Urinary Tumor Markers for Detection and Surveillance in Non-Muscle Invasive Bladder Cancer

Mohamed Jamal Saadh[1], Abeer Mohammad Kharshid[1], Hala Mousa Sbaih[1], Khalil Altaif[1]

[1]Department of Pharmacy, Faculty of Pharmacy, Middle East University, Amman, Jordan

Abstract

Objectives: Cystoscopy still remains the gold standard for diagnosis and follow-up monitoring of bladder cancer (BC). Unfortunately, it is an invasive, and the urine cytology is a highly specific, sensitivity for detection of low grade tumors is only 4% to 31%. The advent of noninvasive urine-based markers as well as other novel modalities has yielded improved diagnostic accuracy.

Material and method: Pubmed/Medline search was conducted to identify original articles, review articles, and editorials regarding urine-based biomarkers for screening, early detection, and surveillance of urothelial carcinoma of the bladder. **Results:** urinary marker shave shown higher sensitivity compared with cytology, and most markers suffer from lower specificity than cytology. In this review, we aimed to summarize the current knowledge on commercially available and promising investigational Urinary tumor markers for detection and surveillance in Non-Muscle Invasive BC. **Conclusions:** biomarkers into clinical decision making will be of value for BC detection and screening in the future.

Keywords: *Bladder cancer (BC) , Biomarkers, Urinary marker.*

Introduction

Bladder cancer (BC), a highly aggressive and heterogeneous disease, is the most common malignancy of the urinary tract. [(1)]. The highest incidence rates of BC are generally found in industrially developed Countries[(2)].

Early diagnosis of BC allows for effective local treatment and optimizes the success of surgical therapy[(3)]. In this review, we use this framework to discuss the urinary biomarkers for early diagnosis of BC.

1. **Cytology**

Urinary cytology has been established as the standard noninvasive urinary marker for detecting BC. High-grade cancers are more likely than low-grade tumors to shed their cells into the urine due to their weaker intercellular attachments [(1)]. Many studies demonstrated a poor sensitivity for low-grade BC (13% to 75%), with improved sensitivity and specificity for high-grade disease. The sensitivity for high-grade cancers has been over 80%. The overall sensitivity of cytology ranges from 25 to 70% [(2)].

2. ImmunoCyt

ImmunoCyt combines cytology with an immunefluorescence assay[(3)]. It detects cellular biomarkers for BC in exfoliated urothelial cells using fluorescent monoclonal antibodies for a high molecular weight form of carcinoembryonic antigen and two bladder tumor cell associated mucins. The test requires trained personnel, it is expensive, and a large number of exfoliated cells are necessary to perform an accurate test[(4)]. ImmunoCyt has a reported overall sensitivity of 50–100% [(5)]. Its specificity has been reported as 69–79%, with a higher false positive rate in patients with benign prostatic hyperplasia or cystitis[(4, 5)].

3. Fluorescence in situ hybridization (FISH)

FISH is a molecular test that utilizes DNA probes to identify the most common urothelial carcinoma related chromosomal changes in exfoliated cells in the

Corresponding Author:
Dr. Mohamed Jamal Saadh
Faculty of Pharmacy- Middle East University
Tel. 00962 786945883
E-mail: msaadeh@meu.edu.jo

urine. The test was designed to detect aneuploidy of chromosomes 3, 7, 17 and the loss of the 9p21 locus in malignant urothelial cells [6].

The sensitivity of the Urovysion FISH test to be between 69 and 85%, with a specificity of 78 to 92% [7, 8]. In a direct comparison of FISH with cytology and NMP-22, FISH was found to have better sensitivity for low grade tumors. The combination of FISH and cytology detected 97.4% of cancers, while the combination of cytology and NMP-22 detected 92.1% [9].

4. Aurora kinase A (AURKA)

The Aurora kinase A (AURKA) gene encodes a serine/ threonine kinase associated with aneuploidy and chromosome instability. This gene has been explored in urine sediment by FISH. A training set was used to establish test conditions. A separate testing set of 100 patients with BC, 92 healthy individuals, and 56 patients with benign urologic disease reported a test sensitivity of 87% and a specificity of 97% [10].

5. Cytokeratins

Cytokeratins are intermediate filaments proteins that are part of the framework of eukaryotic cells; their main function is to enable cells to withstand mechanical stress. In humans 20 different cytokeratin isotypes have been identified. Cytokeratins 8, 18, 19 and 20 have been associated with BC [11]. The urinary BC test detects cytokeratin 8 and 18 fragments in urine. The sensitivity of the urinary BC test varies from 35% to 79% [12].

6. Bladder tumor antigen (BTA)

BTA stat and BTA-TRAK are designed to detect bladder tumor associated antigen in voided urine. This antigen is a human complement factor H–related protein similar to human complement factor H. BTA interacting with complement factor C3b interrupts the complement cascade, potentially conferring a selective growth advantage to cancer cells. In cell culture, normal cells do not express the H-related protein[13].

The sensitivity and specificity of BTA stat ranged from 67 to 70% and 75 to 78%, respectively. The sensitivity and specificity of BTA-TRAK was noted at 66 and 65%, respectively[14]. BTA stat demonstrates improved sensitivity with increasing histological grade; 53, 76, and 90% for grades 1 through 3. It has also been noted to have improved detection and sensitivity for superficial and low-grade tumors compared to urinary cytology; however, at the cost of lower specificity[13]. The infection or urinary lithiasis, may lead to a false positive for BTA. In addition, false positives may be seen in patients with a history of Bacillus Calmette-Guerin (BCG) therapy or urinary diversions utilizing the bowel.

7. LEWIS X

Monoclonal antibodies have also been utilized to detect the Lewis X antigen on exfoliated urothelial cells. The Lewis X is a blood group antigen that is normally absent from urothelial cells in the adult, but is expressed in transitional cell tumors regardless of the secretor status, grade, or stage of the tumor[15]. Commercially available antibodies, such as the P-12 murine monoclonal antibody, have been utilized to target and detect the Lewis X antigen[8].

8. ACCU-DX

The Accu-Dx test was developed as a qualitative point-of-care immunoassay utilizing murine monoclonal antibodies specific for (fibrin/fibrinogen degradation products) FDP. However, since these antibodies have also been noted to react with intact fibrinogen typically found in human serum, the usefulness of the test in the presence of hematuria may be low[16].

9. Hyaluronic acid (HA)

HA is an extracellular glycosaminoglycan that supports tumor cell adhesion and migration, and offers some protection from immune system surveillance in tumor tissues. Small fragments of HA stimulate angiogenesis and are produced by hyaluronidase (HAase). The small HA fragments have been identified in the urine of patients with BC[16]. The importance of HA and HAase in BC pathogenesis has been noted in a study in which staining of BC tissues for HA and HAase correlated with muscle-invasive disease and recurrence[17]. Hyaluronoglucosaminidase-1 (HYAL-1) is a specific HAase that has been identified as a marker for cancer detection, and is a molecular predictor for tumor growth, invasion, and angiogenesis. HYAL-1 mRNA levels have been noted to be elevated 10 to 30 fold higher in bladder cells and tissues that express high HAase activity, as well as in the urine of patients with BC[16].

10. Microsatellite Analysis

One of the most common genetic changes in BC is

loss of heterogeneity in chromosome 9. Chromosomes 4p, 8p, 9p, 11p, and 17p also often display loss of heterogeneity in patients with BC[18].

Microsatellite analysis evaluates for tumor specific genomic alterations in a different manner than FISH. Microsatellite analysis targets highly polymorphic, short tandem repeats. This technique evaluates the shift in the normal ratio of two alleles that occurs with genomic alterations from tumor cell transformation. This loss of heterozygosity can be used as a biomarker of a suspected neoplastic process. It had already been established that microsatellite changes in urine samples matched DNA extracts from tumor tissues[19].

11. BLCA-1 and BLCA-4

BLCA-1 and BLCA-4 are nuclear transcription factors present in BC. BLCA-1 is not expressed in nonmalignant urothelium, whereas BLCA-4 is expressed in both the tumor and adjacent benign areas of the bladder but not in nonmalignant bladders[20].

A nuclear matrix protein specific to BC tissues, termed BLCA-4, has been identified. It holds great promise as a potent urine based bladder tumor marker[5].

12. CEACAM1

Bladder tumor growth and progression depend on angiogenesis. Human carcinoembryonic antigen-related cell adhesion molecule (CEACAM)1 is a cell adhesion molecule with proangiogenic activity. It has previously been observed that CEACAM1, which is ubiquitously expressed in the luminal surface of normal bladder urothelium, is down regulated in BC cells while it is concurrently up regulated in endothelial cells of adjacent blood vesse [5]. This differential switch in CEACAM1 expression is accompanied by an up regulation of proangiogenic and prolymphangiogenic factors. Based on these findings, it was assessed whether CEACAM1 was detectable in urine and whether its levels could help differentiate BC patients from healthy subjects[5].

13. MicroRNA markers

MicroRNAs (miRNAs) are noncoding RNAs that post transcriptionally regulate gene expression[21]. They might serve as an ideal bladder biomarker because they are stable within urine and require little handling care[22], and they are more stable against nuclease degradation due to their small size. Urine contains many nucleases, and assays to examine mRNA expression often fail due to target degradation or require stringent prelaboratory handling of the urine sample. Recently, urinary miRNA expression was reported and the upregulation of miRs-126/182/199a was found to discriminate BC patients from disease-free controls[23].

14. Survivin

Survivin, a novel member of the inhibitor-of-apoptosis gene family, is prominently reexpressed in many types of cancer. Survivin messenger ribonucleic acid (mRNA) is overexpressed in human cancers and can be detected in urine using a bio-dot immunoassay incorporating a rabbit polyclonal antisurvivin antibody[24]. Urinary levels of survivin gene activation, both at the protein and the mRNA level are associated with BC presence, higher grade, and advanced pathologic stage[5].

15. Proteomics and genomics

Advances in the fields of molecular genetics and tissue microarrays have led to the development of many potential novel biomarkers. The chemokine CXCL-1 and the matrix metalloproteinases MMP-2 and 9 have been identified using these immunochemical techniques as potential urine based markers[25].

16. Additional urinary markers

Many other potential biomarkers have been evaluated for their potential usefulness as a urine-based tumor marker. Some of the more promising of these have included cytokeratin CK20, The CK20 assay has 78 to 87% sensitivity and a 56 to 80% specificity for BC detection. A summary of these current and emerging bladder tumor biomarkers are shown in, Table (1)[8].

17. Nuclear matrix proteins (NMP)

NMP-22 is a nuclear mitotic apparatus that is involved in proper distribution of chromatin to daughter cells during cellular replication[25]. It is located in the mitotic spindle during mitosis. NMP-52 and NMP-22 is present at a relatively low level in the interphase nuclear matrix. It is probably released from the nuclei of the tumor cells during apoptosis. NMP-52 and NMP-22 concentration is at least 25 fold greater in BC than in mean levels isolated from normal bladder and also five times greater in urine from patients with pyuria, urolithiasis, hematuria, and cystitis comparing with normal bladder[1, 26].

The NMP marker was identified in the urine of patients with BC at 52 kDa. The dot-ELISA detected the urinary NMP-52 marker in 92% of patients with squamous cell carcinoma, 98% with transitional cell carcinoma, and all six of those with adenocarcinoma of the bladder, with a specificity of 94%[27].

Table (1). Summary of urinary markers and their performance[8].

Urinary Marker	Sensitivity %	Specificity %	Clinical Status
Cytology	12.2-79	78.4-99.4	Laboratory
Quanticyt	42.1-69	67.9-87	Investigational
FISH	69-92.1	89-94.5	Laboratory
NMP-22	49.5-92.1	66-87.3	Laboratory & point of care
BAT-Stat	50-70	67-78	Point of care
Immunocyt	66.7-84.9	62-84.7	Laboratory
FDP (Accu-Dx)	52-68.4	79.6-91	Point of care
Telomrase:			
TRAP	77.4-90	88-93.5	Investigational
Htert	84.8-95	43.8-93.5	
Hyaluronic acid:			
HA	61-83.1	53.6-90.1	Investigational
HYAL-1	57.6-91	78-100	
HAase	81.5	83.8	
HA/HAase	88.1-94	63-84.4	
Lewis X	79.8-84	80-86.4	Investigational
Survivin	75	100	Investigational
LOH	60-97	93	Investigational
BLCA-4	89-94.4	95-100	Investigational
UPK3A	83	83	Investigational

Discussion

Urinary cytology is lake the sensitivity for different grades of BC specifically in low- grade tumors[1, 2]. Urine markers have been investigated; however, to date ,no marker has reached widespread use. Although urinary markers have shown higher sensitivity compared with cytology, most suffer from low specificity[18]. Combination of different markers is promising concept and seems to represent the future. Another limitation is that each setting(screening/early detection and surveillance) suffers from different requirements. Finally,

each marker has also to prove its cost effectiveness and also the advent of noninvasive urine based markers as well as other novel modalities has yielded improved diagnostic accuracy[26].

Conclusions

Future studies are needed to clarify the prospective biological markers in different stage and grade of BC; The combination of urinary markers and urine cytology is a good diagnostic tool for BC in different pathological types of BC.

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