Am J Cancer Res 2019;9(6):1104-1117
www.ajcr.us /ISSN:2156-6976/ajcr0097330

Review Article

Urine protein biomarkers for the detection, surveillance, and treatment response prediction of bladder cancer

Ashish Chakraborty[1], Shobha Dasari[1], Wang Long[2], Chandra Mohan[1]

[1]Department of Biomedical Engineering, University of Houston, Houston, TX, USA; [2]Department of Urology, Xiangya Hospital, Central South University, Changsha 410008, Hunan, China

Received May 21, 2019; Accepted May 29, 2019; Epub June 1, 2019; Published June 15, 2019

Abstract: The "gold standard" diagnostic procedure for bladder cancer is cystoscopy, a technique that can be invasive, expensive, and a possible cause of urinary tract infection. Unlike techniques such as histology, PCR, and staining, assays for protein biomarkers lend themselves well to the creation of efficient point-of-care tests, which are easy to use and yield fast results. A couple of urine-based tests have been approved by the U.S. FDA, but these tests suffer from low sensitivity. Hence, there is clearly a need for more reliable non-invasive biomarkers of bladder cancer. Urinary biomarkers are particularly attractive due to the direct contact of the urine with the urothelial tumor and the ease of sample collection. With these considerations, this review aims to provide a comprehensive listing of the most promising protein biomarkers of bladder cancer in urine. Biomarkers are organized by their potential role in detection, surveillance, or monitoring of treatment response. The purpose of this review is to assess progress towards the goal of identifying ideal urinary proteins for use in each of the above three biomarker applications in bladder cancer.

Keywords: Point-of-care tests, NMP22 test, tumor recurrence, proteomics, NMIBC

Introduction

Bladder cancer (BCa) is the sixth most common type of cancer in the U.S., contributing to 4.7% of cancer cases and resulting in significant morbidity and mortality [1]. BCa manifests as either muscle-invasive or non-muscle-invasive, the latter being the predominant form, comprising about 80% of cases [2]. The "gold standard" diagnostic procedure for BCa is cystoscopy, a technique that can be invasive, expensive, and a possible cause of urinary tract infection [3, 4]. Additionally, bladder mucosa irregularities and small areas of carcinoma in situ (CIS) may contribute to a significant rate of false-negatives due to operator error [5]. Because ten-year recurrence rates of non-muscle-invasive bladder cancer (NMIBC) have been found to be as high as 74.3%, cystoscopy is recommended routinely for surveillance, which contributes to increased expense and a higher risk for urologic disease in the patient [6, 7].

The most reliable non-invasive test for BCa is urine cytology. Although it has reasonably high specificity (~86%), urine cytology suffers from poor sensitivity (~48%), especially in low-grade malignancies (~16%), as well as false positives from benign conditions [8, 9]. The need for a skilled uropathologist coupled with demonstrated inter- and intra-observer variability also significantly diminish the practical utility of this technique [9, 10]. Other tests that are feasible for primary care include the urine dipstick or microscopic urinalysis to detect hematuria. However, these tests are not sufficiently sensitive and have low specificity. Hence, there is clearly a need for more reliable non-invasive biomarkers of BCa.

Urinary biomarkers are particularly attractive due to the direct contact of the urine with the urothelial tumor cells and the ease of sample collection [11]. Nuclear matrix protein 22 (NMP22) is one such urinary biomarker. The NMP22 Bladder Cancer ELISA Test and the NMP22 BladderChek point-of-care (POC) tests have been approved by the U.S. Food and Drug Administration (FDA) [12, 13]. However, these tests suffer from low sensitivity. Wang et al.

compiled ranges in a meta-analysis of 19 studies and found that the overall sensitivity and specificity of NMP22 when used for detection of BCa were 52-59% and 87-89% respectively, with an AUC (area under the receiver operating curve) of 0.83 (**Table 1**). Another well-studied urinary biomarker is the bladder tumor antigen (BTA), also known as human complement factor H related protein (hCFHrp). The two FDA-approved tests for BTA detection are BTA STAT and BTA TRAK, which have 95% CI sensitivities of 64-69% and 62-71%, respectively, and 95% CI specificities of 73-77% and 45-81%, respectively (**Table 1**). In the presence of certain urologic conditions, particularly those associated with hematuria such as urinary tract infection and renal calculi, both the BTA STAT and BTA TRAK tests may yield false positives due to the high presence of complement factor H in blood [14]. Clearly, the current forms of non-invasive protein biomarkers of BCa are lacking in sensitivity and specificity.

Urinary biomarkers have another potential use in predicting BCa treatment response. Conventionally, NMIBC is treated with complete transurethral resection of all visible lesions, followed by induction and maintenance treatment using intravesical therapy if the BCa is classified as intermediate- or high-risk [15]. Failure of intravesical therapy in a patient often requires cystectomy, with early cystectomy resulting in significantly higher 10-year cancer-specific survival rates than deferred cystectomy [15]. Bacillus Calmette-Guerin (BCG) immunotherapy is a typical first-line intravesical therapy. However, because unpredictable failure can occur in 33% of patients after treatment with BCG, biomarkers that predict the effectiveness of BCG in a patient prior to initiating therapy would be invaluable [16]. Such predictive ability would advance the administration of key second-line therapies such as mitomycin C or indicate the need for early cystectomy, thereby reducing the risk of progression to muscle-invasive disease, which is a common outcome of unpredictable BCG failure [16].

Unlike techniques such as histology, PCR, and staining, assays for protein biomarkers lend themselves well to the creation of efficient point-of-care tests, which are easy to use and yield fast results. Testing urinary proteins is also especially convenient due to ease of sample collection, and the proximity of urine to urinary tract tumors. With these considerations taken into account, this review aims to provide a comprehensive listing of the most promising protein biomarkers of BCa in urine. In this review, biomarkers are organized by their potential role in detection, surveillance, or monitoring of treatment response. Where applicable, selected non-protein-based tests, including those that have been FDA-approved or commercialized, are surveyed for comparison. The purpose of this review is to assess progress towards the goal of identifying ideal urinary proteins to be used for each of the above three applications of biomarkers in BCa.

Methods

PubMed was searched for all relevant articles prior to January 31st, 2019. Articles were identified using the following keywords in various combinations: “bladder cancer”, “bladder carcinoma”, “urothelial cancer”, “urothelial carcinoma”, “Nuclear matrix protein 22”, “NMP22”, “Bladder tumor antigen test”, “BTA test”, “UBC”, “UBC rapid”, “effectiveness”, “reliability”, “accuracy”, “bladder cancer”, “bladder carcinoma”, “urothelial cancer”, “urothelial carcinoma”, “diagnosis”, “detection”, “surveillance”, “recurrence”, “urinary biomarker”, “treatment response”, “Bacillus Calmette-Guerin”, “BCG”, “mitomycin C”, “resection”, “TURBT”, and “outcome”. The search was limited to English language articles, but was not time-frame-limited. Only the articles providing metrics detailing the performance characteristics of the candidate biomarkers, such as sensitivity, specificity, an AUC value, or a *p*-value of an outcome association for the biomarker(s) were chosen for inclusion. For ease of interpretation and a more accurate representation of the predictive power of biomarkers, studies reporting metrics of candidate biomarkers used in combination with cystoscopy were excluded.

Results

Potential urinary protein biomarkers for the detection of BCa

Biomarkers for the detection of BCa are defined as those that can predict the presence of any type of BCa, including muscle-invasive bladder cancer (MIBC). An ideal biomarker is one with high sensitivity, specificity, positive predictive

value (PPV), negative predictive value (NPV), and AUC values. Different metrics are valuable for different applications of the biomarker: a high sensitivity could be favored for the detection of high-risk disease, while a high NPV could be favored if the objective is to rule out cystoscopy when the test is negative. **Table 1** summarizes the characteristics of promising detection markers, and was limited to biomarkers found with both sensitivity and specificity greater than or equal to 85%. Biomarkers above this cutoff have comparable or superior metrics compared to the FDA approved urinary protein biomarker tests (NMP22 and BTA, which are included in the table for comparison) and thus may have the potential to be used as alternatives to these tests. PPV, NPV, and AUC values are provided when available. In **Table 1**, a total of 13 biomarker candidates for reliable BCa detection are listed. As a comparison, the NMP 22, BTA STAT, and BTA TRAK tests demonstrated sensitivities of 52-59%, 64-49%, and 62-71%, and specificities of 87-89%, 73-77%, and 45-81% respectively (**Table 1**). In summary, urine Apo-A1, BLCA-4, and hyaluronidase all emerge as promising BCa detection markers, not only because they exhibit high sensitivities (89-95%, 93%, 89-100%) and specificities (85-92%, 97%, 89-91%) that equal or surpass those of the FDA-approved NMP22 and BTA biomarkers (**Table 1**), but also because they have been independently validated. Of promise, several additional urine proteins exhibit sensitivity, specificity, NPV, PPV and AUC values exceeding the FDA-approved tests, but have not yet been independently validated (**Table 1**).

Potential urinary protein biomarkers for the surveillance of BCa

Surveillance biomarkers are those that can predict the recurrence of NMIBC after initial treatment. Although all biomarkers assessed for their ability to detect BCa could theoretically be used as surveillance biomarkers, only studies that directly validated biomarkers specifically for patients at risk for recurrence are included in **Table 2**. When provided, the therapy used in each study is also specified in the table, given its potential influence on recurrence rates. As shown in **Table 2**, four candidate protein markers and marker combinations have been reported. All studies from the search that validated urinary protein biomarkers specifically for surveillance are included. FDA-approved protein tests have also been included from studies that used them to assess their utility for surveillance. It should be stressed however that none of the candidate biomarkers listed in the table have been validated independently. Among the leading candidates are bladder tumor fibronectin (BTF), used as a single marker, that has produced a sensitivity and specificity of 91% and 88%, both of which are higher than or comparable to the FDA-approved tests (**Table 2**), as well as a 10-marker urine protein panel with an AUC value of 0.90, exceeding the performance of the current FDA-approved tests (**Table 2**). Clearly, these promising results warrant independent validation.

Potential urinary protein biomarkers for the prediction of treatment response in BCa patients

Treatment response biomarkers are defined as biomarkers that predict how a patient may respond to a specific treatment. The treatment response studies included in this review primarily present this information by testing the association between a specific post-treatment outcome with the levels of given urinary biomarkers in patients. The administered therapy, tested outcome, and statistical p value are reported for each candidate biomarker interrogated. Most of these studies assessed tumor recurrence following BCG administration (**Table 3**). The search yielded solely proteins, and predominantly cytokines and chemokines among them. All studies that demonstrated statistical significance ($p \leq 0.05$) for potential biomarkers that could gauge response to a specific treatment are included. As listed in **Table 3**, a total of 12 protein markers and marker combinations have been reported as potential biomarkers of treatment response. Several of these urine biomarkers have been independently evaluated with similar results. The potential utility of urine IL-8 and IL-2 have been validated by four more independent groups each, while urine IL-6, IL-18 and TNF-α have been validated by two independent groups each (**Table 3**). Of the studies included, no p value above 0.0209 was observed for IL-8, making it especially promising (**Table 3**). The use of different statistical approaches across the different studies makes comparison between studies challenging.

Table 1. Potential Urinary Biomarkers for the Detection of BCa

	Biomolecule(s)	Method	Reference	Subjects	Sensitivity	Specificity	Accuracy	Notes
FDA-approved	NMP22	NMP22 BladderChek, ELISA	Wang 2017 [36]	5291 patients total	52-59%	87-89%		Meta-analysis of 19 studies AUC = 0.83
	BTA	BTA stat test	Guo 2014 [37]	3462 patients total	64-69%	73-77%		Meta-analysis of 13 studies AUC = 0.75
	BTA	BTA TRAK test	Glas 2003 [38]	829 patients total	62-71%	45-81%		Meta-analysis of 5 studies
Protein	*ANG, APOE, CA-9, IL-8, MMP-9, MMP-10, PAI-1, VEGF*	ELISA	Goodison 2012 [22]	64 BCa, 62 HC	92%	97%	94%	
	Apo-A1	ELISA	Li 2011 [39]	107 BCa, 49 OUC	92%	86%	91%	
		ELISA	Li 2014 [40]	223 BCa, 153 non-BCa	89%	85%	87%	AUC = 0.948
		ELISA	Chen 2010 [41]	126 specimens	95%	92%		AUC = 0.982
	APOE, IL-8, VEGF	ELISA	Goodison 2012 [22]	64 BCa, 62 HC	90%	97%	94%	AUC = 0.968
	Apo-A4 + Coronin-1A + DJ-1/PARK7 + Gamma Synuclein + Semenogelin-2	Western Blot	Kumar 2015 [42]	63 T2/T3 BCa, 110 Ta/T1 BCa, 66 HC	94%	97%	95%	AUC = 0.98
	BLCA-4	ELISA (8 studies) qPCR (1 study)	Cai 2015 [43]	1119 subjects total	93%	97%		Meta-analysis of 9 studies AUC = 0.9607
	CCL18	ELISA	Urquidi 2012 [23]	64 BCa, 63 non-BCa	88%	86%	87%	PPV = 86% NPV = 87% AUC = 0.919
	CPI	Fluorescence spectroscopy	Inoue 2013 [44]	66 BCa, 20 HC	100%	92%	98%	Measured 8 hours after ALA administration AUC = 0.978
	Hyaluronidase	Zymography	Eissa 2015 [45]	94 BCa, 60 OUC, 56 HC	89%	91%	90%	PPV = 89% NPV = 91% AUC = 0.948
		ELISA-like assay	Pham 1997 [46]	22 G1 BCa, 9 G2 BCa, 40 G3 BCa, 48 OUC, 20 HC	100%	89%	93%	Distinguished G2 and G3 (high-grade BCa) from G1 and controls
	HtrA1	ELISA	Lorenzi 2013 [47]	68 BCa, 16 OUC, 68 HC	93%	96%	94%	PPV = 95% NPV = 93% AUC = 0.9839

IL-8	ELISA	Rosser 2014 [48]	31 BCa, 42 OUC	90%	86%	88%	PPV = 82% NPV = 82% AUC = 0.907
ORM1	ELISA	Li 2016 [49]	121 BCa, 21 OUC, 53 HC	92%	94%	93%	AUC = 0.965
Soluble FAS	ELISA	Srivastava 2014 [50]	117 BCa, 46 OUC, 28 HC	88%	89%	88%	AUC = 0.912
UPI	Fluorescence spectroscopy	Inoue 2013 [44]	66 BCa, 20 HC	100%	96%	99%	Measured 8 hours after ALA administration AUC = 0.994

OUC = controls with other urinary conditions, HC = healthy controls. Bolded font indicates a biomarker that has been independently validated by 2+ studies. Italics indicate a sensitivity ≥ 90%, and an underline indicates specificity ≥ 90%.

Table 2. Potential Urinary Biomarkers for the Surveillance of BCa

	Biomolecule(s)	Method	Reference	Subjects	Treatment	Sensitivity	Specificity	Accuracy	Notes
FDA-approved	NMP22	NMP22 BladderChek	Soria 2018 [28]	3353 total from 14 studies	-	11-87.5% (49.5%)	77-100% (91.4%)		PPV = 18.2-100% NPV = 61.9-93.9%
		NMP22 ELISA	Soria 2018 [28]	4650 total from 15 studies	-	24-81% (64%)	49-100% (78.15%)		PPV = 31-100% NPV = 60-91%
	BTA	BTA STAT	Soria 2018 [28]	3064 total from 12 studies	-	40-72% (57%)	29-96% (86%)		PPV = 40-88% NPV = 38-76.9%
		BTA TRAK	Soria 2018 [28]	918 total from 6 studies	-	50-62% (61%)	68-87% (81.5%)		PPV = 45.4% NPV = 88.4%
Protein	Angiogenin, APOE, Carbonic anhydrase 9, IL-8, MMP 9, MMP 10, PAI-1, SDC1, SERPINA1, VEGF-A	ELISA	Rosser 2014 [51]	53 recurrence positive, 72 recurrence negative	Chemotherapy, TURBT	79%	88%	84%	PPV = 82% NPV = 85% AUC = 0.904
	BTF	Chemiluminescent immunometric test	Li 2008 [52]	126 recurrence positive, 41 recurrence negative	TURBT and no intravesical therapy	91%	88%	88%	PPV = 73% NPV = 93%
	cadherin-1, EN2, ErB2, IL-6, IL-8, VEGF-A	ELISA	De Paoli 2016 [53]	27 recurrence negative, 18 recurrence positive	BCG, mitomycin C, TURBT				Test also includes three clinical parameters AUC = 0.91

	sE-cadherin	ELISA	Shariat 2005 [54]	188 under cystoscopy surveillance (of which 122 had BCa), 31 OUC, 10 HC	71%	65%	70%	PPV = 67% NPV = 69% AUC = 0.719

TURBT = transurethral resection of bladder tumor. Italics indicate a sensitivity ≥ 90%, and an underline indicates specificity ≥ 90%. Note: For the FDA-approved tests, ranges are given and medians are included in parentheses.

Table 3. Potential Urinary Biomarkers for the Prediction of Treatment Response in BCa Patients

	Biomolecule(s)	Method	Reference	Subjects	Therapy	Test Performance	Notes
Protein	CEA	RIA	Wahren 1982 [55]	425 BCa, 75 symptom-free, 50 healthy	Radiation treatment	Decreasing levels in patients with symptom-free survival and no local recurrence, p = 0.001	
	GM-CSF	ELISA	Jackson 1998 [56]	34 BCa	BCG	Levels significantly different between patients with good and poor short-term therapeutic outcome, p < 0.05	
	IFN-α	ELISA	Saint 2001 [57]	19 Ta/T1 NMIBC	BCG	Decreasing levels in patients associated with non-recurrence, p < 0.001	
	IFN-α, IL-1ra, IL-2, IL-6, IL-8, IL-12[p70], IL-18, TNF-α, TRAIL	Luminex	Kamat 2016 [24]	130 with intermediate and high-risk NMIBC	BCG	Panel and nomogram results predict likelihood of recurrence	Accuracy: 0.85.5% (95% CI 77.9-93.1%)
	IL-2	ELISA	Saint 2001 [57]	19 Ta/T1 NMIBC	BCG	Increasing levels in patients associated with BCG response, p = 0.01	
		ELISA	Saint 2002 [58]	37 Ta/T1 NMIBC, 13 healthy	BCG	Patients with levels less than 27 pg./micromol. creatinine more likely to have recurrence, p = 0.0009	
		ELISA	Saint 2003 [59]	39 NMIBC or CIS	BCG	Failure to detect during first BCG induction and extended induction cycle correlated with time to recurrence, p = 0.01	
		ELISA, RIA	Watanabe 2003 [60]	20 CIS, 8 OUC	BCG	High levels correlated with BCG treatment efficacy (p < 0.01) as well as tumor recurrence after treatment	
		ELISA	Sanchez-Carbayo 2001 [61]	121 patients, including BCa and OUC	Intravesical therapy	Peaks in IL-2 associated with BCG response, p = 0.041	
		ELISA	de Reijke 1996 [62]	23 TCC	BCG	Presence in urine correlated with tumor recurrence, p = 0.003	

IL-6	ELISA	de Reijke 1996 [62]	23 TCC	BCG	Correlated with early (< 6 months) tumor recurrence, p = 0.040	
	ELISA, RIA	Watanabe 2003 [60]	20 CIS, 8 OUC	BCG	High levels correlated with BCG treatment efficacy (p < 0.05)	
IL-8	ELISA	Sagnak 2009 [63]	41 NMIBC	BCG	Change in levels before first BCG and at 2 hours after BCG correlates with recurrence, cutoff is 12 pg/mL (p = .047)	Sens. = 53%, Spec. = 89%, PPV = 73%, NPV = 77%
	ELISA	Thalmann 2000 [64]	28 NMIBC	BCG	Levels > 4,000 ng. after BCG correlated to higher chance of remaining disease-free (p < 0.05)	Sens. = 62.5%, Spec. = 92%
	ELISA	Kumar 2002 [65]	26 NMIBC	BCG	Levels higher in responders than in nonresponders at 4 hours after BCG (p = 0.001)	Sens. = 100%, Spec. = 88.2%
	ELISA, RIA	Watanabe 2003 [60]	20 CIS, 8 OUC	BCG	High levels correlated with BCG treatment efficacy (p < 0.05)	
	ELISA	Thalmann 1997 [66]	20 BCa	BCG	Levels < 4,000 ng. during first 6 hours after BCG correlated to higher risk of recurrence and progression (p < 0.0002).	Sens. = 100%, Spec. = 90%
IL-10	ELISA, RIA	Watanabe 2003 [60]	20 CIS, 8 OUC	BCG	High levels correlated with BCG treatment efficacy (p < 0.01)	
IL-18	ELISA	Thalmann 2000 [64]	17 NMIBC	BCG	Elevated expression correlated to longer disease-free survival (p < 0.05)	
	ELISA	Eto 2005 [67]	12 TCC	BCG	Disease-free, p < 0.01	
Survivin	CLA	Hausladen 2003 [68]	25 NMIBC	BCG (23), mitomycin C (4)	Posttreatment presence indicates high likelihood of recurrence	Sens. = 100%, Spec. = 78%
TNF-α	ELISA	Shintani 2007 [69]	28 BCa	BCG	Higher levels in non-recurrent group than in recurrent group (p = 0.07)	
	ELISA, RIA	Watanabe 2003 [60]	20 CIS, 8 OUC	BCG	High levels correlated with BCG treatment efficacy (p < 0.05)	
TRAIL	ELISA	Ludwig 2004 [70]	17 NMIBC	BCG	Higher levels present in responders (> 12 months tumor free) than non- responders (p < -0.05)	

BCG = Bacillus Calmette-Guerin immunotherapy, NMIBC = non-muscle-invasive bladder cancer, CIS = carcinoma in-situ, TCC = transitional cell carcinoma, OUC = other urinary conditions. Bolded font indicates a biomarker that has been independently validated by 2+ studies.

Discussion

A hierarchy of phases of biomarker validation, from discovery to incorporation into clinical practice, have been defined, with a special emphasis on bladder cancer [26-28]. This hierarchy consists of four phases: assay development and exploratory studies (phase I), independent validation of accuracy using large cohorts (phase II), external validation studies across multiple institutions and prospective clinical trials (phase III), and post-approval reports (phase IV). While it should be noted that there are several markers that are already commercially available with no intention to seek FDA-approval, this scale provides a useful yardstick for the assessment of biomarker studies and their progress. The 3 different types of BCa marker studies listed in **Tables 1-3** are reviewed below in this context.

Table 1 first lists the validation metrics of several FDA-approved biomarkers for comparison to potential candidates for BCa detection. Assessed by BladderChek and ELISA tests, NMP22 was found to have a sensitivity of 52-59% and a specificity of 87-89% in a meta-analysis of 19 studies (**Table 1**). Using the BTA STAT test, BTA demonstrated a sensitivity of 64-69% and a specificity of 73-77% in a meta-analysis of 13 studies (**Table 1**). The BTA TRAK test showed similar sensitivity of 62-71% but a notably lower specificity of 45-81% in a meta-analysis of 5 studies (**Table 1**). Not included in the table are the UroVysion (sensitivity 57.1%, specificity 87.5%) and uCYT+ (sensitivity 67-100%, specificity 62-84%) FDA-cleared tests, which use fluorescence in situ hybridization and fluorescence immunodetection respectively [27, 28]. A laboratory-developed BCa detection test that meets the regulatory standards of the Clinical Laboratory Improvement Amendments (CLIA) is the Cxbladder Monitor (sensitivity 82%, specificity 85%), which measures the expression of five genes [30]. Additionally, the commercially available UBC Rapid Test (an immunochromatographic method that measures fragments of cytokeratin 8 and 18) has demonstrated a sensitivity of 59% and a specificity of 76% [31]. In general, these phase III-IV markers suffer from subpar sensitivities. Hence, the field would clearly benefit from newer urine-based tests with higher sensitivities and specificities for the detection of BCa.

All urine candidate biomarkers reviewed in **Table 1** demonstrate sensitivities that surpass those of the FDA-approved biomarkers, and many have specificities that are comparable or better. Notably, Apo-A1 has been independently validated in at least three studies, yielding a sensitivity range of 89-100% and a specificity range of 85-92% (**Table 1**). Apo-A1 is the primary protein component of high-density lipoprotein, and is often used as a biomarker of cardiovascular disease. Lipoproteins are theorized to play a role in facilitating tumor survival through kinase activation or in the development of tumor angiogenesis, but the association between lipoproteins and BCa progression is still not well-understood [19, 20]. Further research into this association would help confirm the value of Apo-A1 and other lipoproteins as biomarkers of BCa. Another promising biomarker included in this review is BLCA-4, which Cai et al. found to demonstrate a sensitivity and specificity of 93% and 97% across nine studies. BLCA-4 is a nuclear transcription factor found in bladder tumors in early stages of disease: its association with BCa is clear, but its value as a marker for BCa detection still requires further validation. Hyaluronidase is a third independently validated biomarker that showed promise, as demonstrated by Pham et al. and Eissa et al. Its sensitivity and specificity ranged from 87-100% and 89-98%, respectively. Hyaluronidases catalyze the breakdown of hyaluronic acid, which functions to facilitate cellular proliferation and motility [20]. Besides these three proteins, which represent phase II-III biomarkers, many other proteins have been evaluated as potential biomarkers for the detection of BCa as shown in **Table 1**; although several exhibit promising performance metrics, these await independent validation by other groups.

Head-to-head comparisons of novel urine biomarker against the FDA-approved tests for BCa detection were conducted in some of the reviewed studies. Goodison et al. found that an eight-biomarker panel and a three-biomarker panel produced a sensitivity of 92% and 90%, and a specificity of 97% and 97%, respectively, while the BTA TRAK ELISA test achieved a sensitivity of 78% and a specificity of 83% in the same cohort for BCa detection [20]. Urquidi et al. compared results from a CCL18 assay to the BTA TRAK test. Urine CCL18 exhibited a sensitivity of 88% and a specificity

of 86%, while BTA TRAK exhibited a sensitivity of 80% and a specificity of 84% for BCa detection [22]. These head-to-head comparisons add significant validity to these studies by comparing the proposed biomarkers against the FDA-approved test in the same patient cohort, hence removing possible confounding factors.

One potential use for these detection biomarkers may be for healthy population screening of BCa, not unlike the population screening that is conducted for breast, cervical, and colon cancer. Early detection (i.e., the detection of BCa at an earlier asymptomatic stage) is vital in BCa as it leads to improved survival [17]. However, population-wide screening has generally been deemed impractical as the general prevalence of BCa is low. Instead, screening specific groups with a high risk of BCa may offer improved cost-benefit yield [17]. For example, Steiner et al. used urine dipstick, NMP22, cytology, and UroVysion to screen 183 at-risk patients who had a greater than 40 pack-per-year smoking history [18]. Out of the 75 patients with at least one positive test result, three were diagnosed with non-muscle invasive BCa. Lotan et al. screened 1502 high risk subjects aged over 50 and a greater than ten-year smoking history or a significant occupational exposure to carcinogens such as dyes, petroleum, or chemicals. The NMP22 BladderChek test was used for screening, and 85 patients had a positive test with two being diagnosed with non-muscle invasive BCa [31]. A high-risk population screening study using any of the novel biomarker candidates listed in **Table 1** has yet to be conducted. Promising evidence from such studies would help transition these early-phase markers into the clinical environment.

Another potential risk-factor based evaluation approach would be to test these markers in patients with gross or asymptomatic microscopic hematuria. However, asymptomatic microscopic hematuria is also seen in many patients without BCa, and practitioners must decide which patients need to undergo a complete evaluation for BCa. The current standard evaluation procedure for patients with hematuria is cystoscopy, which has high sensitivity. Because false negative rates can be high with cystoscopy, cytology is often used as an adjunct test to aid in the detection of bladder cancer. However, cytology is only recommended for use in patients with persistent asymptomatic microscopic hematuria or risk factors such as irritative voiding symptoms, tobacco use, and chemical exposure. Therefore, there is a need for a sensitive urine-based marker that can help physicians determine which patients truly need BCa evaluation. Lotan et al. conducted a prospective multi-center study of patients referred for hematuria evaluation, using a nomogram based on age, gender, smoking status, ethnicity, hematuria, and NMP22 BladderChek results to predict whether a patient would develop BCa. In that study, 23 (6%) of 381 patients were found to have BCa, resulting in a predictive accuracy of 0.79 for the model [35]. Further independent evaluation is needed to assess if any of the biomarkers in **Table 1** could outperform current tools for BCa detection in hematuria patients, in terms of their predictive potential.

Candidate biomarkers that have been evaluated for BCa surveillance (to predict recurrence of NMIBC after initial treatment) are fewer than candidates evaluated for BCa detection. A couple of FDA-approved markers have been extensively evaluated for their surveillance potential. A review of multiple studies reported a median sensitivity of 50% and 64% and a median specificity of 91% and 78% for NMP22 BladderChek (14 studies) and NMP22 ELISA (15 studies), respectively (**Table 2**). BTA exhibits a median sensitivity of 57% and 61% and a median specificity of 86% and 82% when assessed with BTA STAT (12 studies) and BTA TRAK (six studies), respectively (**Table 2**). The FDA-approved ImmunoCyt (fluorescence immunohistochemistry) and UroVysion (FISH) tests exhibit median sensitivities of 74% and 60%, and median specificities of 73% and 90% when assessed over 10 studies and 17 studies, respectively [26]. From the same review surveying two studies assessing the CLIA-approved Cxbladder test and five studies assessing the commercial UBC (bladder cancer) test, median sensitivities of 92% and 61% were reported, and a median specificity of 87% was found for the UBC test. With the exception of Cxbladder, for which only two studies were surveyed, sensitivities tended to be consistently low, and although some tests had median specificities above 90%, others have substantial room for improvement. Among the novel urine proteins interrogated, bladder tumor fibronectin (BTF), a tumor-derived glyco-

protein that binds extracellular matrix components, appears to be a promising singular biomarker that exhibits high sensitivity and comparable specificity in comparison to the FDA-approved and commercially available biomarkers. BTF produced a sensitivity and specificity of 91% and 88% for BCa surveillance, respectively (**Table 2**). Until it is validated by independent groups, however, BTF remains a phase I-II marker for BCa surveillance.

Although several urine protein biomarkers for the detection and surveillance of Bca have demonstrated high sensitivity and specificity relative to FDA-approved and other commercial urinary biomarker tests, none have been validated sufficiently to be considered viable alternatives or additions to current laboratory measures. Potential biomarkers for the detection of BCa, three of which are in phase II-III (as discussed above), are closer to this goal than are biomarker candidates for the surveillance of BCa. Until the improved diagnostic metrics of these potential urine protein markers are validated further, combinations of cystoscopy, cytology, and FDA-approved urinary biomarker tests remain the most reliable BCa detection methods.

Many biomarkers have also been tested for their potential in predicting response to treatment. Kamat et al. tested the FDA-approved UroVysion FISH and found that patients with positive FISH results during BCG therapy were more likely to develop recurrent tumors and suffer disease progression ($p < 0.01$) [34]. Other FDA-approved and commercial tests have yet to be extensively researched for their potential to predict treatment response. Among the newer urine protein treatment response biomarkers evaluated, IL-2, IL-6, IL-8 and TNF-α have been validated independently as promising predictors of response to intravesical BCG therapy, and can be considered phase II-III biomarkers. All have functions associated with immune response and inflammation, and are likely to play key functional roles in disease and response to treatment. Clearly, further validation across multiple institutions and in prospective clinical trials are warranted, in order to transition these promising markers to phase III and IV.

Kamat et al. tested a panel of nine biomarkers to assess BCa response to BCG, and concluded that due to the multifactorial nature of the immune response in BCG antitumor activity, the use of multiple biomarkers may be required to reliably predict patient response to intravesical immunotherapy [24]. This conclusion has tentative support from the other studies presented in this review based on the observation that the group's panel, which produced a high AUC of 0.855, included all of the independently validated biomarkers listed above. This suggests the possibility of increased statistical power when combined biomarkers are used to gauge treatment response, although further independent validation of such multi-marker panels are warranted. Finally, the majority of the markers evaluated in this context assessed BCG response, highlighting the lack of studies testing biomarkers' ability to predict response to chemotherapy, radiotherapy, or other forms of intravesical therapy such as mitomycin or epirubicin.

Several intrinsic aspects pertaining to biomarkers and biomarker studies impose limitations. Considerable variation in biomarker levels is often observed due to differences in subjects (ethnicity, age groups, gender, and comorbidities), detection platforms, and sample collection methods. Few of the studies reviewed here have attempted to control for these variables. Even the potential biomarkers that have been validated by independent groups in this study have yet to be tested over a wider spectrum of cohorts and sample collection techniques. Additionally, it is often the case that only studies with positive results are reported, artificially making certain biomarkers appear more promising than they actually are. The reverse could also happen if biomarkers are not tested with a sufficiently sensitive platform; they may be discarded because they are below the detection limits of the particular platform or kit used. Furthermore, many studies are inherently biased by pre-selecting specific molecules to study. In contrast, a transcriptome-wide or proteome-wide approach is a comprehensive and exhaustive method by which to identify candidate molecules that have yet to be studied as potential biomarkers of BCa. Targeted proteomics using antibodies or aptamers as ligands and high sensitivity mass spectrometry may be especially useful in identifying low-abundance but clinically significant protein biomarkers, in a more comprehensive fashion [25].

In conclusion, urine biomarkers of BCa hold great promise, but would necessitate concerted efforts to screen for markers more comprehensively (using various OMICs platforms), standardization of ELISA kits used, controlling for patient demographics, and extensive multi-institution validation. Given that comprehensive OMICs-based screens for urine biomarkers have yet to be reported for BCa, it is likely that the best BCa biomarkers with the highest clinical utility are yet to be discovered. Finally, the utility of biomarker panels composed of leads from proteomic studies and genomic insights warrant investigation.

Disclosure of conflict of interest

None.

Abbreviations

BCa, bladder cancer; BCG, Bacillus Calmette-Guerin; BTF, bladder tumor fibronectin; CIS, carcinoma in situ; CLIA, Clinical Laboratory Improvement Amendments; ELISA, enzyme-linked immunosorbent assay; NPV, negative predictive value; NMIBC, non-muscle invasive bladder cancer; NMP22, Nuclear matrix protein 22; PPV, positive predictive value.

Address correspondence to: Dr. Chandra Mohan, Science & Engineering Research Center, Department of Biomedical Engineering, 3605 Cullen Blvd. Room 2027, Houston 77204, TX, USA. Tel: 713-743-3709; Fax: 713-743-0226; E-mail: cmohan@central.uh.edu

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics. CA Cancer J Clin 2018; 68: 7-30.

[2] Aldousari S, Kassouf W. Update on the management of non-muscle invasive bladder cancer. Can Urol Assoc J 2010; 4: 56-64.

[3] Gogalic S, Sauer U, Doppler S, Preininger C. Bladder cancer biomarker array to detect aberrant levels of proteins in urine. Analyst 2015; 140: 724-35.

[4] Herr HW. The risk of urinary tract infection after flexible cystoscopy in patients with bladder tumor who did not receive prophylactic antibiotics. J Urol 2015; 193: 548-51.

[5] Shariat SF, Karam JA, Lotan Y, Karakiewizc PI. Critical evaluation of urinary markers for bladder cancer detection and monitoring. Rev Urol 2008; 10: 120-35.

[6] Chamie K, Litwin MS, Bassett JC, Daskivich TJ, Lai J, Hanley JM, Konety BR, Saigal CS; Urologic Diseases in America Project. Recurrence of high-risk bladder cancer: a population-based analysis. Cancer 2013; 119: 3219-3227.

[7] Tan WS, Rodney S, Lamb B, Feneley M, Kelly J. Management of non-muscle invasive bladder cancer: a comprehensive analysis of guidelines from the United States, Europe and Asia. Cancer Treat Rev 2016; 47: 22-31.

[8] Leiblich A. Recent developments in the search for urinary biomarkers in bladder cancer. Curr Urol Rep 2017; 18: 100.

[9] Yafi FA, Brimo F, Steinberg J, Aprikian AG, Tanguay S, Kassouf W. Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. Urol Oncol 2015; 33: 66, e25-31.

[10] Têtu B. Diagnosis of urothelial carcinoma from urine. Mod Pathol 2009; 22 Suppl 2: S53-9.

[11] Sapre N, Anderson PD, Costello AJ, Hovens CM, Corcoran NM. Gene-based urinary biomarkers for bladder cancer: an unfulfilled promise? Urol Oncol 2014; 32: 48, e9-17.

[12] Soloway MS, Briggman V, Carpinito GA, Chodak GW, Church PA, Lamm DL, Lange P, Messing E, Pasciak RM, Reservitz GB, Rukstalis DB, Sarosdy MF, Stadler WM, Thiel RP, Hayden CL. Use of a new tumor marker, urinary NMP22, in the detection of occult or rapidly recurring transitional cell carcinoma of the urinary tract following surgical treatment. J Urol 1996; 156: 363-7.

[13] Miyake M, Goodison S, Giacoia EG, Rizwani W, Ross S, Rosser CJ. Influencing factors on the NMP-22 urine assay: an experimental model. BMC Urol 2012; 12: 23.

[14] Duquesne I, Weisbach L, Aziz A, Kluth LA, Xylinas E. The contemporary role and impact of urine-based biomarkers in bladder cancer. Transl Androl Urol 2017; 6: 1031-1042.

[15] O'Donnell M, Brooks N. Treatment options in non-muscle-invasive bladder cancer after BCG failure. Indian J Urol 2015; 31: 312-9.

[16] Ardelt PU, Kneitz B, Adam P, Reiss C, Kocot A, Fensterle J, Chen L, Pasqualini R, Arap W, Gerharz EW, Riedmiller H. Reactive antibodies against bacillus Calmette-Guerin heat-shock protein-65 potentially predict the outcome of immunotherapy for high-grade transitional cell carcinoma of the bladder. Cancer 2010; 116: 600-609.

[17] Clinton T, Lotan Y. Review of the clinical applications to the use of urine-based tumor markers in bladder cancer. Rambam Maimonides Med J 2017; 8.

[18] Steiner H, Bergmeister M, Verdorfer I, Granig T, Mikuz G, Bartsch G, Stoehr B, Brunner A. Early results of bladder-cancer screening in a high-

risk population of heavy smokers. BJU Int 2008; 59: 1026-31.
[19] Gourin CG, Zhi W, Adam BL. Proteomic identification of serum biomarkers for head and neck cancer surveillance. Laryngoscope 2009; 119: 1291-302.
[20] Yang HH, Chen XF, Hu W, Lv DQ, Ding WJ, Tang LJ, Jiang JJ, Ye MH. Lipoprotein(a) level and its association with tumor stage in male patients with primary lung cancer. Clin Chem Lab Med 2009; 47: 452-457.
[21] Toole BP. Hyaluronan in morphogenesis. J Intern Med 1997; 242: 35-40.
[22] Goodison S, Chang M, Dai Y, Urquidi V, Rosser CJ. A multi-analyte assay for the non-invasive detection of bladder cancer. PLoS One 2012; 7.
[23] Urquidi V, Kim J, Chang M, Dai Y, Rosser CJ, Goodison S. CCL18 in a multiplex urine-based assay for the detection of bladder cancer. PLoS One 2012; 7: e37797.
[24] Kamat AM. Corrigendum re: "cytokine panel for response to intravesical therapy (CyPRIT): nomogram of changes in urinary cytokine levels predicts patient response to bacillus calmette-guérin" [Eur Urol 2016;69:197-200]. Eur Urol 2016; 70: e26.
[25] Schiess R, Wollscheid B, Aebersold R. Targeted proteomic strategy for clinical biomarker discovery. Mol Oncol 2009; 3: 33-44.
[26] Goebell PJ, Groshen SL, Schmitz-Dräger BJ. Guidelines for development of diagnostic markers in bladder cancer. World J Urol 2008; 26: 5-11.
[27] Shariat SF, Canto EI, Kattan MW, Slawin KM. Beyond prostate-specific antigen: new serologic biomarkers for improved diagnosis and management of prostate cancer. Rev Urol 2004; 6: 58-72.
[28] Soria F, Droller MJ, Lotan Y, Gontero P, D'Andrea D, Gust KM, Rouprêt M, Babjuk M, Palou J, Shariat SF. An up-to-date catalog of available urinary biomarkers for the surveillance of non-muscle invasive bladder cancer. World J Urol 2018; 36: 1981-1995.
[29] Rosolen DCB, Faria DK, Faria CS, Antonangelo L. Performance of the UroVysion® FISH assay for the diagnosis of malignant effusions using two cutoff strategies. Cancer Med 2018; 7: 1967-1977.
[30] Sullivan PS, Chan JB, Levin MR, Rao J. Urine cytology and adjunct markers for detection and surveillance of bladder cancer. Am J Transl Res 2010; 2: 412-40.
[31] Lotan Y, Elias K, Svatek RS, Bagrodia A, Nuss G, Moran B, Sagalowsky AI. Bladder cancer screening in a high risk asymptomatic population using a point of care urine based protein tumor marker. J Urol 2009; 182: 52-7.
[32] O'Sullivan P, Sharples K, Dalphin M, Davidson P, Gilling 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; 188: 741-747.
[33] Lu P, Cui J, Chen K, Lu Q, Zhang J, Tao J, Han Z, Zhang W, Song R, Gu M. Diagnostic accuracy of the UBC® Rapid test for bladder cancer: a meta-analysis. Oncol Lett 2018; 16: 3770-3778.
[34] Kamat AM, Dickstein RJ, Messetti F, Anderson R, Pretzsch SM, Gonzalez GN, Katz RL, Khanna A, Zaidi T, Wu X, Grossman HB, Dinney CP. Use of fluorescence in situ hybridization to predict response to bacillus Calmette-Guérin therapy for bladder cancer: results of a prospective trial. J Urol 2012; 187: 862-7.
[35] Lotan Y, Svatek RS, Krabbe LM, Xylina E, Klatte T, Shariat SF. Prospective external validation of a bladder cancer detection model. J Urol 2014; 192: 1343-8.
[36] Wang Z, Que H, Suo C, Han Z, Tao J, Huang Z, Ju X, Tan R, Gu M. Evaluation of the NMP22 BladderChek test for detecting bladder cancer: a systematic review and meta-analysis. Oncotarget 2017; 8: 100648-656.
[37] Guo A, Wang X, Gao L, Shi J, Sun C, Wan Z. 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: E347-352.
[38] Glas A, Roos D, Deutekom M, Zwinderman AH, Bossuyt PM, Kurth KH. Tumor markers in the diagnosis of primary bladder cancer. a systematic review. J Urol 2003; 169: 1975-82.
[39] Li H, Li C, Wu H, Zhang T, Wang J, Wang S, Chang J. Identification of Apo-A1 as a biomarker for early diagnosis of bladder transitional cell carcinoma. Proteome Sci 2011; 9: 21.
[40] Li C, Li H, Zhang T, Li J, Liu L, Chang J. Discovery of Apo-A1 as a potential bladder cancer biomarker by urine proteomics and analysis. Biochem Biophys Res Commun 2014; 446: 1047-52.
[41] Chen Y, Chen C, Chen H, Chung T, Wu C, Chen C, Hsu C, Chen M, Tsui K, Chang P, Chang Y, Yu J. Discovery of novel bladder cancer biomarkers by comparative urine proteomics using iTRAQ technology. J Proteome Res 2010; 9: 5803-5815.
[42] Kumar P, Nandi S, Tan TZ, Ler SG, Chia KS, Lim W, Bütow Z, Vordos D, De laTaille A, Al-Haddawi M, Raida M, Beyer B, Ricci E, Colombel M, Chong TS, Chiong E, Soo R, Park MK, Ha HK, Gunaratne J, Thiery JP. Highly sensitive and specific novel biomarkers for the diagnosis of

transitional bladder carcinoma. Oncotarget 2015; 6: 13539-49.
[43] Cai Q, Wu Y, Guo Z, Gong R, Tang Y, Yang K, Li X, Guo X, Niu Y, Zhao Y. Urine BLCA-4 exerts potential role in detecting patients with bladder cancers: a pooled analysis of individual studies. Oncotarget 2015; 6: 37500-10.
[44] Inoue K, Ota U, Ishizuka M, Kawada C, Fukuhara H, Shuin T, Okura I, Tanaka T, Ogura S. Porphyrins as urinary biomarkers for bladder cancer after 5-aminolevulinic acid (ALA) administration: the potential of photodynamic screening for tumors. Photodiagnosis Photodyn Ther 2013; 10: 484-9.
[45] Eissa S, Matboli M, Essawy NO, Kotb YM. Integrative functional genetic-epigenetic approach for selecting genes as urine biomarkers for bladder cancer diagnosis. Tumour Biol 2015; 36: 9545-52.
[46] Pham HT, Block NL, Lokeshwar VB. Tumor-derived hyaluronidase: a diagnostic urine marker for high-grade bladder cancer. Cancer Res 1997; 57: 778-83.
[47] Lorenzi T, Lorenzi M, Altobelli E, Marzioni D, Mensà E, Quaranta A, Paolinelli F, Morroni M, Mazzucchelli R, De Luca A, Procopio AD, Baldi A, Muzzonigro G, Montironi R, Castellucci M. HtrA1 in human urothelial bladder cancer: a secreted protein and a potential novel biomarker. Int J Cancer 2013; 133: 2650-61.
[48] Rosser CJ, Dai Y, Miyake M, Zhang G, Goodison S. Simultaneous multi-analyte urinary protein assay for bladder cancer detection. BMC Biotechnol 2014; 14: 24.
[49] Li F, Yu Z, Chen P, Lin G, Li T, Hou L, Du Y, Tan W. The increased excretion of urinary orosomucoid 1 as a useful biomarker for bladder cancer. Am J Cancer Res 2016; 6: 331-40.
[50] Srivastava AK, Singh PK, Singh D, Dalela D, Rath SK, Bhatt ML. Clinical utility of urinary soluble Fas in screening for bladder cancer. Asia Pac J Clin Oncol 2016; 12: e215-21.
[51] Rosser CJ, Chang M, Dai Y, Ross S, Mengual L, Alcaraz A, Goodison S. Urinary protein biomarker panel for the detection of recurrent bladder cancer. Cancer Epidemiol Biomarkers Prev 2014; 23: 1340-5.
[52] Li LY, Yang M, Zhang HB, Su XK, Xu WF, Chen Y, Shen ZJ, Gao X. Urinary fibronectin as a predictor of a residual tumour load after transurethral resection of bladder transitional cell carcinoma. BJU Int 2008; 102: 566-71.
[53] De Paoli M, Gogalic S, Sauer U, Preininger C, Pandha H, Simpson G, Horvath A, Marquette C. Multiplatform biomarker discovery for bladder cancer recurrence diagnosis. Dis Markers 2016; 2016: 4591910.
[54] Shariat SF, Matsumoto K, Casella R, Jian W, Lerner SP. Urinary levels of soluble e-cadherin in the detection of transitional cell carcinoma of the urinary bladder. Eur Urol 2005; 48: 69-76.
[55] Wahren B, Nilsson B, Zimmerman R. Urinary CEA for prediction of survival time and recurrence in bladder cancer. Cancer 1982; 50: 139-45.
[56] Jackson AM, Ivshina AV, Senko O, Kuznetsova A, Sundan A, O'Donnell MA, Clinton S, Alexandroff AB, Selby PJ, James K, Kuznetsov VA. Prognosis of intravesical bacillus Calmette-Guerin therapy for superficial bladder cancer by immunological urinary measurements: statistically weighted syndrome analysis. J Urol 1998; 159: 1054-63.
[57] Saint F, Patard JJ, Maille P, Soyeux P, Hoznek A, Salomon L, De La Taille A, Abbou CC, Chopin DK. T helper 1/2 lymphocyte urinary cytokine profiles in responding and nonresponding patients after 1 and 2 courses of bacillus Calmette-Guerin for superficial bladder cancer. J Urol 2001; 166: 2142-7.
[58] Saint F, Patard JJ, Maille P, Soyeux P, Hoznek A, Salomon L, Abbou CC, Chopin DK. Prognostic value of a T helper 1 urinary cytokine response after intravesical bacillus Calmette-Guerin treatment for superficial bladder cancer. J Urol 2002; 167: 364-7.
[59] Saint F, Kurth N, Maille P, Vordos D, Hoznek A, Soyeux P, Patard JJ, Abbou CC, Chopin DK. Urinary IL-2 assay for monitoring intravesical bacillus Calmette-Guérin response of superficial bladder cancer during induction course and maintenance therapy. Int J Cancer 2003; 107: 434-40.
[60] Watanabe E, Matsuyama H, Matsuda K, Ohmi C, Tei Y, Yoshihiro S, Ohmoto Y, Naito K. Urinary interleukin-2 may predict clinical outcome of intravesical bacillus Calmette-Guérin immunotherapy for carcinoma in situ of the bladder. Cancer Immunol Immunother 2003; 52: 481-6.
[61] Sanchez-Carbayo M, Urrutia M, Romani R, Herrero M, Gonzalez de Buitrago JM, Navajo JA. Serial urinary IL-2, IL-6, IL-8, TNFalpha, UBC, CYFRA 21-1 and NMP22 during follow-up of patients with bladder cancer receiving intravesical BCG. Anticancer Res 2001; 21: 3041-7.
[62] de Reijke TM, de Boer EC, Kurth KH, Schamhart DH. Urinary cytokines during intravesical bacillus Calmette-Guerin therapy for superficial bladder cancer: processing, stability and prognostic value. J Urol 1996; 155: 477-82.
[63] Sagnak L, Ersoy H, Ozok U, Senturk B, Ercil H, Bahar G, Ozturk E. Predictive value of urinary interleukin-8 cutoff point for recurrences after transurethral resection plus induction bacillus Calmette-Guérin treatment in non-muscle-invasive bladder tumors. Clin Genitourin Cancer 2009; 7: E16-23.

[64] Thalmann GN, Sermier A, Rentsch C, Möhrle K, Cecchini MG, Studer UE. Urinary Interleukin-8 and 18 predict the response of superficial bladder cancer to intravesical therapy with bacillus Calmette-Guérin. J Urol 2000; 164: 2129-33.

[65] Kumar A, Dubey D, Bansal P, Mandhani A, Naik S. Urinary interleukin-8 predicts the response of standard and low dose intravesical bacillus Calmette-Guerin (modified Danish 1331 strain) for superficial bladder cancer. J Urol 2002; 168: 2232-5.

[66] Thalmann GN, Dewald B, Baggiolini M, Studer UE. Interleukin-8 expression in the urine after bacillus Calmette-Guerin therapy: a potential prognostic factor of tumor recurrence and progression. J Urol 1997; 158: 1340-4.

[67] Eto M, Koga H, Noma H, Yamaguchi A, Yoshikai Y, Naito S. Importance of urinary interleukin-18 in intravesical immunotherapy with bacillus calmette-guérin for superficial bladder tumors. Urol Int 2005; 75: 114-8.

[68] Hausladen DA, Wheeler MA, Altieri DC, Colberg JW, Weiss RM. Effect of intravesical treatment of transitional cell carcinoma with bacillus Calmette-Guérin and mitomycin C on urinary survivin levels and outcome. J Urol 2003; 170: 230-4.

[69] Shintani Y, Sawada Y, Inagaki T, Kohjimoto Y, Uekado Y, Shinka T. Intravesical instillation therapy with bacillus Calmette-Guérin for superficial bladder cancer: study of the mechanism of bacillus Calmette-Guérin immunotherapy. Int J Urol 2007; 14: 140-6.

[70] Ludwig AT, Moore JM, Luo Y, Chen X, Saltsgaver NA, O'Donnell MA, Griffith TS. Tumor necrosis factor-related apoptosis-inducing ligand: a novel mechanism for bacillus calmette-guérin-induced antitumor activity. Cancer Res 2004; 64: 3386-90.