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Diagnostic Potential of Urinary α 1-Antitrypsin and Apolipoprotein E in the Detection of Bladder Cancer

Virginia Urquidí*, Steve Goodison*, Shanti Ross, Myron Chang, Yunfeng Dai, and Charles J. Rosser†,‡

Cancer Research Institute (VU, SG, SR) and Section of Urologic Oncology (CJR), M.D. Anderson Cancer Center Orlando, Orlando and Department of Biostatistics, University of Florida (MC, YD), Gainesville, Florida

Abstract

Purpose—The ability to reliably diagnose bladder cancer in voided urine samples would be a major advance. Using high throughput technologies, we identified a panel of bladder cancer associated biomarkers with potential clinical usefulness. In this study we tested 4 potential biomarkers for the noninvasive detection of bladder cancer.

Materials and Methods—We examined voided urine specimens from 124 patients, including 63 newly diagnosed with bladder cancer and 61 controls. Concentrations of proteins were assessed by enzyme-linked immunosorbent assay, including α 1-antitrypsin, apolipoprotein E, osteopontin and pentraxin 3. Data were compared to the results of urinary cytology and the BTA Trak® enzyme-linked immunosorbent assay based bladder cancer detection assay. We used the AUC of ROC curves to compare the usefulness of each biomarker to detect bladder cancer.

Results—Urinary levels of α 1-antitrypsin, apolipoprotein E and bladder tumor antigen were significantly increased in subjects with bladder cancer. α 1-Antitrypsin (AUC 0.9087, 95% CI 0.8555–0.9619) and apolipoprotein E (AUC 0.8987, 95% CI 0.8449–0.9525) were the most accurate biomarkers. The combination of α 1-antitrypsin and apolipoprotein E (AUC 0.9399) achieved 91% sensitivity, 89% specificity, and a positive and negative predictive value of 89% and 90%, respectively. Multivariate regression analysis highlighted only apolipoprotein E as an independent predictor of bladder cancer (OR 24.9, 95% CI 4.22–146.7, $p = 0.0004$).

Conclusions—Alone or in combination, α 1-antitrypsin and apolipoprotein E show promise for the noninvasive detection of bladder cancer (OR 24.9, 95% CI 4.22–146.7, $p = 0.0004$). Larger, prospective studies including more low grade, low stage tumors are needed to confirm these results.

Keywords

urinary bladder; urinary bladder neoplasms; alpha 1-antitrypsin; apolipoproteins A; diagnosis

Bladder cancer is among the 5 most common malignancies worldwide. In the United States the estimated number of new BCa cases in 2012 is 73,500 with 14,880 estimated deaths.¹ Early detection remains one of the most urgent issues in BCa research. Early detection of BCa significantly improves the probability of successful patient treatment. The development of molecular assays that can diagnose the disease accurately or augment current evaluation methods would be a significant advance. Specifically, a molecular assay that is applicable to noninvasively obtained urine would facilitate not only the diagnosis but also the screening of asymptomatic populations at risk.

Using high throughput genomics and proteomics technologies, we performed molecular analyses of voided urine with the goal of identifying novel urinary biomarkers that can improve the accuracy of detecting BCa over that of current urine based assays, eg urinary cytology,² bladder tumor antigen,³ nuclear matrix protein 22⁴ and others.⁵ We derived genomic⁶ and proteomic signatures^{7,8} that achieved highly accurate detection of BCa in noninvasively obtained urine samples. A 14-biomarker signature was identified with 88% sensitivity and 93% specificity.

In this study we monitored the concentration of 4 novel target proteins, including A1AT, APOE, SPP1 (also known as secreted phosphoprotein 1 and bone sialoprotein) and PTX3, in urine samples from a cohort of 124 subjects to determine the diagnostic accuracy of these biomarkers for detecting BCa in voided urine samples. The diagnostic performance of these novel biomarkers was compared with urinary cytology and the BTA Trak assay.

MATERIALS AND METHODS

Patients and Specimen Processing

After receiving institutional review board approval and informed consent, voided urine samples and associated clinical information were collected in a tissue bank. The tissue bank was queried for adequate controls and for subjects with biopsy proven BCa. The control cohort consisted of 61 subjects with no history of BCa, gross hematuria, active urinary tract infection or urolithiasis but with voiding symptoms (37), microscopic hematuria (18) and erectile dysfunction (6). The 63 subjects with newly diagnosed BCa had the urothelial cell carcinoma subtype. According to the International Consensus Panel on Bladder Tumor Markers,⁹ these cohorts served as phase II (validation study). Data are reported using STARD (standards for the reporting of diagnostic accuracy studies) criteria.¹⁰

In our patients with cancer and controls with microscopic hematuria, axial imaging of the abdomen and pelvis was performed with and without intravenous contrast medium. In our subjects with cancer as well as controls with voiding symptoms or microscopic hematuria, office cystoscopy was performed. In subjects with cancer and an abnormality noted on office cystoscopy, formal cystoscopy and bladder tumor resection were done in the operating room using general anesthesia. All subjects with cancer had documented urothelial cell carcinoma, as confirmed by histological examination of excised tumor tissue. Pertinent information on clinical presentation, staging, histological grading^{11,12} and outcome were recorded (table 1).

Before any therapeutic intervention, 50 to 100 ml voided urine were obtained from each subject. Urine (50 ml) was used for clinical laboratory analyses, eg urinary cytology and urinalysis, according to standard procedures. The remaining urine aliquot was assigned a unique identifying number before immediate laboratory processing. Each urine sample was centrifuged at $600 \times$ gravity at 4C for 5 minutes. The supernatant was decanted and aliquoted, and the urinary pellet was snap frozen. The supernatant and pellet were stored at -80°C before analysis. Aliquots of urine supernatants were thawed and analyzed for protein content using a Pierce 660 nm Protein Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts).

Urine Based ELISA

Levels of human A1AT (ab108799, Abcam®), human APOE (KA 1031, Abnova, Walnut, California), human SPP1 (DOST00) and PTX3 (DPTX30, R&D Systems®) were monitored in urine samples using ELISA. The BTA Trak ELISA assay was used to measure BTA levels. Readers of these assays were blinded to disease status. All assays were done according to manufacturer instructions. Calibration curves were prepared using purified standards for each protein assessed. Curves were fit by linear or 4-parameter logistic regression according to manufacturer instructions.

Creatinine Assay

Creatine is converted nonenzymatically to the metabolite creatinine, which diffuses into blood and is excreted into urine by the kidneys at a constant rate. Consequently, urinary creatinine is a useful tool for normalizing the levels of other molecules found in urine.^{13,14} The concentration of all monitored proteins (A1AT, APOE, SPP1, PTX3 and BTA) was normalized to urinary creatinine and these concentrations are reported as a ratio relative to urinary creatinine values.

The creatinine assay (KGE005, R&D Systems) was done according to manufacturer instructions. Briefly, urine supernatants were thawed, diluted with distilled water and treated with alkaline picrate solution. Treated samples were measured on a Synergy™ HT microplate reader at a wavelength of 490 nm. Using purified standards, a standard curve was generated by linear regression. Signal intensities were subsequently converted to concentrations.

Statistical Analysis

We used the Wilcoxon rank sum test to determine the association between each biomarker and BCa. We generated nonparametric ROC curves that plotted the sensitivity value against the false-positive rate ($1 - \text{specificity}$). Each biomarker was normalized to urinary creatinine to account for the variability of voided urine volume and we used cubic root transformation of each biomarker to decrease skewness. We next assessed the relative ability of each biomarker to indicate BCa by calculating the AUC with a higher AUC indicating a stronger predictor. We compared AUCs by the chi-square test.

In addition, we estimated the sensitivity and specificity of each biomarker at the optimal cutoff value defined by the Youden index,¹⁵ ie the cutoff that maximizes the sum of

sensitivity and specificity. The accuracy of a biomarker to predict BCa was defined as the average of sensitivity and specificity. To assess the independent association between biomarkers and BCa, we used logistic regression analysis with BCa status (yes vs no) as the response variable, and age, gender, and the concentrations of A1AT, APOE, SPP1, PTX3 and BTA as explanatory variables. We performed a stepwise selection procedure to identify the most significant combination of biomarkers to predict BCa. Statistical significance in this study was considered at $p < 0.05$ and all reported p values are 2-sided. All analyses were performed using SAS®, version 9.3.

RESULTS

In line with the demographics of the institution, the majority of study subjects were elderly white males. Urinary cytology had 33% sensitivity and 100% specificity. Median followup of the control and cancer cohorts was 11.5 and 12 months, respectively. No controls had abnormal cystoscopy or axial imaging. Furthermore, during followup no control showed BCa or a gross hematuria event. In the cancer cohort 41% of subjects had nonmuscle invasive disease and 19% had low grade disease. Median tumor size was 4.5 cm. Table 1 lists cohort demographic, clinical and pathological characteristics.

Table 2 shows the concentration of 5 urinary proteins measured in urine. Median urinary levels of A1AT (1,209 vs 37.5 ng/ml), APOE (0.07 vs 0.02 ng/ml) and BTA (164.27 vs 13.13 U/ml, each $p < 0.0001$) were significantly higher in subjects with than without BCa. Conversely, median urinary SPP1 levels were increased in subjects without BCa compared to those with BCa (976.45 vs 212.86 ng/ml, $p < 0.0001$). Median urinary PTX3 levels in subjects with BCa were not significantly different from those in subjects without BCa (0.93 vs 0.66 ng/ml, $p = 0.21$).

The ability of those biomarkers to predict the presence of BCa was analyzed using nonparametric ROC analyses (fig. 1). Urinary A1AT (AUC 0.91, 95% CI 0.86–0.96) and APOE (AUC 0.90, 95% CI 0.84–0.95) were accurate biomarkers. Using a cutoff level of 1.24, urinary A1AT had 87% sensitivity, 84% specificity, 85% PPV, 86% NPV and 85% predictive accuracy. Using a cutoff of 0.78, urinary APOE had 90% sensitivity, 74% specificity, 78% PPV, 88% NPV and 82% predictive accuracy. Urinary BTA had the next most favorable parameters (AUC 0.82, 95% CI 0.73–0.90). Using a cutoff level of 0.73, urinary BTA had 79% sensitivity, 83% specificity, 83% PPV, 79% NPV and 81% predictive accuracy. Urinary SPP1 and PTX3 were not accurate biomarkers in this context.

Multivariate logistic regression analysis adjusted for the effects of age and gender demonstrated that increased APOE was an independent predictor of BCa (OR 24.9, 95% CI 4.22–146.7, $p = 0.0004$, table 3). The stepwise selection procedure revealed that the optimal combination of the 4 experimental biomarkers was A1AT plus APOE. Using the Youden index cutoff values for A1AT and APOE, the combination of A1AT and APOE (AUC 0.94, 95% CI 0.90–0.98) achieved 91% sensitivity, 89% specificity, 89% PPV, 90% NPV and 90% predictive accuracy (fig. 2). The AUC of the combination of A1AT and APOE was significantly higher than that of BTA (0.94 vs 0.82, $p = 0.001$).

DISCUSSION

BCa diagnosis hinges on invasive examination of the bladder (cystoscopy) and biopsy of a bladder tumor. A reliable, noninvasive modality to detect de novo or recurrent bladder tumors from voided urine would be of tremendous benefit to patients and health care systems. Thus, we sought to identify novel urine based BCa tumor biomarkers.

The biomarkers chosen for this study were identified in our previous biomarker discovery studies, in which we performed genomic⁶ and proteomic^{7,8} analyses. From these studies we derived highly accurate diagnostic signatures, of which some included A1AT, APOE, SPP1 and PTX3. Using commercially available ELISA kits for these candidate biomarkers, we investigated whether quantitative measurement of these proteins could facilitate the noninvasive detection of BCa in urine samples.

Increased urinary levels of A1AT, APOE and BTA were significantly associated with the presence of BCa. On univariate analyses A1AT and APOE proved to be the most accurate markers associated with BCa with an overall PPV and NPV of 85% and 87%, and 78% and 88%, respectively. Multivariate regression analysis highlighted APOE as an independent predictor of BCa. Furthermore, the combination of A1AT and APOE clearly outperformed individual biomarkers, including BTA, as a target protein for BCa detection by urinalysis (fig. 2).

The most discriminatory protein identified in this study was APOE, which combines with lipids to transport cholesterol and other fats through the bloodstream and, thus, has a critical role in lipid transportation.¹⁶ Data on the role of APOE in cancer are sparse. However, possible mechanisms underlying the APOE role in cancer include enhanced lipid transport into tumor cells and increased cell proliferation. APOE mediates signal transduction upon binding to low density lipoprotein receptor, thus, activating downstream survival signals. This view is supported by a number of studies showing that APOE, upon binding to low density lipoprotein receptor family members, initiates cell signaling and exerts various biological effects.^{17,18} Specifically, in BCa the transitional epithelial response gene TERE1, which is a tumor suppressor gene, reacts with APOE, resulting in increased cell turnover and resistance to apoptosis.^{19,20} Recently, Lindén et al reported that as part of a panel of biomarkers, APOE detected by mass spectroscopy and confirmed by Western blot analysis may be useful for detecting nonmuscle invasive BCa.²¹ In our study APOE was a useful urine based biomarker.

A1AT, also known as SERPINA1, is a member of a family of serine protease inhibitors. Specifically, A1AT irreversibly inhibits trypsin, chymotrypsin and plasminogen activator. Serpins have diverse but critical roles in the cell, including homeostasis regulation, cellular survival and blood clotting. In the oncology literature reports describe genetic aberrations in cancer,²² increased levels in the serum of patients with cancer²³ and a survival disadvantage for tumors expressing A1AT.²⁴ Previously, we reported that A1AT has 74% sensitivity and 80% specificity,⁹ which are slightly decreased from current findings (87% and 84%, respectively). We believe that this difference is due to 1) different ELISA kits used to detect

A1AT and 2) different statistical analyses. In this study we used the Youden index to identify sensitivity and specificity.

SPP1 is a highly phosphorylated sialoprotein with an important role in bone remodeling. However, this protein is expressed in various tissues, including muscle, endothelium, brain and kidney. In a small retrospective study analyzing RNA extracted from solid human bladder tumors, Zaravinos et al noted increased SPP1 expression using whole genome cDNA microarrays.²⁵ In a study that specifically monitored SPP1 levels in upper urinary tract urothelial carcinoma, higher levels correlated with poor prognosis.²⁶

In the current series urinary SPP1 levels were not increased in patients with BCa. Possibly translated SPP1 protein does not follow the mRNA trend in bladder tumor cells or urinary SPP1 was degraded, such that it was not detectable with the commercial ELISA kit that we used.

The pentraxin 3 gene, *PTX3*, is expressed in numerous tissues, such as monocytes, dendritic cells, fibroblasts and endothelial cells.²⁷ While its activity is related to inflammation, its function in cancers is unclear. Numerous studies have shown that PTX3 is a diagnostic biomarker for various cancers, including lung²⁸ and colorectal²⁹ tumors. As described for SPP1, our urothelial cell profiling studies revealed that monitoring PTX3 transcripts may be clinically useful for evaluating patients with BCa. However, soluble urinary PTX3 protein detection was not confirmed as a diagnostic biomarker when monitored with a commercial ELISA kit.

Our study has several limitations. 1) As a tertiary care facility, we tend to see more high grade, high stage disease, which was reflected in our study cohort. To confirm the robustness of A1AT and APOE, subsequent studies must assess larger cohorts including more subjects with low grade, low stage disease. 2) The sensitivity of urinary cytology in our cohort with predominantly high grade disease was lower than would perhaps be expected but in line with our previous experiences with cytology, which had high variability of interpretation among observers as well as poor sensitivity.³⁰ In subsequent studies we could use multiple cytopathologists to better control the interpretive variability of cytology but comparison with the actual recorded clinical evaluation is most appropriate. 3) Urine samples were retrieved from a frozen tissue bank. It is feasible that freshly voided urine samples may provide different results and fresh urine would be the material used for subsequent point of care assays. It is uncertain how the protein composition of urine supernatant may change during frozen storage. Thus, we are currently investigating the performance of select biomarkers in urine processed via a number of different protocols. However, in our experience biomarkers that show promise in tissue banked urine samples perform even better in unprocessed specimens. 4) Our sample size of 124 subjects was small and our 2 groups were relatively homogeneous, ie active cancer or control cases with no active cancer, history of cancer, urinary tract infection, urolithiasis or gross hematuria. The specificity of promising biomarkers such as A1AT and APOE must be tested in cohorts known to be problematic with other urine based assays, eg those with hematuria and urinary tract infection. We are planning a prospective, multicenter phase III trial to assess the clinical usefulness of our candidate diagnostic biomarkers in large, diverse cohorts.

CONCLUSIONS

The development of urine based BCa biomarkers would be of tremendous benefit to patients and health care systems. We found that increased urinary concentrations of A1AT and APOE proteins are strongly associated with the presence of BCa. Larger, prospective studies that include more subjects with low grade, low stage disease are needed to determine the potential role of A1AT and APOE in the evaluation of patients at risk for harboring BCa.

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Abbreviations and Acronyms

A1AT	α 1-antitrypsin
APOE	apolipoprotein E
BCa	bladder cancer
BTA	bladder tumor antigen
ELISA	enzyme-linked immunosorbent assay
NPP	negative predictive value
PPV	positive predictive value
PTX3	pentraxin 3
SPP1	osteopontin

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin*. 2012; 62:10. [PubMed: 22237781]
2. Rife CC, Farrow GM, Utz DC. Urine cytology of transitional cell neoplasms. *Urol Clin North Am*. 1979; 6:599. [PubMed: 505675]
3. Gutiérrez Baños JL, del Henar Rebollo Rodrigo M, Antolín Juárez FM, et al. Usefulness of the BTA STAT Test for the diagnosis of bladder cancer. *Urology*. 2001; 57:685. [PubMed: 11306381]
4. Hwang EC, Choi HS, Jung SI, et al. Use of the NMP22 BladderChek test in the diagnosis and follow-up of urothelial cancer: a cross-sectional study. *Urology*. 2010; 77:154. [PubMed: 20739046]
5. Daniely M, Rona R, Kaplan T, et al. Combined morphologic and fluorescence in situ hybridization analysis of voided urine samples for the detection and follow-up of bladder cancer in patients with benign urine cytology. *Cancer*. 2007; 111:517. [PubMed: 17963263]
6. Rosser CJ, Liu L, Sun Y, et al. Bladder cancer-associated gene expression signatures identified by profiling of exfoliated urothelia. *Cancer Epidemiol Biomarkers Prev*. 2009; 18:444. [PubMed: 19190164]
7. Kreunin P, Zhao J, Rosser C, et al. Bladder cancer associated glycoprotein signatures revealed by urinary proteomic profiling. *J Proteome Res*. 2007; 6:2631. [PubMed: 17518487]
8. Yang N, Feng S, Shedden K, et al. Urinary glyco-protein biomarker discovery for bladder cancer detection using LC-MS/MS and label-free quantification. *Clin Cancer Res*. 2011; 17:3349. [PubMed: 21459797]

9. Lokeshwar VB, Habuchi T, Grossman HB, et al. Bladder tumor markers beyond cytology: International Consensus Panel on bladder tumor markers. *Urology*. 2005; 66:35. [PubMed: 16399415]
10. Bossuyt PM, Reitsma JB, Bruns DE, et al. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *Fam Pract*. 2004; 21:4. [PubMed: 14760036]
11. Greene, FL. *AJCC Cancer Staging Manual*. 6th ed. American Joint Committee on Cancer, American Cancer Society; New York: Springer-Verlag; 2002.
12. Montironi R, Lopez-Beltran A. The 2004 WHO classification of bladder tumors: a summary and commentary. *Int J Surg Pathol*. 2005; 13:143. [PubMed: 15864376]
13. Pisitkun T, Johnstone R, Knepper MA. Discovery of urinary biomarkers. *Molec Cell Proteom*. 2006; 5:1760.
14. Pu FS. Urinary excretion of creatinine in normal subjects. *Chin Pharmaceut J*. 1992; 44:235.
15. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. *Biom J*. 2005; 47:458. [PubMed: 16161804]
16. Chen J, Li Q, Wang J. Topology of human apolipoprotein E3 uniquely regulates its diverse biological functions. *Proc Natl Acad Sci U S A*. 2011; 108:14813. [PubMed: 21873229]
17. Ho YY, Deckelbaum RJ, Chen Y, et al. Apolipoprotein E inhibits serum-stimulated cell proliferation and enhances serum-independent cell proliferation. *J Biol Chem*. 2001; 276:43455. [PubMed: 11551921]
18. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet*. 2000; 1:507. [PubMed: 11701639]
19. Fredericks WJ, McGarvey T, Wang H, et al. The bladder tumor suppressor protein TERE1 (UBIAD1) modulates cell cholesterol: implications for tumor progression. *DNA Cell Biol*. 2011; 30:851. [PubMed: 21740188]
20. McGarvey TW, Nguyen TB, Malkowicz SB. An interaction between apolipoprotein E and TERE1 with a possible association with bladder tumor formation. *J Cell Biochem*. 2005; 95:419. [PubMed: 15782423]
21. Lindén M, Lind SB, Mayrhofer C, et al. Proteomic analysis of urinary biomarker candidates for non-muscle invasive bladder cancer. *Proteomics*. 2012; 12:135. [PubMed: 22065568]
22. Lindor NM, Yang P, Evans I, et al. Alpha-1-antitrypsin deficiency and smoking as risk factors for mismatch repair deficient colorectal cancer: a study from the colon cancer family registry. *Mol Genet Metab*. 2010; 99:157. [PubMed: 19853488]
23. Hamrita B, Chahed K, Trimeche M, et al. Proteomics-based identification of alpha1-antitrypsin and haptoglobin precursors as novel serum markers in infiltrating ductal breast carcinomas. *Clin Chim Acta*. 2009; 404:111. [PubMed: 19306859]
24. Normandin K, Peant B, Le Page C, et al. Protease inhibitor SERPINA1 expression in epithelial ovarian cancer. *Clin Exp Metastasis*. 2010; 27:55. [PubMed: 20049513]
25. Zaravinos A, Lambrou GI, Volanis D, et al. Spot-light on differentially expressed genes in urinary bladder cancer. *PLoS One*. 2011; 6:e18255. [PubMed: 21483670]
26. Ke HL, Chang LL, Yang SF, et al. Osteopontin overexpression predicts poor prognosis of upper urinary tract urothelial carcinoma. *Urol Oncol*. 2011; 29:703. [PubMed: 20022267]
27. Breviario F, d'Aniello EM, Golay J, et al. Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem*. 1992; 267:22190. [PubMed: 1429570]
28. Diamandis EP, Goodglick L, Planque C, et al. Pentraxin-3 is a novel biomarker of lung carcinoma. *Clin Cancer Res*. 2011; 17:2395. [PubMed: 21257721]
29. Mori Y, Oлару AV, Cheng Y, et al. Novel candidate colorectal cancer biomarkers identified by methylation microarray-based scanning. *Endocr Relat Cancer*. 2011; 18:465. [PubMed: 21636702]
30. Nakamura K, Kasraeian A, Iczkowski KA, et al. Utility of serial urinary cytology in the initial evaluation of the patient with microscopic hematuria. *BMC Urol*. 2009; 9:12. [PubMed: 19744317]

Figure 1.

ROC curves of urinary A1AT, APOE, SPP1 and PTX3. *A*, based on ROC AUC Youden index, cutoff values that maximized sum of sensitivity and specificity were determined for each biomarker (red dot). *B*, performance values of each biomarker.

Figure 2.
ROC curves of combination of A1AT and APOE vs BTA

Table 1
Study cohort demographic and clinicopathological characteristics

	NonCa		Ca	
No. pts	61		63	
Median age (range)	61	(30–81)	68	(22–90)
No. men/women	53/8		54/9	
No. race (%):				
White	40	(66)	56	(89)
Black	2	(3)	0	
Other	19	(31)	7	(11)
No. tobacco use (%)	22	(36)	53	(84)
No. gross hematuria (%)	0		47	(74)
No. suspicious/pos cytology (%)	0		21	(33)
Median tumor size (cm)	Not applicable		4.5	
Median followup (mos)	11.5		12	
Clinical stage:	Not applicable			
Tis			2	(3)
Ta			16	(25)
T1			8	(13)
T2			32	(51)
T3			4	(6)
T4			1	(2)
N+			4	(6)
Grade:	Not applicable			
Low			12	(19)
High			51	(81)

Table 2
Concentration of 5 urinary proteins in cancer and noncancer groups

Urinary Proteins	Median	NonCa (range)	Median	Ca (range)
A1AT (ng/ml)	37.5	(5.93–2,448.85)	1,209	(11.18–83,296)
APOE (ng/ml)	0.02	(0.01–0.11)	0.07	(0.01–1.78)
SPP1 (ng/ml)	976.45	(0–11,120)	212.86	(0.27–3,926)
PTX3 (ng/ml)	0.66	(0–2.63)	0.93	(0–12.5)
BTA (U/ml)	13.132	(0.5–36.87)	164.27	(0–24,865.4)

Table 3
Logistic regression analysis of biomarkers in voided urine

	Estimate	SE	OR (95% CI)	p Value
Age	0.036	0.022	1.04 (0.99–1.08)	0.10
Men/women	−1.52	0.92	0.22(0.036–1.33)	0.10
A1AT	0.35	0.45	1.42 (0.59–3.41)	0.44
APOE	3.21	0.91	24.9 (4.22–146.7)	0.0004
SPP1	−0.55	0.39	0.58 (0.27–1.23)	0.15
PTX3	−0.039	0.20	0.96 (0.65–1.41)	0.84
BTA	0.58	0.61	1.79 (0.54–5.94)	0.34