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Clinical-Bladder cancer

Prognostic role of the urokinase plasminogen activator (uPA) system in patients with nonmuscle invasive bladder cancer

Takehiro Iwata, M.D.[a,b], Shoji Kimura, M.D.[a,c], Mohammad Abufaraj, M.D.[a,d], Florian Janisch, M.D.[a,e], Mehdi Kardoust Parizi, M.D.[a,f], Andrea Haitel, M.D.[g], Micheal Rink, M.D.[e], Morgan Rouprêt, M.D.[h], Harun Fajkovic, M.D.[a], Veronica Seebacher, M.D.[i], Peter Nyirady, M.D.[j], Pierre I. Karakiewicz, M.D.[k], Dmitry Enikeev, M.D.[l], Leonid M. Rapoport, M.D.[m], Yasutomo Nasu, M.D.[b], Shahrokh F. Shariat, M.D.[a,l,m,n,o,*]

[a] *Department of Urology, Medical University of Vienna, Vienna, Austria*
[b] *Department of Urology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*
[c] *Department of Urology, Jikei University School of Medicine, Tokyo, Japan*
[d] *Department of Special Surgery, Jordan University Hospital, The University of Jordan, Amman, Jordan*
[e] *Department of Urology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany*
[f] *Department of Urology, Shariati Hospital, Tehran University of Medical Sciences, Teheran, Iran*
[g] *Department of Pathology, Medical University of Vienna, Vienna, Austria*
[h] *Department of Urology, Sorbonne Université, Paris, France*
[i] *Department for Gynaecology and Gynaecologic Oncology, Medical University of Vienna, Vienna, Austria*
[j] *Department of Urology, Semmelweis University, Budapest, Hungary*
[k] *Division of Urology, Cancer Prognostics and Health Outcomes Unit, University of Montreal Health Center, Montreal, QC, Canada*
[l] *Institute for Urology and Reproductive Health, Sechenov University, Moscow, Russia*
[m] *Department of Urology, Weill Cornell Medical College, New York, NY*
[n] *Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX*
[o] *Karl Landsteiner Institute of Urology and Andrology, Vienna, Austria*

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Abstract

Objectives: To assess the role of the urokinase plasminogen activator (uPA) system as a prognostic biomarker in patients with non-muscle invasive bladder cancer (NMIBC) treated with transurethral resection of the bladder (TURB) with or without adjuvant intravesical therapy.

Material and methods: We stained TURB tissue from 827 NMIBC patients with uPA, its receptor (uPAR) and its inhibitor (PAI-1). The status of these markers was categorized as normal vs. overexpressed using the cutoffs of 30% for uPA, 50% for uPAR, and 30% for PAI-1. Multivariable Cox regression analyses were performed to evaluate the prognostic value of these markers.

Results: uPA was overexpressed in 37.7% of patients, uPAR in 44.7% and PAI-1 in 44.6%. Overexpression of these markers was associated with high tumor grade. Within a median follow-up was 60 months (interquartile range: 22−109), uPA (hazard ratio [HR]: 1.40; $P = 0.006$), uPAR (HR: 1.70; $P < 0.001$), PAI-1 (HR: 1.35; $P = 0.014$), and the combination of all 3 markers (HR: 3.38; $P < 0.001$) were associated with recurrence-free survival (RFS); uPA (HR: 1.68; $P = 0.035$) and the combination of all 3 markers (HR: 8.79; $P = 0.005$) were associated with progression-free survival (PFS). The addition of the uPA system to a base model improved the discrimination by 1.3% for RFS and 2.1% for PFS. In subgroup analyses, uPA (HR: 2.19; $P = 0.018$) was associated with PFS in T1G3 patients and its addition to a base model improved the discrimination by 2.5%. uPA (HR: 1.44; $P = 0.019$), uPAR (HR: 1.54; $P = 0.006$), PAI-1 (HR: 1.46; $P = 0.013$) and the combination of all 3 markers (HR: 3.48; $P < 0.001$) were associated with RFS in TaG1-2 patients and their addition to a base model improved the discrimination by 2.1%.

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*Corresponding author. Tel: +4314040026150; fax: +4314040023320.
E-mail address: shahrokh.shariat@meduniwien.ac.at (S.F. Shariat).

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Conclusion: uPA, uPAR, and PAI-1 are overexpressed in one-third to half of patients with NMIBC. Their overexpression is an independent prognosticator of RFS and PFS which improved the predictive accuracy of current clinicopathological characteristics. Biomarkers that capture the biological and clinical behavior of individual tumors may help personalize clinical decision-making in patients with NMIBC.

Keywords: Nonmuscle invasive bladder cancer; Progression; Recurrence; Urokinase plasminogen activator

1. Introduction

Nonmuscle invasive bladder cancer (NMIBC) represents a highly heterogeneous disease with varying biological behavior and clinical prognosis [1]. Despite adequate treatment, approximately 40% to 50% of NMIBC patients experience disease recurrence and 10% to 20% experience disease progression to muscle invasive bladder cancer (MIBC) [1–3]. Current prediction models based on standard clinicopathological features have an insufficient accuracy to allow accurate clinical decision-making for each patient [2,4–6]. Molecular biomarkers that capture the biological and clinical potential of each cancer could help improve the predictive accuracy of current prognostic tools [7]. The urokinase plasminogen activator (uPA) system regulates extracellular matrix degradation which induces tumor progression and metastasis by activating the protease plasmin [8]. The uPA system, which consists uPA, its receptor (uPAR), and its inhibitor type 1 (PAI-1) and type 2, is associated with tumor aggressiveness in various cancers such as breast and prostate cancers [9–11]. In bladder cancer (BCa), preoperative plasma uPA level was shown to be independently associated with poor oncologic outcomes after radical cystectomy [12]. To date, to our knowledge, there is no study evaluating the association of the expression of the uPA system with oncologic outcomes in NMIBC patients.

We hypothesized that the expression of markers of the uPA system is associated with disease recurrence and progression in NMIBC patients. To test this hypothesis, we assessed the expression of uPA, uPAR, and PAI-1 in a large multi-institutional cohort of NMIBC patient treated with TURB with or without adjuvant therapy.

2. Material and methods

2.1. Patient population and management

This was a retrospective multi-institutional study that included 827 patients with NMIBC. No patients had upper tract cancer, prostatic involvement, or preoperatively distant metastasis. All tumors were predominantly urothelial carcinoma. Institutional review board and data-sharing agreements were obtained. All patients underwent TURB for NMIBC according to guideline recommendations at the time. All TURB specimens were staged based on the 2009 TNM classification. Tumor grade was coded according to the 1973 World Health Organization system.

2.2. Immunohistochemistry measurement

We performed uPA, uPAR, and PAI-1 immunohistochemical staining using serial cuts from the same paraffin embedded tissue blocks. Murine IgG1 monoclonal antibodies against uPA (dilution 1:100), uPAR (dilution 1:100), and PAI-1 (dilution 1:50) were utilized (American Diagnostica, Greenwich, CT). Immunostaining was performed by the Dako Autostainer (Carpinteria, CA). Reagents were utilized as supplied in the Envision plus Detection Kit (Carpinteria, CA). Optimum primary antibody dilutions were predetermined using known positive control tissues. Tumor sections with the primary antibodies substituted with rabbit immunoglobulin fraction (normal) or IgG1 monoclonal were utilized as negative controls.

We used bright-field microscopy imaging coupled with advanced color detection software (Automated Cellular Imaging System, ChromaVision Medical Systems Inc., San Juan Capistrano, CA) to detect, classify, and count stained cellular objects based on predetermined color morphology. The status of all markers were categorized into normal vs. overexpressed with the uPA, PAI-1, and uPAR cut-offs of 30%, 30% and 50% immunoreactivity, respectively. The choice of cut-offs were based on preliminary

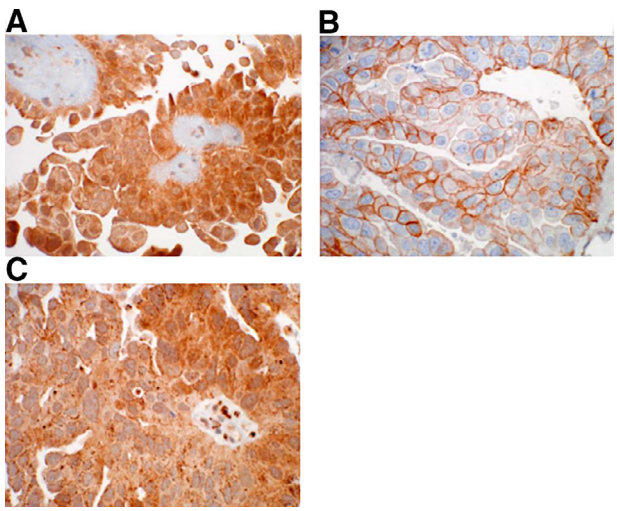

Fig. 1. Immunohistochemical staining of (A) uPA, (B) uPAR, and (C) PAI-1 expression in nonmuscle invasive bladder cancer (× 400 magnification).

Table 1
Association of uPA, uPAR, and PAI-1 expression with clinicopathological characteristics of 827 patients with nonmuscle invasive bladder cancer treated with transurethral resection of the bladder

	uPA			uPAR			PAI-1			Combined (uPA+uPAR+PAI-1)		
	Over expressed (%)	Normal (%)	*P*	Over expressed (%)	Normal (%)	*P*	Over expressed (%)	Normal (%)	*P*	All over expressed (%)	At least one normal (%)	*P*
Median age years (IQR)	68 (59.5–75)	66 (58–74)	0.3	68 (60–75)	66 (58–74)	0.08	68 (58–75)	67 (58–74)	0.3	68 (61–75)	67 (58–74)	0.7
Gender, *n* (%)			0.4			0.487			0.033			0.9
Female	74 (40.4)	109 (59.6)		86 (47.0)	97 (53.0)		69 (37.7)	114 (62.3)		15 (8.2)	168 (91.8)	
Male	238 (37.0)	406 (63.0)		284 (44.1)	360 (55.9)		300 (46.6)	344 (53.4)		52 (8.1)	592 (91.9)	
Pathological T stage, *n* (%)			0.1			0.095			0.2			0.4
pTa	165 (35.6)	298 (64.4)		193 (41.7)	270 (58.3)		196 (42.3)	267 (57.7)		37 (8.0)	426 (92.0)	
pTis	4 (22.2)	14 (77.8)		7 (38.9)	11 (61.1)		6 (33.3)	12 (66.7)		0 (0)	18 (100)	
pT1	143 (41.3)	203 (58.7)		170 (49.1)	176 (50.9)		167 (48.3)	179 (51.7)		30 (8.7)	316 (91.3)	
Pathological grade, *n* (%)			0.016			0.004			0.001			0.000
Grade1	57 (29.2)	138 (70.8)		67 (34.4)	128 (65.6)		64 (32.8)	131 (67.2)		2 (1.0)	193 (99.0)	
Grade2	104 (39.0)	163 (61.1)		126 (47.2)	141 (52.8)		127 (47.6)	140 (52.4)		32 (12.0)	235 (88.0)	
Grade3	151 (41.4)	214 (58.6)		177 (48.5)	188 (51.5)		178 (48.8)	187 (51.2)		33 (9.0)	332 (91.0)	
Concomitant CIS, *n* (%)	16 (36.4)	28 (63.6)	0.8	17 (38.6)	27 (61.4)	0.4	18 (40.9)	26 (59.1)	0.6	1 (2.3)	43 (97.7)	0.1
Prior recurrence, *n* (%)	55 (35.3)	101 (64.7)	0.5	70 (44.9)	86 (55.1)	0.9	65 (41.7)	91 (58.3)	0.4	12 (7.7)	144 (92.3)	0.8
Intravesical therapy, *n* (%)			0.3			0.5			0.4			0.5
Early-single instillation	77 (39.5)	118 (60.5)		80 (41.0)	115 (59.0)		79 (40.5)	116 (59.5)		11 (5.6)	184 (94.4)	
Adjuvant chemotherapy	8 (36.4)	14 (63.6)		12 (54.6)	10 (45.5)		12 (54.6)	10 (45.4)		2 (9.1)	20 (90.9)	
Adjuvant BCG	41 (30.4)	94 (69.3)		63 (46.7)	72 (53.3)		58 (43.0)	77 (57.0)		10 (7.4)	125 (92.6)	
Tumor size, *n* (%)			0.1			0.061			0.2			0.083
<1 cm	105 (33.7)	207 (66.4)		132 (42.3)	180 (57.7)		137 (43.9)	175 (56.1)		20 (6.4)	292 (93.6)	
1–3 cm	142 (39.3)	219 (60.7)		156 (43.2)	205 (56.8)		153 (42.4)	208 (57.6)		28 (7.8)	333 (92.2)	
≥3 cm	65 (42.2)	89 (57.8)		82 (53.2)	72 (46.8)		79 (51.3)	75 (48.7)		19 (12.3)	135 (87.7)	
Number of tumors, *n* (%)			0.4			0.04			0.4			0.5
Single	213 (39.4)	327 (60.6)		233 (43.1)	307 (56.9)		233 (43.1)	307 (56.9)		48 (8.9)	492 (91.1)	
2–7	69 (34.5)	131 (65.5)		104 (52.0)	96 (48.0)		98 (49.0)	102 (51.0)		14 (7.0)	186 (93.0)	
≥8	30 (34.5)	57 (65.5)		33 (37.9)	54 (62.1)		38 (43.7)	49 (56.3)		5 (5.7)	82 (94.3)	
Smoking, *n* (%)			0.6			0.5			0.6			0.9
Nonsmoker	79 (39.1)	123 (60.9)		86 (42.6)	116 (57.4)		87 (43.1)	115 (56.9)		17 (8.4)	185 (91.6)	
Ever smoker	233 (37.3)	392 (62.7)		284 (45.4)	341 (54.6)		282 (45.1)	343 (54.9)		50 (8.0)	575 (92.0)	

CIS = carcinoma in situ; IQR = interquartile range; PAI-1 = plasminogen activator inhibitor-1; uPA = urokinase plasminogen activator; uPAR = urokinase plasminogen activator receptor.

serial Kaplan-Meier analyses revealing them to be the optimal ones for the endpoints of recurrence-free survival (RFS) (data not shown).

2.3. Follow-up

Due to the retrospective design, follow-up schedule was not standardized. Patient received clinical and radiological follow-up based on final pathology, guidelines at the time and physician discretion [1]. Generally, patients underwent physical examination, urine cytology and cystoscopy every 3 months within the first 2 years, every 6 months from the third to the fifth year and then annually. In case of suspicious lesions in the cystoscopy, patients underwent re-biopsy. If urine cytology was positive but cystoscopy was unremarkable, bladder and prostatic urethra biopsies in addition to upper urinary tract examination were performed. Disease recurrence was defined as first tumor relapse of any stage or grade in the bladder. Disease progression was defined as muscle invasive BCa relapse. Tumor recurrence in the upper urinary tract was not considered tumor recurrence but rather as a second primary tumor. Cause of death was attributed through chart or death records reviews [13].

2.4. Statistical analysis

The Mann-Whitney test and chi-square test were used to evaluate the statistical significance of differences in medians and proportions, respectively. Follow-up time was calculated from the date of TURB. Patients still alive were censored at the date of their last follow-up. Kaplan-Meier curves were applied to assess the correlation between the uPA system and RFS and progression-free survival (PFS). The log-rank test was used to evaluate the statistical difference in RFS and PFS according to the uPA system status. Univariable and multivariable Cox proportional hazard analyses were performed to evaluate the association of the uPA system with RFS and PFS after adjusting for the effects of confounders. Discrimination was evaluated using Harrell's concordance index [14]. In subgroup analyses, we investigated the RFS and PFS in patients with T1G3 and TaG1-2. Statistical significance was set at $P < 0.05$ and all tests were two-sided. All statistical analyses were performed using STATA v.14.1 (StataCorp LP, College Station, TX).

3. Result

3.1. Association of the uPA system with patients characteristics

The overexpression of uPA, uPAR, and PAI-1 were found in 312 (37.7%), 370 (44.7%), and 369 (44.6%) patients, respectively. Representative immunohistochemical stainings of all 3 markers are shown in Fig. 1. Overexpression of 1 of the 3 markers, 2 of the 3 and all 3 were seen in 360 (43.5%), 245 (29.6%), and 67 (8.1%) patients, respectively. Overall, 672 (81.3%) patients exhibited overexpression of at least 1 marker. Association of the uPA system with the clinicopathological features of the 827 NMIBC patients is shown in Table 1. Overexpression of uPA, uPAR, PAI-1 and the combination of all 3 markers were associated with tumor grade.

3.2. Association of the uPA system with disease recurrence and progression

Within a median follow-up of 60 months (interquartile range [IQR]: 22–109), 280 (33.9%) patients experienced disease recurrence and 71 (8.9%) patients experienced disease progression. The median time to disease recurrence was 24.9 months (IQR: 7–64) and the median time to disease progression was 55 months (IQR: 20-98). The 5-year RFS rates for patients with none of the markers overexpressed, one overexpressed, 2 overexpressed and all 3 overexpressed were 81.6% ± 3.4% (SE), 65.3% ± 2.9% (SE), 53.2% ± 3.8% (SE) and 30.0% ± 6.6% (SE), respectively (Fig. 2A). The 5-year PFS rates for patients with none of the markers overexpressed, one overexpressed, 2 overexpressed and all

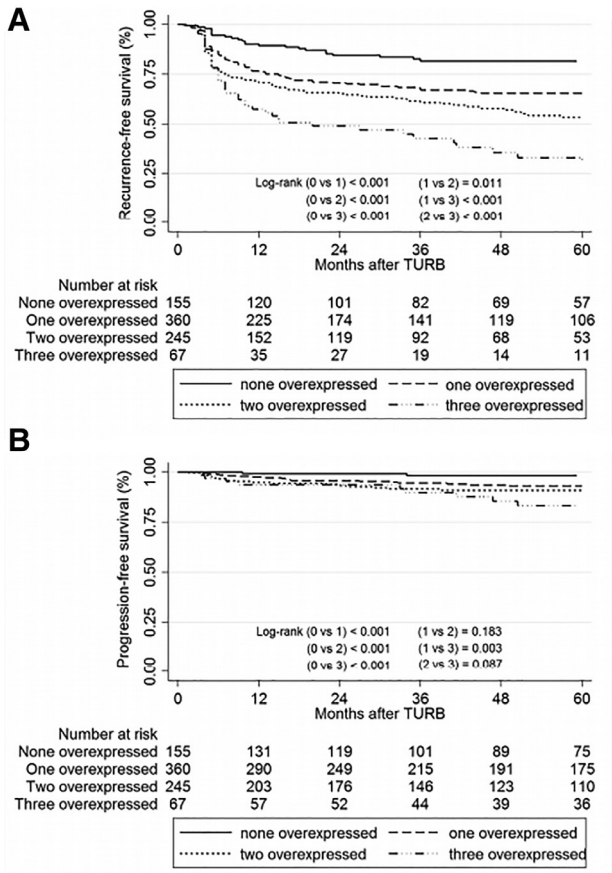

Fig. 2. Kaplan-Meier estimates for (A) recurrence-free survival and (B) progression-free survival according to the urokinase plasminogen activator system status in 827 patients with nonmuscle invasive bladder cancer treated with transurethral resection of the bladder.

3 overexpressed were 98.3% ± 1.2% (SE), 93.1% ± 1.6% (SE), 91.0% ± 2.0% (SE) and 83.2% ± 5.2% (SE), respectively (Fig. 2B).

On multivariable analyses, uPA (HR: 1.40; $P = 0.006$), uPAR (HR: 1.70; $P < 0.001$), PAI-1 (HR: 1.35; $P = 0.014$) and the combination of all 3 markers (HR: 3.38; $P < 0.001$) were independently associated with RFS (Table 2). uPA (HR: 1.68; $P = 0.035$) and the combination of all 3 markers (HR: 8.79; $P = 0.005$) were also independently associated with PFS (Table 3). The addition of the uPA system to a base prediction model of disease recurrence comprising patient age, pathological T-stage, tumor grade, tumor size, number of tumors, prior recurrence history, and intravesical therapy improved its discrimination from 73.0% to 74.3%. It further improved the models discrimination for disease progression from 77.6% to 79.7%. Overexpression of uPAR and the combination of all 3 markers were associated with cancer-specific survival but no markers was associated with overall survival on multivariable analyses (data not shown).

3.3. Association of the uPA system with disease recurrence and progression in T1G3 patients

A total of 346 (41.8%) patients had T1G3 tumor. Within a median follow-up of 43 months (IQR: 14−85), 88 (25.4%) of these patients experienced disease recurrence and 40 (11.6%) experienced disease progression. The 5-year RFS rates for patients with none of the markers overexpressed, 1 overexpression, 2 overexpression and all 3 overexpression were 94.2% ± 3.3% (SE), 78.5% ± 4.0% (SE), 61.7% ± 4.9% (SE) and 51.3% ± 11.5% (SE), respectively (Fig. 3A). The 5-year PFS rates for patients with none of the markers overexpressed, one overexpressed, 2 overexpressed and all 3 overexpressed were 97.9% ± 2.1% (SE), 88.6% ± 3.3% (SE), 85.4% ± 3.5% (SE) and 77.5% ± 9.1% (SE), respectively (Fig. 3B).

On multivariable analyses, uPA (HR: 1.64; $P = 0.022$), uPAR (HR: 2.00; $P = 0.002$), and the combination of all 3 markers (HR: 6.12; $P = 0.006$) were independently associated with RFS. uPA (HR: 2.19; $P = 0.018$) was also independently associated with PFS (Table 4). The addition of the uPA system to a base prediction model for disease recurrence comprising patient age, tumor size, number of tumors and intravesical therapy improved its discrimination from 72.6% to 75.7%. The addition of the uPA system to a base prediction model for disease progression comprising patient age, tumor size, number of tumors and prior recurrence history improved its discrimination from 76.0% to 78.5%.

Table 2
Univariable and multivariable Cox regression analyses for prediction of recurrence-free survival in 827 patients treated with transurethral resection of the bladder

	Univariable			Multivariable[a]		
	HR	95%CI	*P*	HR	95%CI	*P*
Age	1.03	1.02−1.04	<0.001	1.02	1.01−1.03	<0.001
Female (ref. male)	1.01	0.76−1.34	0.936	-	-	-
Pathological T stage (ref. pTa)						
pTis	0.72	0.30−1.75	0.5	1.53	0.60−3.94	0.4
pT1	0.59	0.46−0.76	<0.001	0.33	0.17−0.61	<0.001
Pathological grade (ref. Grade 1)						
Grade 2	2.41	1.73−3.36	<0.001	1.68	1.19−2.37	0.003
Grade 3	1.15	0.82−1.63	0.4	2.92	1.49−5.71	0.002
Concomitant CIS	0.84	0.47−1.50	0.6	-	-	-
Tumor size (ref. <3 cm)						
≥3 cm	3.05	2.37−3.92	<0.001	2.23	1.70−2.92	<0.001
Number of tumors (ref. single)						
2−7	1.51	1.15−1.97	0.003	1.65	1.24−2.19	0.001
≥8	0.99	0.65−1.50	0.96	1.20	0.77−1.84	0.4
Prior recurrence history	0.56	0.39−0.79	0.001	0.75	0.51−1.09	0.1
Intravesical therapy	0.36	0.27−0.48	<0.001	0.42	0.31−0.58	<0.001
Smoking status (ref. nonsmoker)						
Ever-smoker	1.19	0.90−1.56	0.2	-	-	-
Harrell's C index				0.7297		
uPA	1.60	1.26−2.02	<0.001	1.40	1.10−1.78	0.006
uPAR	1.85	1.46−2.34	<0.001	1.70	1.33−2.17	<0.001
PAI-1	1.47	1.26−1.86	0.001	1.35	1.06−1.71	0.014
uPA+uPAR+PAI-1	4.90	3.00−8.02	<0.001	3.38	2.04−5.60	<0.001
Harrell's C index				0.7431		

[a] Adjusted for age, pathological T stage, pathological grade, tumor size, number of tumors, prior recurrence history, intravesical therapy.CI = confidence interval; CIS = carcinoma in situ; HR = Hazard ratio; PAI-1 = plasminogen activator inhibitor-1, uPA = urokinase plasminogen activator; uPAR; urokinase plasminogen activator receptor.

Table 3
Univariable and multivariable Cox regression analyses for prediction of progression-free survival in 827 patients treated with transurethral resection of the bladder

	Univariable			Multivariable[a]		
	HR	95%CI	*P*	HR	95%CI	*P*
Age	1.04	1.02−1.06	<0.001	1.04	1.01−1.06	0.002
Female (ref. male)	0.77	0.42−1.41	0.4	-	-	-
Pathological T stage (ref. pTa)						
pTis	3.39	0.80−14.3	0.1	1.97	0.41−9.51	0.4
pT1	2.33	1.44−3.77	0.001	0.72	0.27−1.89	0.5
Pathological grade (ref. Grade 1)						
Grade 2	16.7	2.26−123.4	0.006	11.47	1.53−85.9	0.018
Grade 3	29.4	4.05−213.2	0.001	25.65	2.91−226.4	0.003
Concomitant CIS	1.88	0.75−4.68	0.2	-	-	-
Tumor size (ref. <3 cm)						
≥3 cm	2.21	1.36−3.60	0.001	1.87	1.09−3.21	0.023
Number of tumors (ref. single)						
2−7	1.51	1.15−1.97	0.003	1.39	0.80−2.44	0.2
≥8	0.99	0.65−1.50	0.96	2.15	1.11−4.15	0.023
Prior recurrence history	2.19	1.30−3.70	0.003	2.23	1.21−4.15	0.010
Intravesical therapy	1.15	0.71−1.85	0.6	-	-	-
Smoking status (ref. nonsmoker)						
Ever-smoker	1.68	0.90−3.12	0.1	-	-	-
Harrell's C index				0.7764		
uPA	1.95	1.22−3.11	0.005	1.68	1.04−2.74	0.035
uPAR	1.97	1.22−3.17	0.005	1.48	0.90−2.41	0.1
PAI-1	1.82	1.13−2.92	0.013	1.45	0.90−2.34	0.1
uPA+uPAR+PAI-1	15.9	3.61−70.0	<0.001	8.79	1.96−39.4	0.005
Harrell's C index				0.7973		

[a] Adjusted for age, pathological T stage, pathological grade, tumor size, number of tumors, prior recurrence history.CI = confidence interval; CIS = carcinoma in situ; HR = Hazard ratio; PAI-1 = plasminogen activator inhibitor-1uPA = urokinase plasminogen activator; uPAR; urokinase plasminogen activator receptor.

3.4. Association of the uPA system with disease recurrence and progression in TaG1-2 patients

A total of 447 (54.1%) patients had TaG1-2 tumor. Within a median follow-up of 76 months (IQR: 29-134), 177 (39.6%) patients experienced disease recurrence and 25 (5.6%) patients experienced disease progression. The 5-year RFS rates for patients with none of the markers overexpressed, one overexpressed, 2 overexpressed and all 3 overexpressed were 75.6% ± 4.9% (SE), 57.9% ± 3.9% (SE), 43.7% ± 5.8% (SE) and 11.3% ± 6.8% (SE), respectively (Fig. 4A). The 5-year PFS rates for patients with none of the markers overexpressed, one overexpressed, 2 overexpressed and all 3 overexpressed were 100%, 96.2% ± 1.5% (SE), 98.0% ± 1.4% (SE) and 90.2% ± 5.4% (SE), respectively (Fig. 4B).

On multivariable analyses, uPA (HR: 1.44; $P = 0.019$), uPAR (HR: 1.54; $P = 0.006$), PAI-1 (HR: 1.46; $P = 0.013$) and the combination of all 3 markers (HR: 3.48; $P < 0.001$) were independently associated with RFS (Table 4). The addition of the uPA system to a base prediction model for disease recurrence comprising patient age, tumor size, number of tumors, prior recurrence history and intravesical therapy improved its discrimination from 72.3% to 74.4%. A total of 16 (1.9%) patients had TaG3 tumor. No marker was associated with disease recurrence and progression on univariable analyses (Supplementary table).

4. Discussion

NMIBC is a highly heterogeneous disease with varying biological and clinical behavior [15]. The EAU guidelines recommend to stratify NMIBC population into the risks of disease recurrence and progression according to clinicopathological features [1]. In this multi-institutional study, we investigated biomarkers related to the biological and clinical behavior of NMIBC patients treated with TURB with or without adjuvant intravesical therapy. We found that overexpression of the uPA system was associated with higher tumor grade. In our study, grade 3 tumor was more overexpressed than grade 1 in all 3 markers; risk ratios were uPA (1.19, 95% confidence interval [CI] 1.06−1.34, $P = 0.005$), uPAR (1.22, 95% CI 1.08−1.37, $P = 0.001$), PAI-1 (1.25, 95% CI 1.11−1.41, $P = 0.0003$) and the combination of all 3 markers (1.49, 95% CI 1.34−1.66, $P = 0.0002$). Tumor grade has been unanimously associated with disease recurrence and progression in NMIBC. Therefore, overexpression of the uPA system might predict disease recurrence and progression which are more important endpoints in NMIBC patients.

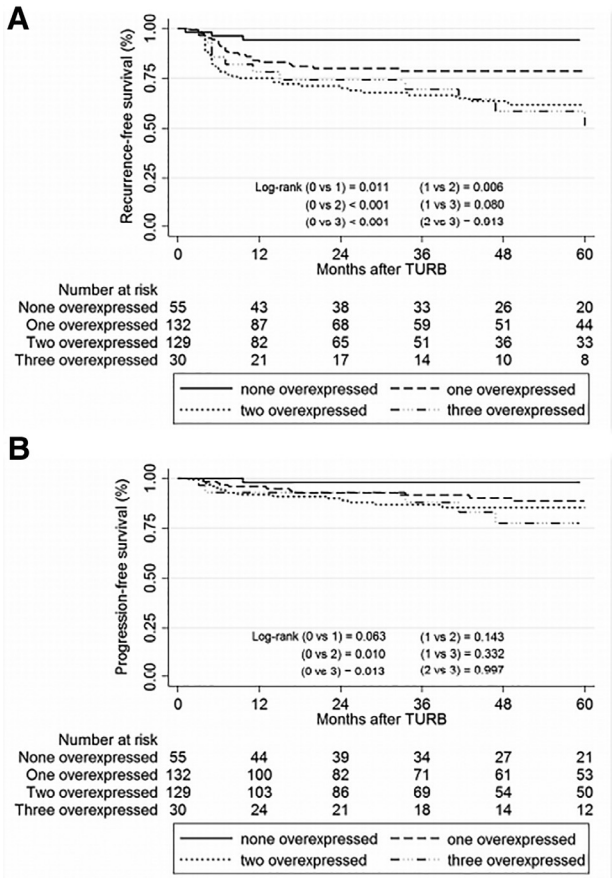

Fig. 3. Kaplan-Meier estimates for (A) recurrence-free survival and (B) progression-free survival according to the urokinase plasminogen activator system status in 346 patients treated with transurethral resection of the bladder for pathological T1 and Grade3 non-muscle invasive bladder cancer.

NMIBC patients experienced high rates of disease recurrence and progression which compel a rigorous surveillance protocol [16]. Predicting disease recurrence and progression is important to identify the patients most likely to benefit from intensified therapy such as adjuvant chemo- and/or immunotherapy as well as early radical cystectomy [17,18]. We found that all 3 markers were independently associated with RFS and that uPA and the combination of all 3 markers were independently associated with PFS. The addition of the uPA system to a base model for disease recurrence and progression improved its discrimination of the latter by 1.3% and 2.1%, respectively. The European Organization for Research and Treatment of Cancer and the Spanish Urological Club for Oncological Treatment developed risk models to help clinicians and their patients in the clinical decision-making [2,19]. These tools are based on established clinicopathological features; however, the accuracy of these models remains unsatisfactory [4−6,20]. Their performance could potentially be complemented by adding factors such as molecular markers (i.e., the uPA system) [7,20].

The main function of the uPA system was thought to be limited to the degradation of extracellular matrix and basement membrane required for local invasion and spread to distant metastasis of malignant cells [21]. However, it has become clear that the uPA system affects multiple aspects of the acquisition of functional competences including self-sufficiency in growth signals, insensitivity to antigrowth signals, limitless replicative potential, sustained angiogenesis, and tumor invasion and metastasis [21,22]. Actually, in breast cancer, high level of evidence supports that uPA and PAI-1 are clinically useful biomarkers for assessing prognosis in

Table 4
Multivariable Cox regression analyses for prediction of recurrence-free survival and progression-free survival in 346 patients treated with transurethral resection of the bladder (TURB) for pathological T1 and Grade 3 and in 447 patients treated with TURB for pathological Ta and Grade 1 and 2 nonmuscle invasive bladder cancer

	RFS[a]			PFS[b]		
T1G3	HR	95%CI	*P*	HR	95%CI	*P*
uPA	1.64	1.07−2.51	0.022	2.19	1.15−4.18	0.018
uPAR	2.00	1.29−3.12	0.002	-	-	-
PAI-1	1.29	0.84−2.00	0.2	-	-	-
uPA+uPAR+PAI-1	6.12	1.70−22.1	0.006	7.60	0.88−65.8	0.065
Harrell's C index		0.7567 (base model: 0.7256)			0.7853 (base model: 0.7596)	
	RFS[c]			PFS[d]		
TaG1-2	HR	95%CI	*P*	HR	95%CI	*P*
uPA	1.44	1.06−1.94	0.019	-	-	-
uPAR	1.54	1.13−2.09	0.006	-	-	-
PAI-1	1.46	1.08−1.97	0.013	-	-	-
uPA+uPAR+PAI-1	3.48	1.93−6.27	<0.001	-	-	-
Harrell's C index		0.7438 (base model: 0.723)		-		

[a] Adjusted for age, tumor size, number of tumors and intravesical therapy.
[b] Adjusted for age, tumor size, number of tumors and prior recurrence history.
[c] Adjusted for age, tumor size, number of tumors, prior recurrence history and intravesical therapy.
[d] Not performed.CI = confidence interval; HR = hazard ratio; PFS = progression-free survival; RFS = recurrence-free survival; PAI-1 = plasminogen activator inhibitor-1; uPA = urokinase plasminogen activator; uPAR; urokinase plasminogen activator receptor.

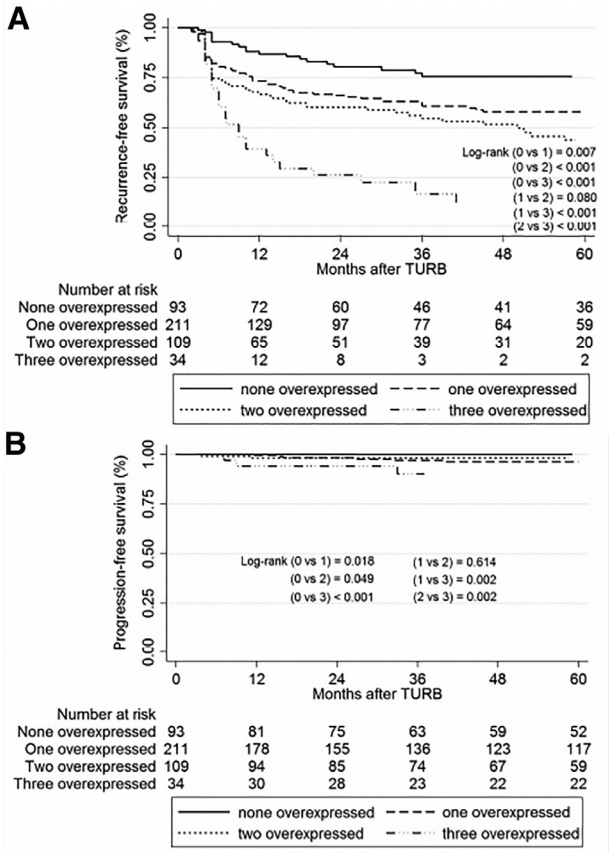

Fig. 4. Kaplan-Meier estimates for (A) recurrence free survival and (B) progression free survival according to urokinase plasminogen activator system status in 447 patients treated with transurethral resection of the bladder for pathological Ta and Grade1-2 nonmuscle invasive bladder cancer.

early disease; these markers help identify high-risk patients most likely to benefit from adjuvant chemotherapy [23,24]. In BCa, previous studies reported on the utility of the uPA system as a clinical biomarker [12,25–27]. For example, Seddinghzadeh et al. [26] demonstrated that the mRNA levels of uPA and uPAR were associated with cancer-specific death in 194 muscle invasive BCa patients. However, only few studies investigated the prognostic value of the uPA system in NMIBC.

Hasui et al. [28] reported that uPA overexpression in NMIBC tissue was associated with disease progression and death in 52 NMIBC patients; uPA was, indeed, overexpressed in 32.7% of patients. To our knowledge, our study is the first study reporting the association of the uPA system with disease recurrence in NMIBC. Our results support the cumulative evidence that the uPA system was associated with oncologic outcomes in BCa. Furthermore, we performed subgroup analyses in T1G3 and TaG1-2 patients. We found uPA was independently associated with both RFS and PFS in T1G3 patients and its addition to a base model for PFS improved the discrimination of the latter by 2.5%. All 3 markers were also independently associated with RFS in TaG1-2 patients and their addition to a base model for RFS improved the discrimination of the latter by 2.1%. The tissue expression of the uPA system can thus identify NMIBC patients who are most likely to experience disease recurrence and progression despite adequate local therapy. The improvement in discrimination beyond that obtained by standard clinicopathological features suggests that clinicians could improve their clinical decision-making regarding intravesical chemotherapy, BCG immunotherapy or early radical cystectomy by using the information afforded by these markers. However, the number of patients in overexpression of the combination of all 3 markers was small, it is difficult to unequivocally conclude that the combination of all 3 markers provides a more accurate prediction of disease recurrence and progression compared to a single biomarker. In the future, we should evaluate the prognostic value of the uPA system in a larger prospective study.

Our study is limited by its retrospective and multi-institutional design. We could not control for surgeon and pathologic variances, differences of follow-up scheduling. Additionally, we could not account for the type and duration of intravesical therapy due to the heterogeneity of the maintenance therapy scheduling among multi-centers. TURB specimens were not evaluated by central pathological review. In addition, the pathological information such as lymphovascular invasion and variant histology were not available in our analyses [29,30]. Moreover, we could not account for disease progression in TaG3 or T1 cohorts treated with early cystectomy which might affect our results in this study. Our results should be interpreted with caution, because we could not exclude the patients treated with early cystectomy. Immunohistochemistry has inherent limitations such as reproducibility and reliability; however, we used an autostainer and a standardized, automated scoring system with advanced color detection software to minimize variation. Despite these limitations, the large sample size is an important strength of our study that distinguishes from previous studies and increases the generalizability of our results.

5. Conclusions

The uPA system was overexpressed in approximately 80% of NMIBC patients. Overexpression of the uPA system was independently associated with disease recurrence and overexpression of uPA and the combination of all 3 markers were independently associated with disease progression in NMIBC patients treated with TURB with or without adjuvant intravesical therapy. Biomarkers that improve the accuracy of current predicting tools for disease recurrence and progression can inform clinical decision-making for individual patient.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

None.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2019.05.019.

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