as a group of tumors with uniform differentiation (classic or mixed, defined as 'classic plus one non-classic'). This group included 52 patients, 43 men and nine women (Table 1C).

Although adjusting the model of survival to the data is not as good as defining the border value for NDN on level 2 (Fig. 1A), the group of patients with NDN ≤ 1 certainly had a more favorable prognosis (Fig. 1B). In the six months after cystectomy (n = 103), patient survival in these groups (pure classic differentiation [NDN = 0] and mixed [NDN = 1]) was statistically significantly different (P = 0.02 and P = 0.03, respectively) from the survival of patients with more than one non-classic type of differentiation (NDN >1) (Fig. 1C). In the six months after cystectomy, there was no essential difference in the lengths of survival in groups NDN = 0 and NDN = 1, which were 3.32 and 3.17 years, respectively (P = 0.82).

DISCUSSION

In this study, we observed a statistically significant (P = 0.024) difference in the survival of patients with invasive urothelial-cell cancer of the bladder in groups NDN ≤ 2 and NDN > 2, for whom a survival model adjusted to the data was the best (Fig. 1A). These results are similar to those published by us previously [1] and confirm the prognostic value of NDN in a larger group of patients (n = 133). The presence of ≤2 non-classic types of differentiation in urothelial tumors is associated with a better prognosis (Fig. 1A). In the group examined, a negative correlation between the length of survival and T classification (r = –0.35) was observed, which is consistent with the observations of other authors [3]. The commonly used way of evaluating non-classic differentiation in urothelial tumors considers only prominent differentiation, which defines the so-called 'urothelial cancer variant', on which prognosis depends to a greater (nested, micropapillary, plasmacytoid/lymphoma-like, lipoid cell, sarcomatoid, small-cell, and large-cell undifferentiated variants) or lesser degree (other variants) [5, 13-19]. When there are several prominent patterns of differentiation in one tumor, it is possible to describe them as the so-called 'mixed pattern' [4]. Reuter proposed that tumors in which the number of prominent non-classic types of differentiation is >1 should be called 'mixed cancers'. The importance of the non-prominent occurrences of differentiation in the background is unknown and they are usually omitted from routine diagnostics, although their presence in variant cases is known [5]. The results of some examinations have shown that the mixed component of a tumor might arise from metaplastic foci within the already existing tumor [20]. According to Reuter, 'To the best of our knowledge ... microscopic areas of glandular or squamous metaplasia ... commonly seen in otherwise typical high-grade UC ... are clinically irrelevant and may be ignored...' [4]. The methodology

of the evaluation of the presence of non-classic differentiation in urothelial cancers allows the selection of cases with uniform non-classic differentiation (NDN = 1), classified in the appropriate range of NDT%, starting from trace representations. In this way, a group of 31 cases was separated from the mixed types of differentiation: classic plus one non-classic (NDN = 1). This differs from cases with variant differentiation, known from the reports of other authors, in that in our series, the value of NDN was exactly 1. In our 31 patients with variant differentiation there was no other type of non-classic differentiation in the background, even in trace amounts. In this way, the prognostic influence of NDN was eliminated and we propose that the prognosis for the whole group with NDN = 1 is not statistically significantly different from the prognosis of patients with pure classic differentiation (NDN = 0) (Table 1B). This observation may indicate that single types of non-classic differentiation arising in a urothelial tumor are probably not a factor associated with a worse prognosis. In the NDN = 1 group, we observed only some types of non-classic differentiation: squamous, glandular, nested, microcystic, micropapillary, giant cell, lymphoid, and undifferentiated (Table 1A). However, at least two of them (nested and micropapillary) are described as very malignant [5]. In the range of NDT% >50%, the average survival of six patients was 3.01 years (Table 1A), longer than the average survival of the whole NDN = 1 group (2.44 years; n = 31). Our observations suggest that the type of histopathological differentiation of the tumor, determined with the NDN parameter, is the most important prognostic element in urothelial cancer (Figs. 1B and 1C) compared with the presence of a single, even very extensive occurrence of non-classic differentiation. The clearly shorter survival in patients with differentiation in the NDT% ranges 10-20% and 20-30% (Table 1A) might be associated with death from causes independent of the disease. The survival of only two patients (2.66 and 3.79 years) in the NDT% range 10-20% was longer than the average survival of the entire group. In the other four cases in this range and in one case from the NDT% range 20-30%, death took place within six months of cystectomy (0.1, 0.2, 0.4, 0.5, and 0.4 years). One patient from the NDT% range 20-30% died in the eighth month and one, with T1 classification, in the ninth month after cystectomy. It cannot be excluded that the causes of these patients' deaths, in a high proportion of cases in both NDT% ranges, might have been independent of their bladder cancer. All cases of uniform classic and/or non-classic differentiation (n = 52) showed that the length of survival depended on T classification (Table 1C), which testifies to the representativeness of the group. The average survival of the whole group of uniformly differentiated tumors (2.7 years; n = 52) was essentially greater (P = 0.0055) than that of the whole sample (2.1 years; n = 133).

The real occurrence of non-classic differentiation was 84%, which is consistent with our previous reports in which the percentage of the NC pattern was 81% [13]. Data published by other authors concerning the predominant types of differentiation included squamous epithelial and glandular metaplasias, present in about 25% of patients with urothelial cancer [3, 5, 21]. According to Wasco et al., 'Any amount and type of divergent differentiation appears to be significant and therefore should be reported', and the non-classic pattern of differentiation is indeed more frequently recognized in patients (30.5%) [2]. In our data, cases with NDN = 1 only comprised 23% of all the tumors. Wasco et al. found more than four times as many pure urothelial cancers (69.5%) than we found in our study (16% of tumors had NDN = 0) [2]. In some proposed systems of evaluating the components of non-classic differentiation in the tumor, the authors defined ranges theoretically from 0% to 100% (NC ranges <10%, 10-50%, and >50% corresponding to 'local', 'moderate and high or local', 'moderate and extensive' categories of Wasco et al. or Samaratunga et al.,

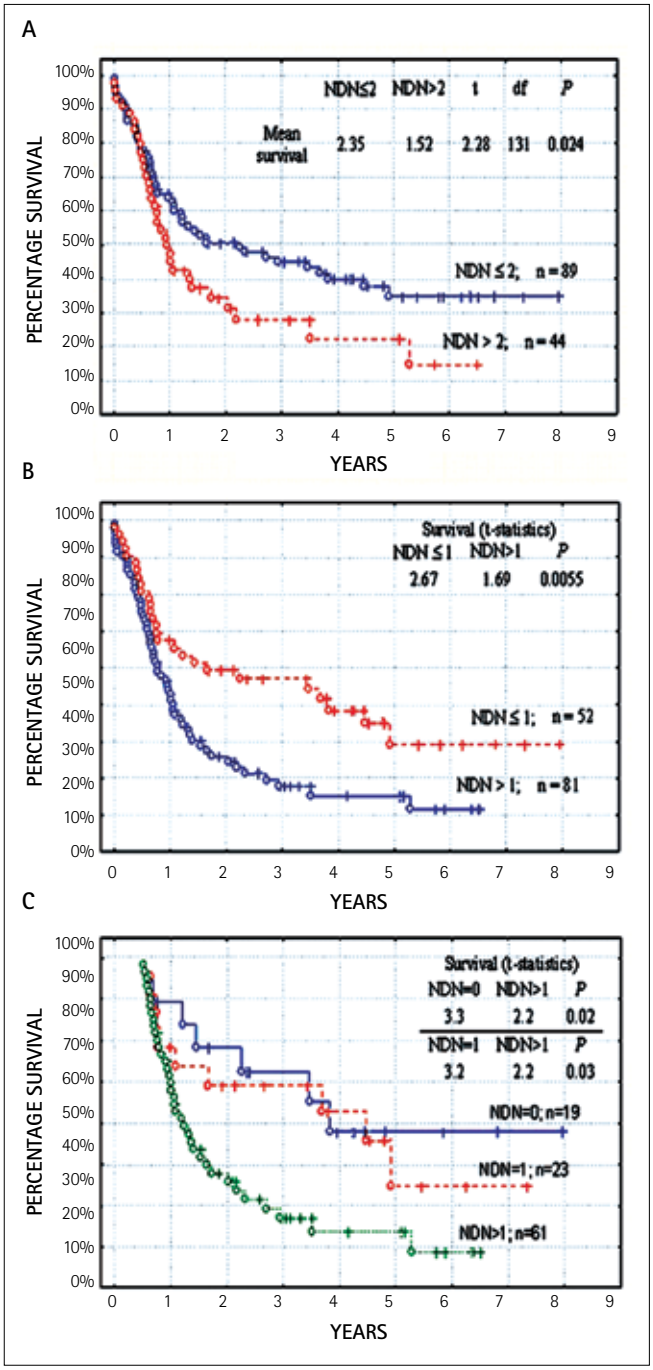

Fig. 1. A. The dependence of survival on NDN. The best adjustment of the survival model to the data was obtained for NDN ≤2 (Wilcoxon's test statistic with the Gehan modification: 1.479509, P = 0.13900); **B.** Although the test statistics do not show the optimum adjustment of the survival model to the data (according to Wilcoxon with the Gehan modification: –2.22712, P = 0.02594), uniform differentiation in the primary tumor (i.e., classic, or mixed = classic plus one nonclassic) was associated with a more favorable prognosis; **C.** Six months after cystectomy, the mean survival of each group (NDN = 0 and NDN = 1) was independently significantly longer than that in patients with tumors of NDN >1.

respectively, but the regions of differentiation below the declared threshold of 10% were probably omitted [2, 22]. Thus, both the predominance and the uniformity of non-classic differentiation remain vaguely defined. In our results, patients with a single non-classic type of differentiation (NDN = 1) represented a population of above 23%, assuming that all instances of NDT were considered, even in 'trace' amounts (that is starting from NDT% = 1%). Consequently, in published cases of variant differentiation at rates

Table 1. A. Survival and types of non-classic differentiation in tumors with NDN = 1, according to NDT% differentiation; **B.** Statistics showed no significant difference in the survival of the groups of patients with pure classic (NDN = 0) and mixed (classic plus one non-classic, NDN = 1) differentiation patterns; **C.** T-advancement categories, average survival, and age according to NDT% differentiation in patients with NDN ≤1.

A

NDT \ NDT%	≤1	1–3	3–6.5	6.5–10	10–20	20–30	30–40	40–50	50–100	All
	Number of cases									
Squamous	2	–	1	–	1	2	–	–	–	6
Glandular	–	–	1	–	–	–	–	–	3	4
Nested	–	1	–	1	1	–	–	–	1	4
Microcystic	2	2	–	1	1	–	–	–	–	6
Micropapillary	–	–	1	–	1	1	–	–	–	3
Giant cell	1	1	1	–	–	–	–	–	–	3
Lymphoid	–	–	–	–	1	–	–	–	1	2
Undifferentiated	–	–	–	1	1	–	–	–	1	3
All	5	4	4	3	6	3	0	0	6	31
Survival	2.53	3.11	2.55	4.25	1.27	0.61	–	–	3.01	2.44

B

Mean survival time (years)		t	df	P	Number of cases	
NDN = 1	NDN = 0				NDN = 1	NDN = 0
2.44	3.01	–0.93	50	0.357	31	21

C

	NDT%											Survival	Age
	0	≤1	1–3	3–6.5	6.5–10	10–20	20–30	30–40	40–50	50–100	All		
	Number of cases												
T1	6	–	1	–	1	–	1	–	–	–	9	4.3	63.4
T2	3	2	1	2	1	–	1	–	–	3	13	2.7	63.0
T3	7	2	2	2	0	5	0	–	–	3	21	2.2	61.7
T4	5	1	–	–	1	1	1	–	–	0	9	1.9	68.3
ALL	21	5	4	4	3	6	3	0	0	6	52	2.7	63.5

above 23%, the 'variant character' of the tumor is not 'prominent' at all (because it must be 'trace') or the variant types of differentiation are not uniform differentiations (single). Alternatively, the prognosis might depend more on NDN than on variant NDT.

CONCLUSIONS

Because the histopathological evaluation of urothelial tumor differentiation may still be imprecise, there may also be inaccuracies when tumors are subjected to scientific examination. This might intensify the effects of the incomparability and unrepeatability of the results. Because the NDN influences the prognosis, omitting it from a diagnosis or subjecting the material to scientific examination might constitute a serious methodological mistake. Therefore, further studies are required that identify the rules of co-occurrence of histological types in urothelial tumors with non-uniform differentiation (NDN >1) and that evaluate the influence of the type of differentiation on prognoses.

REFERENCES

1. Jozwicki W, Domaniewski J, Skok Z et al: *Usefulness of histologic homogeneity estimation of muscle-invasive urinary bladder cancer in an individual prognosis: a mapping study.* Urology 2005; 66: 1122-1126.
2. Wasco MJ, Daignault S, Zhang Y et al: *Urothelial carcinoma with divergent histologic differentiation (mixed histologic features) predicts the presence of locally advanced bladder cancer when detected at transurethral resection.* Urology 2007; 70: 69-74.
3. Lopez-Beltran A, Sauter G, Gasser T et al: *Infiltrating urothelial carcinoma.* In: Eble JN, Sauter G, Epstein JI et al, eds. WHO Classification of Tumours. Pathology and Genetics. Tumours of the Urinary System and Male Genital Organs. Lyon: IARC Press, 2004, pp. 97-104.
4. Reuter VE: *The pathology of bladder cancer.* Urology 2006; 67: 11-18.
5. Lopez-Beltran A, Cheng L: *Histologic variants of urothelial carcinoma: differential diagnosis and clinical implications.* Hum Pathol 2006; 37: 1371-1388.
6. Cheng L, Neumann RM, Nehra A et al: *Cancer heterogeneity and its biologic implications in the grading of urothelial carcinoma.* Cancer 2000; 88: 1663-1670.
7. Stoehr R, Zietz S, Burger M et al: *Deletions of chromosomes 9 and 8p in histologically normal urothelium of patients with bladder cancer.* Eur Urol 2005; 47: 58-63: 58-63.
8. Dozmorov MG, Kyker KD, Saban R et al: *Analysis of the interaction of extracellular matrix and phenotype of bladder cancer cells.* BMC Cancer 2006; 6: 12.
9. Smith BA, Kennedy WJ, Harnden P et al: *Identification of genes involved in human urothelial cell-matrix interactions: implications for the progression pathways of malignant urothelium.* Cancer Res 2001; 61: 1678-1685.
10. Syrigos KN, Harrington KJ, Pignatelli M: *Role of adhesion molecules in bladder cancer: an important part of the jigsaw.* Urology 1999; 53: 428-434.
11. Rebel JM, Thijssen CD, Vermey M et al: *Modulation of intra-epithelial expansion of human T24 bladder-carcinoma cells in murine urothelium by*

growth factors and extracellular-matrix components. Int J Cancer 1995; 60: 707-711.
12. Domanowska E, Jozwicki W, Domaniewski J et al: *Muscle-invasive urothelial cell carcinoma of the human bladder: Multidirectional differentiation and ability to metastasize*. Hum Pathol 2007; 38: 741-746.
13. Nassar H: *Carcinomas with micropapillary morphology: clinical significance and current concepts*. Adv Anat Pathol 2004; 11: 297-303.
14. Mai KT, Park PC, Yazdi HM et al: *Plasmacytoid urothelial carcinoma of the urinary bladder: report of seven new cases*. Eur Urol 2006; 50: 1111-1114.
15. Lopez-Beltran A, Luque RJ, Vicioso L et al: *Lymphoepithelioma-like carcinoma of the urinary bladder: a clinicopathologic study of 13 cases*. Virchows Arch 2001; 438: 552-557.
16. Ikegami H, Iwasaki H, Ohjimi Y et al: *Sarcomatoid carcinoma of the urinary bladder: a clinicopathologic and immunohistochemical analysis of 14 patients*. Hum Pathol 2000; 31: 332-340.
17. Lopez-Beltran A, Pacelli A, Rothenberg HJ et al: *Carcinosarcoma and sarcomatoid carcinoma of the bladder: clinicopathological study of 41 cases*. J Urol 1998; 159: 1497-1503.
18. Johansson SL, Borghede G, Holmang S: *Micropapillary bladder carcinoma: a clinicopathological study of 20 cases*. J Urol 1999; 161: 1798-1802.
19. Holmang S, Johansson SL: *The nested variant of transitional cell carcinoma - a rare neoplasm with poor prognosis*. Scand J Urol Nephrol 2001; 35: 102-105.
20. Kunze E, Francksen B: *Histogenesis of non-urothelial carcinoma of the urinary bladder from pre-existent transitional cell carcinomas: a histopathological and immunohistochemical study*. Urol Res 2002; 30: 66-78.
21. Sakamoto N, Tsuneyoshi M, Enjoji M: *Urinary bladder carcinoma with a neoplastic squamous component: a mapping study of 31 cases*. Histopathology 1992; 21: 135-141.
22. Samaratunga H, Khoo K: *Micropapillary variant of urothelial carcinoma of the urinary bladder; a clinicopathological and immuno-histochemical study*. Histopathology 2004; 45: 55-64.

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