Urine as an emerging liquid biopsy for bladder cancer biomarkers

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INTRODUCTION

Bladder cancer (BC) is the sixth most prevalent cancer worldwide in both men and women, with incidence and mortality increasing year by year[1]. BC can originate in the urothelial cells lining inside the organ or other parts of the urinary tract[2].

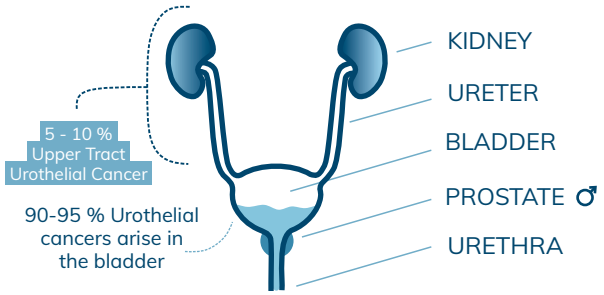

Image adapted from https://www.hopkinsmedicine.org/greenberg-bladder-cancer-institute/utuc/#:~:text=While%20the%20majority%20of%20urothelial,that%20kidney%20to%20the%20bladder

Figure 1: Urinary Tract

In general, most types begin as non-muscle invasive urothelial carcinoma of the urinary bladder (UCB), which are localized to a region and do not invade the muscular wall and can be treated effectively. However, recurrence rates are high, with progression to muscle invasion UCB seen in 15% of patients[3].

Given the high rate of recurrence, regular follow-ups are necessary to monitor progression[4]. Guidelines from the European Association of Urology (EAU) and the American Urological Association (AUA) suggest a combination of cystoscopy, cytology and imaging for surveillance of patients with UCB[5], procedures that can be invasive and expensive. This intensive follow-up program makes BC one of the most expensive cancers to monitor[1].

Consequently, finding novel diagnostic strategies, that are non-invasive as well as cost-effective, can improve the quality of life of patients with BC. This white paper focuses on urine as sample type in BC research and how urinary biomarkers can be used for initial detection and follow-up of the disease.

CURRENT DETECTION METHODS ARE INVASIVE AND/OR OFFER POOR SENSITIVITY

Urinary biomarkers can improve detection. Currently, two methods are commonly used to detect BC:

Cystoscopy is the gold standard method for BC diagnosis, and involves inserting a cystoscope to observe any abnormal changes in the bladder. While cystoscopy can offer sensitivity of up to 90%, it can miss detection of small tumors[2]. Additionally, the procedure is invasive, can be highly uncomfortable for the patient[1] and requires the experience of a urologist or nurse[5].

Urine cytology involves observing a urine sample under a microscope to check for abnormal cells. Together with cystoscopy, this test is used in the diagnosis and follow-up of BC patients. Benefits of urine cytology are that it is non-invasive, inexpensive and offers a specificity of up to 98% for high-grade tumor detection. However, urinary cytology has an overall low sensitivity (less than 40%), making it challenging to use as a diagnostic tool for BC suspicion[2]. Additionally, voided urine cytology requires trained cyto-pathologists and can be subject to inter-observer variability[5].

As cystoscopy and urine cytology may not always be practical or feasible and is a costly procedure to follow repeatedly, finding alternative ways to detect as well as monitor BC is actively being researched[2].

There is an increased interest in non-invasive urinary biomarkers for initial detection and follow-up of the disease[5]. Given the function of the bladder and its close proximity to the urinary tract, urine as a sample type is particularly exciting as it can contain a reliable source of cancer biomarkers[6].

Biomarker detection in urine for BC is also attractive as it allows non-invasive collection, as well as offers the possibility of repeated sampling. Table 1 shows the range of commercially urinary biomarker tests and kits that are available for the detection of BC.

Test	FDA/CE	Starting material	Biomarker type	Sensitivity	Specificity
uCyt+	Y Y	Exfoliated cells	Antigens/ Metabolites	73%	66%
NMP22	Y Y	Exfoliated cells	Peptides	40%	99%
UroVysion	Y Y	Exfoliated cells	DNA	72%	83%
BTA stat / BTA Track	Y Y	Exfoliated cells	Proteins	70%	75%
CxBladder	N N	Exfoliated cells	mRNA	82%	85%
Xpert Detection	N Y	Exfoliated cells	mRNA	76%	85%
Uromonitor	N Y	Exfoliated cells	Tumor cell DNA	74%	93%

Table 1: Commercially available urine tests for bladder cancer detection

TYPES OF BIOMARKERS IN URINE

Current FDA-approved tests have poor sensitivity or specificity, especially for low-grade and early-stage BC tumors and recurrent diagnoses. Therefore, other urinary biomarkers for BC diagnostics are currently being investigated[7].

Possible biomarker candidates for BC in urine include exfoliated bladder cancer cells (EBCCs), cell-free DNA (cfDNA) and exosomes[8]. Exosomes, which are a class of extracellular vesicles released by all cells, and containing DNA, RNA, and proteins, are particularly promising as they represent a fingerprint of the cell of origin[7,9]. Recent advancements in OMICs technologies, including genomics, epigenomics, proteomics, transcriptomics, and metabolomics have also improved our understanding of the molecular landscape causing cancers[10].

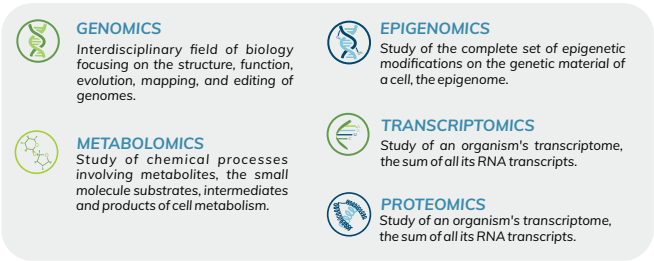

Figure 2: Definitions of omics

Genomic biomarkers

One particularly interesting biomarker that has shown to be a game-changer for disease monitoring and early detection of recurrence in BC is telomerase reverse transcriptase (TERT) promotor mutations. These mutations are extremely specific to BC and are not present in inflammatory or urinary infections, which are current drawbacks with the current non-invasive assays. These biomarkers are currently being included in BC urine-based tests, Uromonitor, Uromonitor-V2, and UroSEEK[1].

In low-grade non-muscle invasive bladder cancer (NMIBC) tumors, mutations in the fibroblast growth factor receptor 3 (FGFR3) oncogene are frequent. One study showed the sensitivity for detecting BC by the FGFR3 mutation was 58%[11]. Alternatively, in high-grade NMIBC tumors, mutations in p53 genes, which can cause dysregulation of the RAS-MAPK (mitogen-activated protein kinase) pathway are seen more often. Mutations in these genes are a strong indicator for BC. Mutations in RAS (Rat sarcoma) oncogenes occur in 13% of all BC tumors, providing valuable urinary biomarker candidates[7].

Epigenomic biomarkers

Epigenetic factors also play an important role in the development of BC and can be useful biomarkers for disease detection and monitoring. Several studies have revealed the role of DNA methylation and methylated genes in influencing gene expression, ultimately leading to the development and progression of BC. However, further validation of these markers is required[7].

DNA methylation is a biological process by which methyl groups are added to the DNA molecule. Methylation can change the activity of a DNA segment without changing the sequence

- ***Hypermethylation*** *– an increase in the epigenetic methylation of DNA*
- ***Hypomethylation*** *– a decrease in the epigenetic methylation of DNA*

EpiCheck, a urine-based assay that analyses 15 DNA methylation markers commonly altered in BC, is currently the only developed product that looks at these biomarkers[1].

Transcriptomic biomarkers

Several studies have shown the potential of miRNAs in the detection of BC. While some studies used a single and others a panel of up and/or downregulated miRNAs, all of them showed sensitivity ranging from 72% to 90% and specificity ranging from 82% to 90%[1].

Long non-coding RNAs (lncRNAs) are important regulators of genetic and epigenetic expression and can interact with miRNAs promoting or repressing its activity

A recent meta-analysis concluded lncRNAs in urine may serve as non-invasive diagnostic biomarkers for BC, but more work is needed in this space[12].

Proteomic biomarkers

Several urinary protein biomarkers for BC have been identified. One study comparing 46 patients with BC and 40 healthy controls reported urinary calprotectin can detect the cancer type with 80% sensitivity at 92% specificity. The median calprotectin level was 10-fold higher in patients than healthy controls[13].

Other studies reported similar findings with a higher abundance of proteins in urine samples of BC patients ($p < 0.05$) compared with matched controls[14].

For example, urinary proteins, stathmin-1 (also known as oncoprotein-18) and CD147 (also known as basigin or EMMPRIN) have also shown potential in BC detection. In a group of 30 patients and 30 controls, stathmin-1 had a sensitivity and specificity of 90% and 87% respectively, while CD147 a 97% and 100% respectively[15]. Additionally, increased levels of Reg-1 (lithostathine-1-alpha) were also found in urine of BC patients[16].

Urothelial bladder carcinoma 1 (BLCA-1) and urothelial bladder carcinoma 4 (BLCA-4) are nuclear matrix proteins (NMPs) which can be elevated early in the development of BC, thereby can be used to detect the cancer in its initial stages, even before the appearance of a visible tumor[7].

Table 2 shows a list of possible urine protein biomarkers that have been investigated in BC detection[7]:

Calprotectin	Cytokeratins 8, 18, 19	APOC2
Stathmin-1	Survivin	APOC3
CD147	ProEGF	APOE
Reg-1	SAA4	CCL18
BLCA-1	APOA1	PAI-1
BLCA-4	APOA2	CD44
Hyaluronidase	APOB	

Table 2: Protein biomarkers investigated for bladder cancer detection

Metabolomic biomarkers

A number of urinary metabolites for BC have been identified. A study profiling urine metabolites found 12 differential metabolites that distinguished the disease from control groups with a sensitivity of 91.3% and specificity of 92.5%[17].

Further, a recent study performed urine metabolic profiling on two subject cohorts with and without BC in three independent platforms. A set of candidate biomarkers for BC including palmitoyl sphingomyelin, lactate, gluconate, adenosine, 2-methylbutyryl-glycine and guanidinoacetate were suggested[18].

FUTURE PERSPECTIVES

Given the wide array of biomarkers, urine is a promising sample type that can change the way the disease is detected and monitored in the future. Urine sampling also offers several other benefits as it is easy, quick and non-invasive.

However, for effective clinical applications, standardization of pre-analytical conditions for the handling of urine specimens is required. More work needs to be done to better understand if variables such as urine collection, urine fractions, use of protease inhibitors, storage, and shipping conditions can influence sample quality and/or have an impact on BC biomarker detection[6].

Novosanis' Colli-Pee®, a urine collection device prefilled with preservative, allows for volumetric collection of different urine volumes, which can facilitate and standardize detection and stabilization of urinary biomarkers in cancer research.

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