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Clinical Significance of Serological and Urological Levels of Bladder Cancer-Specific Antigen-1 (BLCA-1) in Bladder Cancer

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Background: The aim of this study was to determine the clinical significance of the expression levels of bladder cancer-specific antigen-1 (BLCA-1) in the diagnosis of bladder cancer (BC). The study also determined the relationship between BLCA-1 expression levels and the clinical manifestation of BC.

Material/Methods: Patient samples were derived from 66 cases of BC that presented at the Department of Urology, Affiliated Hospital of Chengde Medical University, were recruited from April 2014 to May 2015, and 64 healthy control cases. Serum and urine BLCA-1 levels were detected by enzyme-linked immunosorbent assay (ELISA).

Results: Urine BLCA-1 levels in BC patients were significantly higher than that found in healthy controls ($P<0.01$). BLCA-1 levels in the urine of patients without mucus membrane invasion (Ta) were significantly different from urine levels found in patients with mucus membrane invasion (T1–T4; $P=0.022$). BLCA-1 levels in the serum of patients without muscular coat invasion (Ta–T1) were significantly different than serum levels of patients with muscular coat invasion (T2–T4; $P=0.042$).

Conclusions: BLCA-1 is involved in the appearance and development of BC. Clinical detection of serum and urine BLCA-1 protein levels showed a high level of sensitivity and specificity in diagnosing BC. Further study of the functional expression of BLCA-1 levels as a valuable and novel diagnostic marker in BC is clearly warranted.

MeSH Keywords: Diagnosis • Tumor Markers, Biological • Urinary Bladder Neoplasms

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Background

Bladder cancer is one of the most common urinary system tumors in China [1]. In addition, BC adversely affects the physical well-being and mental health of the patient. Early diagnosis of BC is helpful in improving the prognosis of the patient presenting with BC. At present, the clinical diagnosis of BC is via the perceived self-reported symptoms of the presenting patient, from detailed analysis of tissue biopsy specimens by cystoscopy, and from voided urine cytology. However, cystoscopy is an invasive examination, and voided urine cytology has a low level of sensitivity, which might provoke the reporting of a misdiagnosis. For patients without gross hematuria or other clinical symptoms, in these situations screening methods should be inexpensive and can be invasive; however, simultaneously, it is very important that the screening methodologies are sensitive and specific with credible negative and positive differential prognostic values. Unfortunately, all of the approaches described above lack these important criteria of sensitivity and specificity.

In 1996, Getzenberg et al., from the University of Pittsburgh, successfully isolated and identified 6 novel BC-specific nuclear matrix proteins [2]. This was achieved by comparing epithelial cells of 17 BC patients with that of healthy controls by Western immunoblot assays [2]. Bladder cancer specific nuclear matrix proteins (BLCAs) are specifically expressed in BC tissues, which can then be released into the peripheral circulation when cancer cells are lysed. Previously published studies have suggested that the BLCA family of proteins display a high level of sensitivity and specificity in the diagnosis of BC [2–4], and have an inherently high potential utility in clinical diagnostic applications.

Feng et al. previously studied the possible association between the levels of BLCA-1 and the clinically important pathological parameters of BC [5]. They assayed 77 BC tissue specimens for BLCA-1 expression by immunohistochemical staining and semi-quantitative evaluation [5], reporting that BLCA-1 expression was associated with the expression of a number of inflammatory cytokines and mediators, including VEGF, MMP9, IL-1α, and IL-8, although BLCA-1 expression was not correlated with the secretion of TNFα [5].

In a related study by Myers-Irvin et al. (2005), BC-specific nuclear structural alterations were identified; in particular, the functional expression of BLCA-1 in tissue specimens and urine samples were assayed in the setting of BC as compared with apparently healthy normal control subjects [4]. By Western immunoblot analysis and ELISA studies, BLCA-1 expression was found in tissues from patients with BC but it was absent from adjacent areas of normal bladder tissue and was absent from normal, apparently healthy control donor bladder tissue. Interestingly, by urinalysis and with a set cut-off of 0.025 optical density units, BLCA-1 was detected in 20 of 25 BC urine specimens, but was detected in only 6 of 46 normal, high risk prostate or renal cancer specimens that were tested at the same time. Importantly, these analyses resulted in an assay system with a sensitivity of detection of 80% and an assay specificity of 87% [4]. In essence, this study suggests that BLCA-1 has clinical utility as a urological marker of BC, with diagnostic importance in the detection of this condition.

The present study aimed to reveal the clinical significance of the expression levels of BLCA-1 in the diagnosis of BC, as well as to determine the relationship between the urological levels of BLCA-1 and the clinical manifestation of BC.

Material and Methods

Patients

We recruited 66 patients diagnosed with primary urinary bladder epithelia cancer by pathology at the Department of Urology of the Affiliated Hospital of Chengde Medical University (Chengde, People's Republic of China) from April 2014 through May 2015. Inclusion criteria were: patients were aged over 18 years and not previously treated with radiotherapy or chemotherapy. Exclusion criteria were: patients presenting with other urinary system tumors, with severe disease evident in other systems, or with autoimmune diseases that included systemic lupus erythematosus, rheumatic disease, and others. Bladder cancers were classified according to the WHO 2004 grading system, and the stages were determined according to the previously published guidelines of the 2009 TNM staging criteria. The control group included 64 normal, apparently healthy volunteers who were matched by age and sex. This study conformed to the Helsinki Declaration and approved was approved by the Ethics Committee of Chengde Medical University. All subjects signed an informed consent document prior to recruitment and inclusion into the study.

Enzyme-linked immunosorbent assay

Venous blood (5–10 ml) and fresh mid-stream urine (10–20 ml) were collected in the morning (6: 00 a.m.) on the first day following admission to the clinic. All samples were centrifuged at 3000 rpm for 15 min, and the supernatants were immediately stored at –80°C. BLCA-1 levels in serum and urine specimens were detected by ELISA (BLCA-1 kit, CSB-E14974h (Cusabio Biotech Co., Ltd., Wuhan, China), and recorded as ng/ml. Each sample was detected twice. BTA levels in urine specimens were detected by ELISA (BTA kit, Per Grande Co., Ltd., Beijing, China). This BTA kit is the first CFDA (China Food and Drug Administration)-approved domestic urine-based biomarker for

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Table 1. Comparison of clinical characteristics between bladder cancer patients and controls.

	Non-cancer (N=64) (median, range)	Cancer (N=66) (median, range)	p Value
Median Age (range, y)	59 (26~86)	53 (43~90)	0.922
Male: Female ratio	32: 32	38: 28	0.386
Clinical Stage			
Ta	n/a	11 (16%)	–
T1	n/a	21 (31%)	–
T2	n/a	24 (37%)	–
T3+T4	n/a	10 (15%)	–
Grade			
Low	n/a	33 (50%)	–
High	n/a	33 (50%)	–
Urine BLCA-1	0.539 (0.174–4.057)	1.203 (0.198–7.217)	P<0.001
Serum BLCA-1	4.16 (1.682–20.893)	5.480 (0.544–17.035)	P<0.001

BC diagnosis. Clinical trials have compared it with BTA TARK at the General Hospital of the People's Liberation Army, Beijing Friendship Hospital Capital Medical University, and the Jiangsu Cancer Hospital. The results showed that the BTA kit was in good agreement with the BTA TARK (R=0.9318).

Statistical analysis

Continuous variables that were normally distributed are described as the mean ±SD, and continuous variables that were not normally distributed are described as the median and range. Continuous variables that were normally distributed were analyzed by the Student's *t* test, and continuous variables that were not normally distributed were analyzed by the Mann-Whitney U test. Categorical variables were analyzed by chi-square test. Diagnostic values of the markers were analyzed by ROC. All data were analyzed using SPSS v.19.0 statistical software (SPSS Inc., USA). Alpha values of $P<0.05$ were considered statistically significant.

Results

In this study, 66 BC cases were confirmed by analytical pathology, which included 38 male and 28 female cases, with a median age of 53 years (range of 43–90 years). Urinary bladder epithelial cancer included the following: 28 cases of patients with a low-grade tumor, and 38 cases of patients with a high-grade tumor. Ta was seen in 11 cases; a T1 stage was seen in 21 cases; a T2 stage was seen in 24 cases; and T3+T4 stages were seen in 10 cases. In the normal control group, there were 64 cases, with a mean age of 59 (range of 26–86 years old). There were no significant differences in terms of age or sex of both groups ($P>0.05$; Table 1).

Comparison of clinical characteristics between BC patients and controls

The median concentration of the urine and serum levels of BLCA-1 for the cancer group were 1.203 ng/ml and 5.480 ng/ml, respectively, and the median in the control group was 0.539 ng/ml and 4.160 ng/ml, respectively. Urine BLCA-1 levels in the cancer group was significantly higher than that of the control group ($P<0.01$; Table 1).

Association of urinary or serum BLCA-1 levels and clinical features in BC

Urine BLCA-1 levels were significantly different between patients with no mucus membrane invasion (Ta) and in patients with mucus membrane invasion (T1–T4; $P=0.022$). Serum BLCA-1 levels were significantly different between patients with no muscular coat invasion (Ta–T1) and patients that exhibited muscular coat invasion (T2–T4; $P=0.042$; Table 2).

Sensitivity and specificity of serum and urine BLCA-1

The ROC curve was drawn. When the cut-off value was set at 0.899 ng/ml, the urine-borne BLCA-1levels displayed superior sensitivity (67%, 44/66) and specificity (92%, 59/64). In

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Table 2. Association between urine or serum BLCA-1 level and clinical feature in bladder cancer patients.

	Urine BLCA-1 (ng/ml) (median, range)	Serum BLCA-1 (ng/ml) (median, range)
Connective tissue invasion		
Ta (n=11)	0.487 (0.235–4.692)	4.658 (2.980–7.143)
T1–T4 (n=55)	1.391 (0.199–7.218)	5.620 (1.682–20.893)
p Value	0.022	0.200
Muscle invasion		
Ta–T1 (n=32)	0.937 (0.199–7.007)	4.835 (2.136–20.893)
T2–T4 (n=34)	1.435 (0.401–7.218)	6.081 (1.682–15.595)
p Value	0.130	0.042

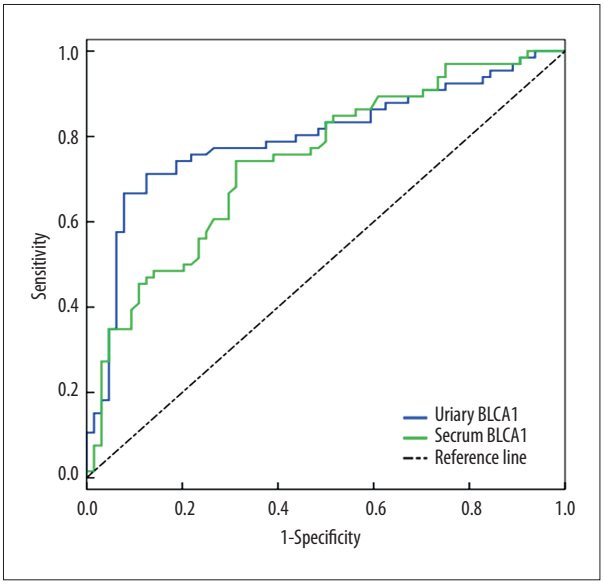

Figure 1. Receiver operating characteristic (ROC) curves of BLCA-1 that were applied in the diagnosis of bladder cancer. The area under the ROC curves of urine-borne BLCA-1 was 0.797, and serum-borne BLCA-1 was 0.743. Key: BLCA-1 (bladder cancer specific antigen-1).

addition, when the cut-off value was set at 4.529 ng/ml, the serum-borne BLCA-1levels displayed superior sensitivity (74%, 49/66) and specificity (68.75%, 44/64; Figure 1).

ROC analysis showed that the urine levels of BLCA-1 AUC in BC patients was 0.797 (95% CI: 0.717–0.877), the serum-borne levels of BLCA-1 AUC was 0.743 (95% CI: 0.659-0.827), and the urine BTA AUC was 0.635 (95% CI: 0.539–0.731; Table 3). The urine BLCA-1 AUC exceeded that of the BTA AUC (P=0.004); their sensitivities in the diagnosis of BC patients were 67% (44/66) and 60% (40/66), respectively; and their specificities were 92% (59/64) and 67% (43/64), respectively (Table 3).

Table 3. Sensitivity and specificity of urine or serum BLCA-1 detection.

	Urine BLCA-1	Serum BLCA-1
AUC (95%CI)	0.797 (0.717–0.877)	0.743 (0.659–0.827)
Cut-off (ng/ml)	0.899	4.529
Sensitivity	67%	74%
Specificity	92%	69%
Youden's index	59	43

Discussion

In this case-control study, we demonstrate that urine BLCA-1 levels in patients presenting with BC were markedly higher than levels found in normal, apparently healthy controls. Moreover, the levels of BLCA-1 in the urine of patients without mucus membrane invasion were significantly different from the urine levels found in patients with mucus membrane invasion. We also showed that the levels of BLCA-1 in the serum of patients without muscular coat invasion were significantly different than the serological levels of BLCA-1 found in patients with muscular coat invasion. Collectively, our observations strongly suggest that BLCA-1 is involved in the appearance and subsequent development of BC. We submit that further study of the functional expression of BLCA-1 would show diagnostic value as a novel marker in BC.

Indeed, a previously published study by Myers-Irvin et al. (2005) showed that when the cut-off value of the absorbance was set at 0.025, the sensitivity and specificity of detecting the functional expression of BLCA-1 were 80% and 87%, respectively [4]. Feng et al. examined the bladder tissues of 77 patients diagnosed with BC using pathological techniques including

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immunohistochemistry and Western blot analyses [5]. The results showed that BLCA-1 was unrelated to the occurrence and size of the tumor, but was positively associated with tumor stage, grade of the tumor, and its relative developmental aggression, showing that higher levels of detected functional expression of BLCA-1 are found in tumors with higher staging, grade, and relative aggressiveness of BC.

In this study, urine BLCA-1 levels were significantly higher in BC patients than in the apparently normal healthy controls. Within the 66 cases of BC patients recruited into this study, we found that urine-borne levels of BLCA-1 in 44 patients exceeded the cut-off value.

Moreover, in 64 cases of apparently normal healthy controls, urine BLCA-1 levels in 59 of the cases were lower than the cut-off value. Detection of urine BLCA-1 in the diagnosis of BC showed a high degree of sensitivity and specificity. Our results suggest the potential clinical application of urine BLCA-1. However, there was no available standard for the detection of BLCA-1 by ELISA, and there is no widely accepted cut-off value. For these reasons, there is an urgent need for a higher volume of experimental data on the biology and clinical value of BLCA-1 by research investigators all over the world.

As a nuclear matrix, BLCA-1 could be theoretically released into blood after lysis of cancer cells. Most studies on BLCA-1 have used urine samples, and there is no published report on using the serum BLCA-1 test for the diagnosis of bladder cancer in a Chinese population. Therefore, we investigated whether BLCA-1 could be detected in serum and whether its high expression in serum indicated a high possibility of bladder cancer, similar to the role of prostate-specific antigen (PSA) in prostate cancer.

The present study shows that urine BLCA-1 levels are related to invasion of the tumor mucosa, and serum BLCA-1 levels were related to the invasion of the tumor muscular coat, which indicated that BLCA-1 might play a significant role in tumor progression by participating in the secretory process of the tumor microenvironment and improving the invasive ability of bladder tumors. However, further study is required to determine whether functional expression of BLCA-1 interacts with angiogenic factors and subsequently leads to cancer progression and recurrence. Is there any correlation between urine levels and serum levels of BLCA-1 in all patients? As mentioned above, we have made intensive attempts at serum BLCA-1 detection. Also, a larger sample size could yield a more reliable result in the correlation analysis. We performed the analysis but the result was unsatisfactory, perhaps due to insufficient sample size. We plan to investigate this further in the future.

It should be noted that the BLCA-1 gene sequence is similar to the cancer metastasis-associated genes TI-227H [6–8]. BLCA-1 expression is related to some angiogenic factors, including the functional expression of vascular endothelial growth factor (VEGF), and matrix metalloproteinase (MMP)-9, which collectively indicate that BLCA-1 expression might alter the expression of the discussed biological factors.

Currently, for the reliable diagnosis and monitoring of BC, 2 assay systems are available – the relatively mature BTA-stat test and the BTA-Trak test – which are recommended by the U.S. FDA [9]. However, BTA assay cannot distinguish bladder mucosal damage that might have been caused by benign lesions. In subjects who present with other diseases of the urinary system, the specificity was lower. In that study, the rate of missed diagnosis reached a level of 20% through combined detection by BTA and urine cytology [10]. Leyh et al. performed the BTA test with 71 cases of BC and reported a false-positive rate of 10% [11]. In addition, many published studies have reported that detection of urine-borne BTA is susceptible to the influence of other benign urinary system diseases, including hematuria and calculus, among others [12–15].

We recognize that our study has several limitations. Despite the clinical utility of the clinical discovery of the high level of sensitivity and specificity of BLCA-1 in the diagnosis of bladder cancer, the value of determining the utility of BLCA-1 levels as a diagnostic marker in BC is tempered by important limitations in the experimental design of our study. For example, the study was dependent on a relatively small population size of 66 cases of BC that presented at a local urology clinic. Thus, observations from this study warrant independent verification and validation between laboratories of university medical center departments of urology. In addition, to strengthen the statistical confidence in the outcomes of this study, a larger cohort of study subjects is needed to confirm the clinical value of BLCA-1 as a urological biomarker in BC.

Furthermore, it might have been useful to compare the analyses of this study with that of the traditional BTA-TRAK assay, which is essentially an ELISA that is designed to detect particular recurrent antigens that are allegedly expressed at higher levels in BC. However, the value of such an approach also has its own inherent limitations due to its poor specificity and sensitivity, which historically was unable to replace traditional cystoscopy and other diagnostic strategies in the setting of BC. For this reason, although the BTA-TRAK assay was widely considered a major breakthrough for the early detection of BC in the late 1990s and early 2000s, the BTA-TRAK assay has since fallen into disuse; therefore, its value as a comparative analytical benchmark in understanding the utility of BLCA-1 as a biomarker requires careful interpretation and appropriate controls.

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Conclusions

We conclude that detection of urine-borne BLCA-1 has value in the diagnosis of BC and exhibits a high degree of sensitivity and specificity. Thus, the detection of BLCA-1 is expected to serve as a novel BC biomarker. Finally, for BLCA-1 to be considered a novel tumor biomarker, multi-center, standardized, prospective studies are needed and should include a larger sample size than was reported in the current study.

Conflicts of interests

None.

References:

1. Bin Z, Li XH: Advances in epidemiology of bladder cancer. International Journal of Urology and Nephrology, 2007; 27: 469–76
2. Getzenberg RH, Konety BR, Oeler TA et al: Bladder cancer-associated nuclear matrix proteins. Cancer Res, 1996; 56: 1690–94
3. Konety BR, Nguyen TS, Dhir R et al: Detection of bladder cancer using a novel nuclear matrix protein, BLCA-4. Clin Cancer Res, 2000; 6: 2618–25
4. Myers-Irvin JM, Landsittel D, Getzenberg RH: Use of the novel marker BLCA-1 for the detection of bladder cancer. J Urol, 2005; 174: 64–68
5. Feng C, Wang L, Ding G et al: BLCA1 expression is associated with angiogenesis of bladder cancer and is correlated with common pro-angiogenic factors. Int J Clin Exp Med, 2015; 8: 16259–65
6. Santoni M, Catanzariti F, Minardi D et al: Pathogenic and diagnostic potential of BLCA-1 and BLCA-4 nuclear proteins in urothelial cell carcinoma of human bladder. Adv Urol, 2012; 2012: 397412
7. Ishiguro T, Nakajima M, Naito M et al: Identification of genes differentially expressed in B16 murine melanoma sublines with different metastatic potentials. Cancer Res, 1996; 56: 875–79
8. Ishiguro T, Nagawa H, Naito M, Tsuruo T: Analysis of novel metastasis-associated gene TI-227. Jpn J Cancer Res, 2000; 91: 390–94
9. Fei YL, Zhi LZ: [Research status of the relationship between BLCA-1 and BLCA-4 and bladder cancer.] Chinese Journal of Clinicians, 2013: 8393–96 [in Chinese]
10. Sarosdy MF, deVere White RW, Soloway MS et al: Results of a multicenter trial using the BTA test to monitor for and diagnose recurrent bladder cancer. J Urol, 1995; 154: 379–83; discussion 383–84
11. Leyh H, Marberger M, Conort P et al: Comparison of the BTA stat test with voided urine cytology and bladder wash cytology in the diagnosis and monitoring of bladder cancer. Eur Urol, 1999; 35: 52–56
12. Edgren M, Lennernas B, Larsson A, Nilsson S: Serum concentrations of VEGF and b-FGF in renal cell, prostate and urinary bladder carcinomas. Anticancer Res, 1999; 19: 869–73
13. Miyake M, Goodison S, Rizwani W et al: Urinary BTA: indicator of bladder cancer or of hematuria. World J Urol, 2012; 30: 869–73
14. Guo A, Wang X, Gao L et al: Bladder tumour antigen (BTA stat) test compared to the urine cytology in the diagnosis of bladder cancer: A meta-analysis. Can Urol Assoc J, 2014; 8: E347–52
15. Chou R, Gore JL, Buckley D et al: Urinary biomarkers for diagnosis of bladder cancer: A systematic review and meta-analysis. Ann Intern Med, 2015; 163: 922–31

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