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Urine-TPA (Tissue Polypeptide Antigen), flowcytometry and cytology as markers for tumor invasiveness in urinary bladder carcinoma*

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Summary. Urine-Tissue Polypeptide Antigen (U-TPA) was measured in 81 patients with a previously diagnosed bladder carcinoma. U-TPA was elevated in 74% of the patients with invasive bladder cancer as compared to only 15% of the patients with superficial tumors. Only one patient without a tumor recurrence had an elevated U-TPA level (4%). The results were compared with cytological grading and flow-DNA measurements in a multivariate analysis with T-category as the result variable. U-TPA and grade showed each, independently, a significant relation ($P \ll 0{,}001$) to T-category whereas the result of the DNA measurements did not explain the variation in T-category when U-TPA and grade were already in the equation. For diagnostic purposes U-TPA seems to be of limited value but may serve as an indicator of tumor recurrence in bladder cancer patients.

Key words: Bladder cancer – TPA – DNA – Cytology

Introduction

The natural course of human bladder carcinoma is highy variable. The therapeutic choices vary from repeated fulguration of isolated tumors to radical removal of the entire bladder. The therapeutic strategy is based on the estimated malignant potential of the tumor, primarily reflected by histopathology and cytological examination. For tumors which have clearly low or high grade malignancy the choice of therapy is easily decided. The majority of patients, however, have cancers of an intermediate degree of differentiation, a group in which the aggressiveness of the tumor is more difficult to assess. As important is it not to carry out unnecessary mutilating surgery, as it is to give the patient a chance of definite cure by means of radical surgery before the tumor has advanced to an incurable stage. Apart from tumor cell DNA-content as measured by flow-cytometry [9], also loss of ABO(H)-antigen [12] has been proposed to provide additional prognostic information.

Tissue polypeptide antigen (TPA) was first isolated from "membranes" of various human carcinomas [2]. TPA is break-down product of cytokeratins. The serum content of TPA has been found to be elevated due to various malignant disorders [3] as well as in some inflammatory conditions like Crohn's disease and hepatitis [13]. The serum content of TPA has been reported to parallel tumor progression of urothelial cancer [1] and also the urine content of TPA has been reported to be correlated to tumor stage and to survival [7].

TPA is present in normal, inflammatory as well as in malignant urothelium [6]. The elevated levels of TPA in serum and urine which can be found in malignant conditions are probably due to an increased cell turnover and autolysis [6].

We here report on a multivariate analysis of urine-TPA, cytology and DNA-measurements as related to tumor invasiveness. Our intention was to evaluate the usefulness of urine-TPA as a marker of the biologial activity of the tumor in the monitoring of patients with bladder carcinoma.

Materials and methods

A total of 81 patients admitted for cystoscopic control with a known diagnosis of bladder carcinoma were evaluated. To avoid diurnal variations, only 24 h urine specimens were used [11]. Therefore only patients staying in hospital were included. Since elevated U-TPA levels can be caused by inflammatory disorders patients with urinary tract infections were excluded from the study. The patients were examined by cystoscopy and bimanual palpation. Bladder washings

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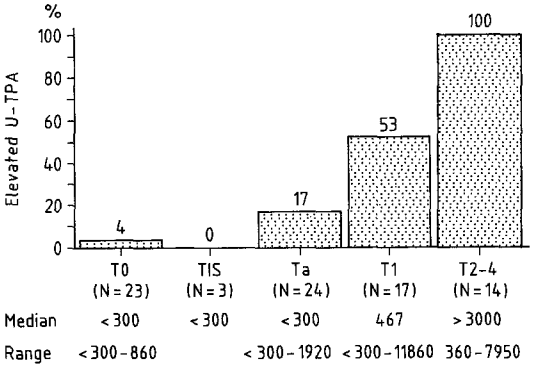

Fig. 1. Patients with elevated U-TPA in the respective T-categories. Range and median U-TPA values are also shown

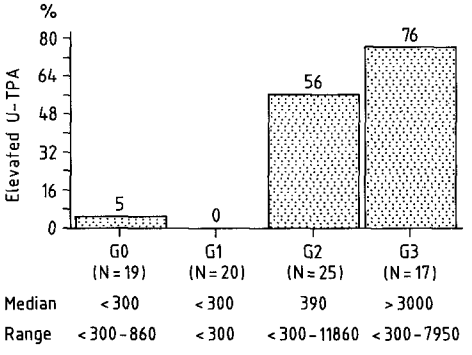

Fig. 2. Patients with elevated U-TPA in relation to cytological grading. Range and median U-TPA values are also shown

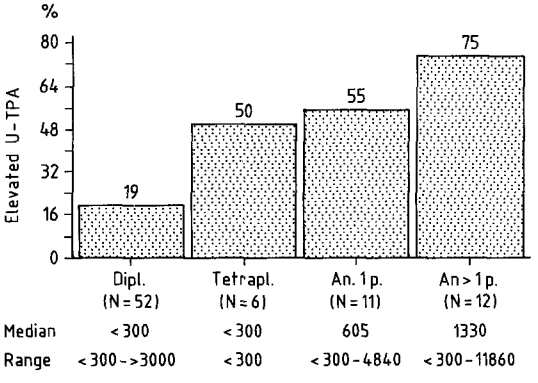

Fig. 3. Patients with elevated U-TPA in relation to cytological flow-DNA measurements. These were classified as diploid (Dipl.), tetraploid (Tetrap.), aneuploid with one peak (An 1p.), or aneuploid with more than one peak (An>1p.). Range and median U-TPA values are also shown

were taken for cytological examination, according to Esposti [8], and DNA measurements by means of flow-cytometry as described by Tribukait et al. [14]. Biopsies were taken when tumor recurrences were found.

Table 1. Cytological grading as related to T-category. Figures within brackets indicate the number of patients with elevated U-TPA levels

T-category	Cytological findings				
	0	1	2	3	Total
T0	18 (1)	3	2	–	23 (1)
TIS	–	–	–	3	3 (0)
Ta	1	14	7 (3)	2 (1)	24 (4)
T1	–	3 (1)	12 (7)	2 (2)	17 (10)
T2	–	–	3 (3)	5 (5)	8 (8)
T3	–	–	1 (1)	3 (3)	4 (4)
T4	–	–	–	2 (2)	2 (2)
Total	19 (1)	20 (1)	25 (14)	17 (13)	81 (29)

Laboratory method

Urine-TPA was analyzed by means of radiomimmunoassay using a commercially available kit (Prolifigen® – Sangtec, Bromma, Sweden). From 24 h specimen [11] of urine 50 ml was taken and stored at −18°C until analyzed. Each specimen was incubated with antiserum against TPA (horse anti-He La serum) which binds the TPA present. [125I]TPA was added. The remaining anti-TPA antibody thus labelled, was precipitated with rabbit-anti (horse IgG) antibody and the radioactivity measured in a gamma counter. The upper normal limit was set at 300 U/l according to recommendations by the manufacturer and based on studies of urine from healthy controls (unpublished data). Since the Prolifigen® kit was originally designed for serum assays and not urine, comparative analyses were also performed using a serum matrix with similar results.

Statistical methods

Multiple logistic regression with T-category as the result variable was used [12]. T-category: {0, A = 0}, {≥ 1 = 1}. The background factors were grade, ploidy and U-TPA coded as follows: Grade: {0, 1 = 0}, {2, 3 = 1}, ploidy: {diploidy = 0}, {aneuploidy = 1}, and U-TPA: {< 300 U/l = 0}, {≥ 300 U/l = 1}.

As the result variable herein was discrete and with few categories, we chose to dicotomize it into two groups; non-invasive and invasive tumors. TIS cases were excluded. Logistic regression was then the natural choice for the multiple analysis. Odds ratios (OR) are also shown as an approximation of the relative risk to have elevated U-TPA-levels, grade two or three cellular atypis, and non-diploid DNA pattern respectively.

Results

Three patients (5%) had tumors classified as TIS, 24 (41%) as Ta, 17 (29% as T1 and 14 (24%) as T2–4. The remaining 23 patients seemed to be free from recurrence of their bladder cancer (T0). Two of three patients with tumor in situ (TIS) had a negative cystoscopy, but all three patients were found to have malignant cytology. In patients with invasive carcinoma (T1–4) 90% (28/31) had malignant cells in their bladder washings,

Table 2. DNA-findings bladder washings as related as related to T-category. Figures within brackets indicate the number of patients with elevated U-TPA levels

T-category	Diploid	Tetraploid	Aneuploid 1 peak	Aneuploid >1 peak	Total
T0	21	1	1 (1)	–	23 (1)
TIS	–	–	1	2	3 (0)
Ta	19 (3)	2	1 (1)	2	24 (4)
T1	9 (5)	1 (1)	5 (2)	2 (2)	17 (10)
T2	1 (1)	2 (2)	2 (2)	3 (3)	8 (8)
T3	1 (1)	–	–	3 (3)	4 (4)
T4	1 (1)	–	1 (1)	–	2 (2)
Total	52 (11)	6 (3)	11 (7)	12 (8)	81 (29)

Table 3. Bivariate analysis with T-category as the dependent variable with U-TPA, grade and DNA-content as predictors. Patients with carcinoma in situ were excluded. Odds ratios (OR) are also shown

	T-category		P value	OR
	Ta	T 1–4		
Percent patients with U-TPA ≥ 300 U/l	16.7 (4/24)	77,4 (24/31)	≤0.001	17.1
Percent patients with grade > 1	35.7 (9/24)	90,3 (28/31)	≤0.001	15.5
Percent patients with aneuploidy	20,8 (5/24)	61,3 (19/31)	<0.001	6,0

Table 4. Logistic regression with T-category as the result variable. Odds ratios (OR) and their confidence intervals (CI) are also shown

Factor	Beta coeff. β	P vlaue	OR	95% CI (multiple)
U-TPA	2.39	<0.001	10,9	2,7–43,6
Grade	2.08	0.007	8,0	1,8–36,6
Intercept	−2.72			

whereas cytology was "positive" in 37% in patients with superficial (Ta) tumors (Table 1).

Cystoscopy was normal in 24 patients but two of these had a carcinoma in situ, and one of 57 patients with a cystoscopic suspicion of tumor recurrence had only normal urothelium in histological and cytological examination, thus, making the number of T0 patients 23. In one patient with negative cystoscopy an elevated U-TPA value was found, however, this patient was on intermittent treatment with intravesical chemotherapy. Two of the three patients who were on such a protocol exhibited markedly elevated U-TPA levels.

47% (27/57) of the patients who had a cystoscopic suspicion of a recurrence of their bladder tumors, had elevated U-TPA levels as compared to 4% (1/24) in the group exhibiting normal cystoscopy.

With regard to T-categry elevated U-TPA levels were found in 74% of the patients with invasive (T1–4) bladder cancer (Fig. 1). All patients with tumors T2–4 had elevated levels of U-TPA. In contrast elevated U-TPA levels were found only in 17% (4/24) of the patients with superficial tumors (Ta). No patient with TIS had elevated U-TPA.

In patients with no or only slight cellular atypia (G0–1) only 3% (1/49) had elevated U-TPA, whereas patients with moderate (G2) and strong (G3) cellular atypia had elevated U-TPA concentration in 56 and 76% respectively (Fig. 2).

DNA-content was regarded as diploid in bladder washings from 52 patients where of 10 (19%) had elevated U-TPA levels (Table 2 and Fig. 3). In patients with non-diploid DNA-pattern in bladder washings U-TPA was elevated in 62% (18/29). U-TPA was elevated in 50% in tetraploid cases, in 55% in aneuploid cases with one aneuploid peak in the DNA histogram, and in 75% in cases with more than one aneuploid peak.

Bivariate analysis

In the bivariate analysis, DNA-measurements, U-TPA, and cytological grading, were all strongly associated to T-category (Table 3).

Multivariate analysis

U-TPA and grade showed each, independently, a significant relation to T-category (Table 4). In contrast DNA did not contribute significantly to explain the variation in T-category (result variable) when U-TPA and grade were already in the equation.

Discussion

Elevated concentrations of TPA in serum (S-TPA) have been found in metastatic urinary cancers and measurements of S-TPA can be used for monitoring of the patient's response to treatment [1, 10]. Increasing urinary TPA levels have been reported to parallel increasing T-category [7] and our study confirms these data (Fig. 1). In the monitoring of patients with superficial bladder tumors, serum-TPA is of limited value, since TPA in the serum is usually not elevated until the tumor has progressed to an invasive stage and metastasized [10].

As a diagnostic test, measurement of U-TPA seems to be of limited importance since only 47% of patients with cystoscopically proven bladder tumors were found to have elevated U-TPA values.

Cytological grading serves as a complement to histological grading [5], which is the most important parameter when investigating bladder carcinoma patients. In this study, however, we have compared the three parameters; U-TPA, cytological grading, and flow DNA measurements as these three reflect cellular properties of the tumor.

As far as tumor invasiveness is concerned, U-TPA seems to provide similar information as the cytological grading with a detection rate of 74 and 90% respectively in patients with invasive bladder cancer, and 15 and 37% respectively in superficial tumors. U-TPA should not replace cytological grading, but it is a simple and objective laboratory test and can be useful as complement when cytological expertise is not readily available. U-TPA measurement may be used as a complement to the histological staging since elevated levels indicate invasive cancer (74% as compared to 15% in non-invasive tumors).

In this study we found U-TPA-levels better related to tumor invasiveness than DNA-pattern as calculated by means of multivariate analysis. The importance of such data must be related to the patients' survival before any definite conclusions can be drawn with regard to the prognostic information obtained from U-TPA and flow-DNA measurements.

In conclusion, measurement of U-TPA is easily carried out with commercially available RIA-kits; U-PA values seem to be strongly associated to T-category, similar to cytological grading and DNA measurements, hence, measurement of U-TPA can be used as a tumor indicator in the follow-up of patients with urothelial carcinoma.

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