Urinary Tumor Markers Could Predict Survival in Bladder Carcinoma

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Abstract The early diagnosis of bladder cancer is important for effective treatment of the disease. This study aimed to evaluate the nuclear matrix protein 22 (NMP 22), soluble epithelial cadherin (E-cadherin), cathepsin-D and total protein with clinico-pathological features of bladder cancer, and to determine the relation between each marker and tumor progression after treatment. The study includes 65 patients with bladder cancer, 14 benign urinary diseases and 11 healthy volunteers. Patients were categorized according to bilharzial infestation, T stage, tumor grade, size and the presence of lymph node metastasis. Forty patients were followed for disease progression after surgery. There was a significant increase of NMP22, E-cadherin, cathepsin-D and total protein detected in cancer group compared to healthy and benign groups. It was found that NMP 22 and E-cadherin had highest sensitivity (84.4, 76.9 %, respectively) while, total protein showed highest specificity (77.4 %). Tumor size correlated with urinary NMP22 ($r = 0.3$, $p = 0.02$), although, E-cadherin, cathepsin-D and total protein correlated with tumor size ($r = 0.3$, 0.28 , 0.2 ; $p = 0.01$, $p = 0.01$, 0.04 , respectively) and lymph node metastasis ($r = 0.32$, 0.34 , 0.2 ; $p = 0.003$, 0.005 , 0.04 , respectively). Elevated pretreatment urinary

NMP22, E-cadherin and total protein levels was associated significantly with bladder cancer recurrence ($p = 0.02$, 0.001 , 0.005 , respectively). In conclusion, determination of urinary NMP22, E-cadherin and total protein in bladder cancer patients or persons at risk of developing bladder cancer will help in early detection of the disease and prediction of recurrence. The use of a combination of tumor markers is markedly useful than the assessment of single one.

Keywords NMP22 · Cadherin · Cathepsin D · Total protein · Bladder carcinoma

Introduction

Carcinoma of the bladder is the most prevalent cancer in Egypt and in most African countries [1]. The two major types of bladder cancer in Egypt are bilharzial and non bilharzial. The non bilharzial type is similar to the western type but the bilharzial type is commonly squamous cell carcinoma. The continuous need for developing non-invasive methods for early diagnosis and monitoring of bladder cancer resulted in various assays that determine soluble markers in voided urine specimen [2].

The nuclear matrix (NM) contains proteins including enzymes and transcription factors that contribute to the preservation of nuclear shape and organization [3]. The United States Food and Drug Administration had been approved nuclear matrix protein NMP (NMP22) for the detection or rapidly recurring bladder tumor [4]. Cadherin had an important role in molecular pathogenesis of bladder cancer and may be responsible for development of muscle invasion and metastasis. Promoter hypermethylation had been identified as an important epigenetic event associated

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with the loss of E-cadherin gene expression during cancer progression [5]. Thus, cadherin cell adhesion molecules represent biological and molecular targets for preventing disease progression [6]. Immuno-histochemical studies have indicated that cathepsin-D, independently of its proteolytic activity, stimulates not only cancer cell proliferation, but also tumor angiogenesis [7]. Tumors arising from growth alterations of cells in the epithelium lining the bladder are expected to excrete proteins and desquamate fragments of membrane into the bladder cavity where they mix with the urine. The proteinuria in bladder cancer patients could be caused by a decreased tubular reabsorption of proteins [8]. In addition, the urine of bladder cancer patient may also contain degradation products of the extracellular matrix (ECM) derived from the proteolytic activity of invasive tumors [9].

This study evaluates the urinary levels of NMP 22, soluble E-cadherin, cathepsin D and total protein in primary bladder cancer.

Patients and Methods

This study is a retrospective case-controlled, hospital based study. It included 90 subjects in the period from May, 2006 to May, 2009 in the South Egypt Cancer Institute and urology department, faculty of medicine, Assiut University, Egypt. The study was approved by the local institutional review board and informed consent was obtained from each subject. The study comprised 65 patients who had primary bladder cancer (clinically and radiologically) admitted for pathological and surgical evaluation, 14 patients with noncancerous urological conditions (benign prostate hyperplasia, urinary tract infection or urolithiasis), and 11 healthy subjects. Patients with other genitourinary malignancies, hepatic insufficiency or renal failure were excluded from the study.

Voided urine samples were collected before cystoscopic evaluation in all patients, centrifuged at $1,000 \times g$ for 15 min and the supernatant aliquotted and stored at -70°C until assayed. Nuclear matrix protein 22 was determined by kit from Matritech, MATRITECH Inc., Newton, USA. Determination of NMP22 based on enzyme immunoassay (EIA) for the in vitro quantitative determination of the nuclear mitotic apparatus protein (NuMA) in stabilized voided urine. The assay employs two monoclonal antibodies raised against NuMA. Quantitative sandwich enzyme immunoassay technique was used for determination of E-cadherin in urine by Quantikine, R&D systems Inc., Minneapolis USA. A monoclonal antibody specific for E-cadherin has been pre-coated onto a microplate for the quantitative determination of soluble human epithelial cadherin (sE-cadherin) concentrations in urine. Cathepsin-D activity was determined by the

following chemical method. Briefly, 0.05 ml of serum or blank was added to 0.05 ml of 0.1 M sodium formate, 0.15 ml of water and 0.05 ml of hemoglobin substrate 1 %, and then incubated at 45°C for 60 min. The reaction was terminated by adding 1 ml of 3 % w/v trichloroacetic acid. The absorbance difference was measured at 280 nm. [10]. Urinary total protein and creatinine determined by commercial kit, Stan Bio, Germany.

Patients categorized into subgroups according to the following: Bilharzial or non-bilharzial cancer, pathological type (squamous, transitional and adenocarcinoma), tumor stage, tumor grade, tumor size and state of node metastasis. Forty patients were followed for disease progression for 3 years after cystectomy and transurethral resection or until local recurrence or distant metastasis had occurred.

Statistical Analysis

Statistical Package for Social Science (SPSS) program for windows, version 16 was used for data analysis. Data is expressed as mean $\pm$ SE, corrected for mg creatinine. Mann–Whitney *U* test used to compare the means of two groups of cases on one variable with $p \leq 0.05$ considered statistically significant. Correlation between variables was performed using by Pearson correlation coefficients and a two-tailed test of significance. Receiver operating characteristics (ROC) curves calculating the true positive fraction (sensitivity) and false-positive fraction (100-specificity) of markers at several cutoff points [11]. Sensitivity (percent positive cases of the 65 malignant cases) and specificity (percent negative cases of the control group including benign and normal controls) were calculated. The Kaplan–Meir test detects the recurrence-free interval in relation to the level of urinary markers (it was defined as time from surgery until the documented time of local recurrence or distant metastasis).

Results

The study population characteristics are shown in Table 1. NMP22, E-cadherin, cathepsin D and total protein levels were highest in the cancer group followed by benign group then the healthy control group (Table 2). NMP22 showed a significant difference between cancer group and the benign and health group ($p = 0.01$), but, no significant difference was found with E-cadherin, cathepsin-D or total protein in this group. A significant difference was observed between the benign and the healthy groups in NMP22 ($p = 0.007$), E-cadherin ($p = 0.003$), cathepsin-D ($p = 0.005$) and total protein ($p = 0.000$). No significant difference was found in any of the studied markers between the different

pathological types, bilharzial and non-bilharzial cancer, different T stages, grades, sizes or lymph node involvement, except in E-cadherin and cathepsin-D where there was a significant difference in the presence of lymph node metastasis (Table 2). The ROC curves detect the sensitivity and specificity of the studied urinary markers in all patients groups and these area demonstrated in Table 3 and Figs. 1, 2, 3 and 4.

Elevated pretreatment urinary NMP22, E-cadherin and total protein were associated significantly with bladder cancer recurrence ($p = 0.02$, 0.001 , 0.005 , respectively) (Figs. 5, 6 and 7). However, elevated pretreatment urinary cathepsin-D was not associate with bladder cancer recurrence ($p = 0.3$) (Fig. 8). There were positive weak correlations between different tumor variables and urinary markers. Tumor size correlated with urinary NMP22 ($r = 0.3$, $p = 0.02$), E-cadherin, ($r = 0.3$, $p = 0.01$), cathepsin-D ($r = 0.28$, $p = 0.01$) and total protein ($r = 0.2$; 0.04). Lymph node metastasis correlated with E-cadherin, cathepsin-D and total protein ($r = 0.32$, 0.34 , 0.2 ; $p = 0.003$, 0.005 , 0.04 , respectively).

Table 1 Demographic data of the studied subjects

Mean $\pm$ SD	54 $\pm$ 14
Age (years)	
Maximum	88
Minimum	24
Study groups	
Normal Urinary tract	11 (12.2 %)
Bladder Cancer	65 (72.2 %)
Other urinary disease (benign lesion)	14 (15.6.8 %)
Microscopic picture of bladder cancer	
TCC	32 (49.2 %)
Squamous	29 (44.6 %)
Adenocarcinoma	4 (6.2 %)
Tumor grade	
Grade I	15 (23.1 %)
Grade II	24 (36.9 %)
Grade III	26 (40 %)
Tumor stage	
T1	11 (16.9 %)
T2	10 (15.4 %)
T3	38 (58.5 %)
T4	6 (9.2 %)
Lymph node	
N0	56 (86.1 %)
N1	9 (13.9 %)
Tumor size	
Up to 3 cm	16 (24.6 %)
3–7 cm	30 (46.2 %)
More than 7 cm	19 (29.2 %)

Discussion

The journey towards a noninvasive test for diagnosing bladder cancer, in order to replace or extend time between cystoscopy, has been ongoing for more than a decade. However, only a handful of tests that aid in clinical decision making are commercially available [12]. In this study a significant increase in NMP22 levels was detected in the malignant group and not in either benign group or healthy controls. These findings are in agreement with other authors [13, 14]. However, urinary tract infection, inflammation and malignancy associated with other genitourinary organs were the primary cause for false positive tests in the NMP22 evaluation [15, 16]. A negative result of NMP22 test in 49 % of 130 patients with biopsy confirmed bladder cancer was reported. So, the false negative rate was high and thus NMP22 test could not replace cystoscopy for diagnosis of bladder cancer [17]. In this study, the sensitivity and specificity of NMP22 was 84.4 and 59.3 % of 65 patients with biopsy confirmed bladder cancer and there was a non-significant increase in NMP22 levels in non-bilharzial than bilharzial patients. This could be attributed to lower NMP22 levels in squamous cell carcinoma group. In contrast, the sensitivity of NMP22 was in bilharzial and not in non-bilharzial bladder cancer. NMP22 positive rates in bladder squamous cell carcinoma group were higher than those in the TCC group but without a statistical difference [13]. Multivariate analysis revealed that tumor size affected the urinary NMP22 values and the positive rate by tumor size was 42.3, 59.1 and 85.0 % for tumors of <10, 10–30 and >30 m respectively [18]. The sensitivity of NMP22 was 50.0 % [19] which is in agreement with this study. Similar to our findings, several studies did not find the quantitative value of NMP22 to be useful in differentiation low from high grade and stage [20] while, other reported a significant association [21]. These differences in results could be attributed to first: the different threshold used in these studies, second: not all studies use both normal and benign urological lesions as control groups, third: most studies use the absolute marker level and did not correct the marker level to urinary creatinine level. Also, the false positive rates of NMP22 increase with age indicating that age related reference intervals should be used [22].

In this study, elevated pretreatment urinary NMP22 was significantly associated with bladder cancer recurrence was consistent with the findings of Hutterer et al. [23] who reported a better ability of a NMP22 level of ≥ 10 units/ml to predict non TCC recurrence compared to urinary cytology suggesting that NMP22 may have a role in the surveillance of patients at risk of non-TCC recurrence.

In this study, E-cadherin, cathepsin-D and total protein levels were significantly higher in cancer and benign

Table 2 Urinary NMP22, E-cadherin, cathepsin D and total protein (units/mg creatinine) in all studied groups

	NMP22	<i>p</i> value	E-cadherin	<i>p</i> value	Cathepsin D	<i>p</i> value	Total protein	<i>p</i> value
Cancer group	17.88 ± 1.00	0.000	43.23 ± 2.85	0.000	.04 ± .01	0.007	.22 ± .01	0.004
Healthy control*	8.50 ± .86		21.82 ± 4.07		.01 ± .00		.10 ± .01	
Benign control	13.20 ± 1.47		39.95 ± 4.12		.03 ± .01		.20 ± .02	
TCC*	18.53 ± 1.19	NS	42.0 ± 3.05	NS	.04 ± .01	NS	.22 ± .01	NS
Squamous	17.88 ± 1.78		45.35 ± 5.23		.03 ± .01		.22 ± .02	
Adenocarcinoma	12.69 ± 3.17		37.78 ± 10.15		.06 ± .03		.15 ± .04	
Bilharzial	17.72 ± 1.27	NS	44.56 ± 3.62	NS	.04 ± .01	NS	.22 ± .02	NS
Non-Bilharzial*	18.28 ± 1.49		39.77 ± 4.10		.04 ± .01		.23 ± .02	
Grade I*	18.55 ± 2.90	NS	46.61 ± 7.90	NS	.04 ± .01	NS	.22 ± .04	NS
Grade II	17.52 ± 1.32		40.04 ± 3.75		.05 ± .01		.20 ± .02	
Grade III	17.83 ± 1.47		44.23 ± 4.37		.03 ± .00		.23 ± .02	
T1*	19.14 ± 1.95	NS	45.57 ± 5.76	0.07	.05 ± .01	NS	0.23 ± .03	NS
T2	18.24 ± 2.51		31.19 ± 3.42		.04 ± .01		0.22 ± .03	
T3	17.37 ± 1.46		44.98 ± 4.33		.04 ± .01		0.22 ± .02	
T4	18.13 ± 2.38		47.94 ± 5.31		.03 ± .01		0.21 ± .03	
N0*	17.42 ± 1.11	NS	41.24 ± 3.16	0.007	.03 ± .01	0.01	.21 ± .01	NS
N1	20.68 ± 1.97		55.61 ± 4.25		.07 ± .02		.25 ± .02	
Up to 3 cm*	16.45 ± 1.48	NS	36.43 ± 4.58	NS	.04 ± .01	NS	.22 ± .02	NS
3–7 cm	18.41 ± 1.99		41.35 ± 4.12		.03 ± .01		.21 ± .02	
More than 7 cm	19.99 ± 1.81		52.53 ± 5.86		.06 ± .01		.24 ± .03	

* Compared to this group as done by ANOVA test or Mann–Whitney U test

Table 3 Sensitivity and specificity of the studied urinary markers in all patients groups

	NMP22		E-cadherin		Cathepsin D		Total protein	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
All cancer patient	84.4	59.3 %	76.9	50 %	43.1	64.5	50.8	77.4
T.C.C	87.5		78.1		50.0		59.4	
Squamous carcinoma	85.7		72.4		37.9		44.8	
Adenocarcinoma	50		100.0		25.0		25.0	
Bilharzial cancer	82.6		78.7		40.4		46.8	
Non-Bilharzial cancer	88.9		72.2		50.0		61.1	
Stage T1	86.4		50.0		63.6		63.6	
Stage T2	80		81.8		40.0		50.0	
Stage T3	78.4		73.7		36.8		47.4	
Stage T4	100		100		50.0		50.0	
Grade I	66.7		73.3		46.7		53.3	
Grade II	91.7		70.8		41.7		45.8	
Grade III	87.5		76.9		42.3		57.7	
N0 (no node metastasis)	81.8		69.6		35.7		46.4	
N1 (node metastasis)	100		100		88.9		77.8	

Cut off value = 10 (units/mg creatinine) for NMP22, cut off value = 26 ng/mg creatinine for E-cadherin, cut off value = 0.030 unit enzyme activity/min/mg/ml creatinine for cathepsin D, cut off value = 0.20 mg/mg creatinine for total protein

Fig. 1 ROC curve for calculating sensitivity and specificity of urinary NMP22. Area under the curve = 0.781

Fig. 2 ROC curve for calculating sensitivity and specificity of urinary E-cadherin. Area under the curve = 0.664

Fig. 3 ROC curve for calculating sensitivity and specificity of urinary cathepsin D. Area under the curve = 0.548

Fig. 4 ROC curve for calculating sensitivity and specificity of urinary total protein. Area under the curve = 0.702

Fig. 5 Kaplan–Meier survival curve testing recurrence interval in relation to NMP22 levels

Fig. 6 Kaplan–Meier survival curve testing recurrence interval in relation to E-cadherin levels

Fig. 7 Kaplan–Meier survival curve testing recurrence interval in relation to cathepsin D levels

Fig. 8 Kaplan–Meier survival curve testing recurrence interval in relation to total protein levels

groups compared to the healthy control group. This in agreement with several studies that found plasma levels of E-cadherin was significantly higher in bladder cancer patients than in healthy controls [24]. The level of urinary E-cadherin was higher in patients with TCC than normal controls or patients with benign disorders of the urinary system and correlated with tumor size and lymph node metastasis. In contrast, urinary E-cadherin levels strongly correlated with tumor grade but showed no significant correlation with the stage, size and number of the tumors. The urinary E-cadherin level was significantly higher in the recurrent group than the primary group [25]. E-cadherin found in the urine of patients with bladder cancer came from direct release of cells in the urinary epithelium, and may result from blood filtered through the kidney. From this study, E-cadherin, cathepsin-D and total protein, were significantly helpful to recognize the lesion in the urinary tract whatever bladder cancer or any benign disorder (stones, cystitis and benign prostatic hyperplasia). It is

worthy noted that E-cadherin, cathepsin-D and total protein could be significantly helpful to recognize lymph node metastasis and correlated with tumor size. Further evaluation of E-cadherin and total protein in bladder cancer patient were valid in testing recurrence since elevated pre treatment urinary level of E-cadherin and total protein were significantly associated with bladder cancer recurrence, whereas, cathepsin-D did not detect recurrence. This is consistent with the findings of others, as cathepsin expression associated with bladder cancer progression [26]. Also, it expressed aspartyl lysosomal protease that played an important role in tumor invasion and metastasis [27]. Among the measured markers, total protein showed the highest specificity (77.4 %) indicating its value in confirming the negative cases. The combination of E-cadherin, total protein plus NMP22 improves the detection of UCC and identifies clinically significant disease. Trials of a commercially developed E-cadherin and total protein assay suitable for an end-user laboratory alongside NMP22 are required to assess their potential clinical utility in improving diagnostic and surveillance care pathways. Of the one hundred twenty four polypeptides identified in bladder cancer urines, most corresponded to plasma proteins and their variants [28]. Molecular-based detection techniques represent potentially attractive strategies for noninvasive detection of aggressive bladder cancer using urine as the specimen source. Identification of new markers and development of novel platforms may improve detection in the future.

Conclusions

NMP22 is a useful marker for detection of both squamous and transitional cell carcinoma of the bladder cancer, while E-cadherin is the best in detecting adenocarcinoma. E-cadherin, cathepsin-D and total protein will be significantly helpful to recognize lymph node metastasis and also, correlated with tumor size as NMP22. In addition, NMP22, E-cadherin and total protein levels were valid in testing recurrence. Combined use of NMP22, E-cadherin and total protein will improve the diagnosis of all types of bladder cancer with high sensitivity and specificity.

Conflicts of interest No conflicts of interest are declared.

References

1. el-Mawla NG, el-Bolkainy MN, Khaled HM. Bladder cancer in Africa: update. *Semin Oncol.* 2001;28:174–8.
2. Shariat SF, Casella R, Monoski MA, Sulser T, Gasser TC, Lerner SP. The addition of urinary urokinase-type plasminogen activator to urinary nuclear matrix protein 22 and cytology improves the detection of bladder cancer. *J Urol.* 2003;170:2244–7.

3. Sjakste N, Sjakste T, Vikmanis U. Role of the nuclear matrix proteins in malignant transformation and cancer diagnosis. *Exp Oncol*. 2004;26:170–8.
4. Bredel M, Pollack IF, Hamilton RL, Birner P, Hainfellner JA, Zentner J. DNA topoisomerase IIalpha predicts progression-free and overall survival in pediatric malignant non-brainstem gliomas. *Int J Cancer*. 2002;99:817–20.
5. Chang HW, Chow V, Lam KY, Wei WI, Yuen A. Loss of E-cadherin expression resulting from promoter hypermethylation in oral tongue carcinoma and its prognostic significance. *Cancer*. 2002;94(2):386–92.
6. Bryan RT, Tselepis C. Cadherin switching and bladder cancer. *J Urol*. 2010;184(2):423–31.
7. Berchem G, Glondou M, Gleizes M, Brouillet JP, Vignon F, Garcia M, Liaudet-Coopman E. Cathepsin-D affects multiple tumor progression steps in vivo: proliferation, angiogenesis and apoptosis. *Oncogene*. 2002;21:5951–5.
8. Hardwicke J. Proteinuria. *Sci Basis Med Annu Rev*. 1970;1:211–29.
9. Zhau HY, Babaian RJ, Hong SJ. A new 180 kDa urine protein marker associated with bladder cancer. *J Urol*. 1990;144:47–52.
10. Kuhn SH, de Kock MA. A preliminary study of elevated alkaline phosphatase and cathepsin in bronchial aspirates of patients with lung cancer and bronchitis. *Chest*. 1975;68:326–30.
11. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med*. 1978;8:283–98.
12. Reinert T. Methylation markers for urine-based detection of bladder cancer: the next generation of urinary markers for diagnosis and surveillance of bladder cancer. *Adv Urol*. 2012;2012:503271.
13. Eissa S, Swellam M, Sadek M, Mourad MS, El Ahmady O, Khalifa A. Comparative evaluation of the nuclear matrix protein, fibronectin, urinary bladder cancer antigen and voided urine cytology in the detection of bladder tumors. *J Urol*. 2002;168:465–9.
14. Gödekmerdan A, Yahşi S, Semerciöz A, İlhan F, Akpolat N, Yekeler H. Determination of nuclear matrix protein 22 levels in cystitis, urothelial dysplasia and urothelial carcinoma. *Turk J Med Sci*. 2006;36:93–6.
15. Sanchez-Carbayo M, Urrutia M, Silva JM, Romani R, De Buitrago JM, Navajo JA. Comparative predictive values of urinary cytology, urinary bladder cancer antigen, CYFRA 21-1 and NMP22 for evaluating symptomatic patients at risk for bladder cancer. *J Urol*. 2001;165:1462–7.
16. Sawczuk IS, Bagiella E, Sawczuk AT, Yun EJ. Clinical application of NMP22 in the management of transitional cell carcinoma of the bladder. *Cancer Detect Prev*. 2000;24:364–8.
17. Casella R, Huber P, Blochlinger A, Stoffel F, Dalquen P, Gasser TC, Lehmann K. Urinary level of nuclear matrix protein 22 in the diagnosis of bladder cancer: experience with 130 patients with biopsy confirmed tumor. *J Urol*. 2000;164:1926–8.
18. Akaza H, Miyanaga N, Tsukamoto T, Ishikawa S, Noguchi R, Ohtani M, Kawabe K, Kubota Y, Fujita K, Obata K, et al. Evaluation of urinary NMP22 (nuclear matrix protein 22) as a diagnostic marker for urothelial cancer-NMP22 as a urinary marker for surveillance of bladder cancer. NMP22 study group. *Gan To Kagaku Ryoho*. 1997;24:829–36.
19. O'Sullivan P, Sharples K, Dalphin M, Davidson P, Gillig P, Cambridge L, Harvey J, Toro T, Giles N, Luxmanan C, Alves CF, Yoon HS, Hinder V, Masters J, Kennedy-Smith A, Beaven T, Guilford PJ. A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. *J Urol*. 2012 [Epub ahead of print].
20. Sumi S, Arai K, Kitahara S, Yoshida KI. Preliminary report of the clinical performance of a new urinary bladder cancer antigen test: comparison to voided urine cytology in the detection of transitional cell carcinoma of the bladder. *Clin Chim Acta*. 2000;296:111–20.
21. Sanchez-Carbayo M, Herrero E, Megias J, Mira A, Soria F. Evaluation of nuclear matrix protein 22 as a tumour marker in the detection of transitional cell carcinoma of the bladder. *BJU Int*. 1999;84:706–13.
22. Horstmann M, Todenhöfer T, Hennenlotter J, Aufderklamm S, Mischinger J, Kuehs U, Gakis G, Stenzl A, Schwentner C. Influence of age on false positive rates of urine-based tumor markers. *World J Urol*. 2012. [Epub ahead of print].
23. Hutterer GC, Karakiewicz PI, Zippe C, Ludecke G, Boman H, Sanchez-Carbayo M, Casella R, Mian C, Friedrich MG, Eissa S, et al. Urinary cytology and nuclear matrix protein 22 in the detection of bladder cancer recurrence other than transitional cell carcinoma. *BJU Int*. 2008;101:561–5.
24. Matsumoto K, Shariat SF, Casella R, Wheeler TM, Slawin KM, Lerner SP. Preoperative plasma soluble E-cadherin predicts metastases to lymph nodes and prognosis in patients undergoing radical cystectomy. *J Urol*. 2003;170:2248–52.
25. Shi B, Laudon V, Yu S, Dong D, Zhu Y, Xu Z. E-cadherin tissue expression and urinary soluble forms of E-cadherin in patients with bladder transitional cell carcinoma. *Urol Int*. 2008;81:320–4.
26. Staack A, Tolic D, Kristiansen G, Schnorr D, Loening SA, Jung K. Expression of cathepsins B, H, and L and their inhibitors as markers of transitional cell carcinoma of the bladder. *Urology*. 2004;63:1089–94.
27. Fehrenbacher N, Jaattela M. Lysosomes as targets for cancer therapy. *Cancer Res*. 2005;65:2993–5.
28. Rasmussen HH, Orntoft TF, Wolf H, Celis JE. Towards a comprehensive database of proteins from the urine of patients with bladder cancer. *J Urol*. 1996;155:2113–9.