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REVIEW

The Development of Non-Invasive Diagnostic Tools in Bladder Cancer

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Abstract: Bladder cancer is a common urinary tract cancer with a difficult clinical course. With frequent recurrence, patients with a history of bladder cancer often undergo surveillance that involves invasive cystoscopies and biopsies. Not only is this financially burdensome for patients but it is also mentally and physically intensive. Given this predicament, the field has shifted towards the use of non-invasive urinary tests to detect bladder cancer earlier in the disease course and to avoid unnecessary procedures. The first non-invasive test developed was urine cytology; however, that was found to have a low sensitivity, especially for low-grade lesions. There are many tests that are available that utilize common protein biomarkers to enhance the sensitivity of detection. However, many of these tests lack the specificity seen with cytology. With recent technological and research advancements, there are newer detection systems such as RNA sequencing and microfluidics along with novel bladder cancer biomarkers including mRNAs, methylation patterns and exosomes, which have potential to be used in clinical practice. The aim of this review is to highlight established non-invasive bladder cancer diagnostic tests as well as innovative methodologies that are on the horizon for use in bladder cancer detection.

Keywords: bladder, cancer, diagnostic, cystoscopy, invasive, biomarkers

Introduction

Bladder cancer is the 10th most common cancer in the world with an increasing incidence in developed nations.[1,2] In the US, it is the fourth most common cancer among men and 10th among women.[3] It is estimated that there will be about 83,730 new cases and about 17,200 deaths from bladder cancer among men and women in 2021.[4] Similarly, in Europe, the mortality rates per 100,000 person-years are 3.2 for men and 0.9 for women.[5] Bladder cancer is the most common malignancy of the urinary tract with an incidence rate four times higher in men compared to women.[6] As the world population ages, incidence rates in both sexes are expected to increase in the US and European countries.[7] Well-established risk factors include tobacco smoking and occupational exposure to aromatic amines and hydrocarbons.[8] Other risk factors are dietary (artificial sweeteners, coffee, and meat consumption) and genetic factors.[9] Bladder cancer represents a debilitating clinical entity with a high financial burden, and a substantial impact on one's quality of life, with studies showing a significant detrimental effect on physical, mental, and social well-being.[3,10]

At first presentation, about 75% of all bladder cancers are non-muscle invasive bladder cancer (NMIBC).[8] There are various stages of NMIBC including carcinoma in situ (CIS), confinement to the mucosa (Ta) and confinement to the submucosa (T1).[8] About 70% of NMIBC are Ta, 20% T1, and 10% CIS.[11] The gold standard treatment of NMIBC often depends on the stage but commonly involves transurethral resection (TUR) of the tumor and adjuvant intravesical chemotherapy or immunotherapy such as Bacillus Calmette–Guérin (BCG) to reduce recurrence rate and risk of progression; specifically, these therapeutic measures such as BCG are hypothesized to correct the immune system disequilibrium occurring during carcinogenesis through immune-stimulation with a detrimental effect on tumor cells.[8,12] The gold standard for diagnosis and surveillance for bladder cancer is white light cystoscopy (WLC) and urinary cytology. WLC has a sensitivity of 85–90% for papillary tumors and 67% for CIS.[13] Unfortunately, it has limited

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detection of subtle changes such as small, low-grade tumors, with up to 20% commonly missed, which may result in early recurrence.[13] WLC is also limited in distinguishing benign versus malignant lesions with individuals who had a prior TUR or chemotherapy.[13] Furthermore, not only does WLC have poor detection of low-grade and CIS tumors but can also be uncomfortable for patients and increases risk of urinary tract infections and urethral stenosis.[10] Due to these limitations, NMIBC has a high recurrence rate (50–70%) with up to 20% progressing to muscle invasive bladder cancer (MIBC).[13] Multiple international guidelines and panels have recommended a surveillance schedule based on recurrence risk and degree of progression.[11,14,15] Therefore, NMIBC requires constant monitoring and surveillance based on the initial stage at diagnosis.

With recent technological advancements, efforts were made to improve this distinction with methods such as fluorescence cystoscopy, narrow band imaging and confocal laser endomicroscopy. However, these are still invasive and expensive techniques.[6,13] Therefore, urine cytology has become increasingly relied upon for non-invasive detection and surveillance due to its low cost and ease of use. Although urine cytology has high specificity (~86%), it is limited due to its low sensitivity (48%), especially with low-grade NMIBC.[16] Frequent screenings with WLC can be mentally, physically, and financially burdensome for patients. Due to the invasiveness of the procedure, there is a need for a non-invasive and reliable alternative. Many novel urinary biomarkers are under investigation and six assays are commercially available for use with WLC; however, none are currently adopted into routine clinical practice due to high cost or poor sensitivity. Combinations of these biomarkers are underway to increase specificity and/or sensitivity to avoid unnecessary WLC for low-grade NMIBC tumors. Table 1 provides a general overview of the various assays utilized in the diagnosis of bladder cancer.

This review aims to highlight the development and implementation of novel non-diagnostic tools in the evaluation of bladder cancer and to delineate future methods that may play a role in the evolving landscape of bladder cancer diagnosis.

Table 1 Bladder Cancer Assays

Test	Mechanism	Sensitivity (%)	Specificity (%)	Reference
WLC	Examine bladder with cystoscope	67–90	83	[13]
WLC with biopsy	Examine bladder with cystoscope	67–90	83	[13]
Cytology	Examine urothelial cells under microscope	48 (16–84)	86	[17]
NMP22 ELISA	Detect NMP22 protein	69	88	[19]
NMP22 BladderChek	Detect NMP22 protein	58	88	[19]
BTA-Stat	Detect human complement factor H-related protein	56–83	72–86	[20,21]
BTA-Trak	Detect human complement factor H-related protein	66–77	69	[6,23]
Urovysion	FISH chromosome abnormalities	72	83	[25]
ImmunoCyt	Immunofluorescence antigen detection	73	66	[32]
Xpert BC Assay	5 mRNAs detection	78	84	[35]
CxBladder	5 mRNAs detection	90	91	[39,40]
ADXBLADDER	MCM5 proteins detection	45–73	70–88	[43–45]
Bladder EpiCheck	DNA methylation patterns	90	83	[48]
Uromonitor	Detect genetic mutations	93	85	[54]
Assure MDx	Detect genetic mutations and methylation patterns	97	93	[55]
UBC test	Cytokeratin detection	30–87	91	[59]

Non-Invasive Bladder Cancer Diagnostic Tests

Cytology

Since 1945, urine cytology has served as an easy and reliable non-invasive measure of bladder cancer, with a specificity found to be as high as 95%.[17] However, it is limited by its low sensitivity to low-grade tumors. Yafi et al found a sensitivity of 84% for high-grade tumors, but only 14% for low-grade tumors.[17] Interpretation is highly user dependent and can be difficult to distinguish based on the specimen quality such as if the tissue environment is heavily inflamed.[18]

Due to these limitations, many have advocated the use of complementary urinary biomarkers using immunofluorescence and protein expression.[18] Yafi et al found improvement in cytology sensitivity through combinations with urinary biomarkers including the Hemoglobin Dipstick, BTA Stat, NMP22 BladderChek, and ImmunoCyt. Of these combinations, the optimal combination was cytology with the NMP22 BladderChek, yielding a sensitivity of 94% and specificity of 84% for high-grade tumors and 31% sensitivity for low-grade tumors.[17]

NMP22 BladderChek

Nuclear matrix proteins (NMPs) provide support for the cell nucleus. One protein, NMP22, has been found to be overexpressed in urothelial tumors and released into the urine following apoptosis of tumor cells.[16] Consequently, it has been found to be up to 25 times greater in bladder cancer cell lines than normal urothelium.[17] Thus, it can presumably be used for the diagnosis and subsequent surveillance of bladder cancer.

There are two FDA-approved tests, including the NMP22 Bladder Cancer ELISA and NMP22 BladderChek tests, which utilize this marker. The ELISA, or quantitative test, is performed in a lab, while the BladderChek, or qualitative test, can be done as a point-of-care (POC) assay. Both analyze voided urine for NMP22 markers. In 2015, Chou et al performed a meta-analysis and found an overall specificity of 88% and sensitivity of 69% for the ELISA test and overall specificity of 88% and sensitivity of 58% for the BladderChek test.[19] Based on the tumor type, Yafi et al found BladderChek to have a sensitivity of 25% for low grade and 92% for high-grade tumors.[17] Bladderchek could be useful clinically to distinguish which patients will need cystoscopy.[6] Unfortunately, it has been found to have a fairly high false-positive rate for urinary tract infections (UTIs), calculi, foreign bodies, and other genitourinary cancers.[13]

Bladder Tumor Antigen Assays

Bladder tumor antigen (BTA) assays involve the use of immunoassays that detect human complement factor H-related protein in urine.[16] This protein is released by cancerous cells to interfere with the complement cascade, providing survival advantage for the tumors. Currently, there are two FDA-approved assays, which analyze BTA quantitatively (BTA-Stat) and qualitatively (BTA-Trak).

BTA-Stat is a POC assay, which provides results in 5 minutes, while BTA-Trak is more specialized and consequently takes longer to result. Both tests are relatively easy to perform.[9] BTA-stat's overall specificity is 72–85.7% and sensitivity is 56–83%.[20,21] For high-grade tumors, BTA-stat's sensitivity was found to be around 64–69%.[22] Similarly, BTA-Trak's overall specificity is 69% and sensitivity is 66% with a higher sensitivity of approximately 77% for high-grade tumors.[6,23] In a meta-analysis of 13 studies conducted by Guo et al, BTA-stat was found to be superior to cytology only in sensitivity (67% vs 43%), whereas specificity was inferior to cytology.[22] Few clinical studies have used BTA-Trak as it requires more equipment and is not as quick. For that reason, BTA-Stat is preferred over BTA-Trak. Overall, BTA assays have been shown to be more sensitive than cytology but are limited by their lack of specificity and are only used as an adjuvant in clinical practice.

Urovysion

The Urovysion test is a multitarget, multicolor fluorescence in situ hybridization (FISH) assay. Its detection is based on specific chromosomal abnormalities on chromosomes 3, 7, and 17 found on exfoliated urothelial cells.[6] In urothelial cancers, these exfoliated urothelial cells are seen in higher frequency.[24] The assay incorporates multiple probes to specific chromosomal abnormalities to increase its sensitivity.[24] It is the most expensive FDA-approved test for the diagnosis and surveillance of bladder cancer recurrence.

Through a pooled meta-analysis of 2477 FISH tests, Urovysion was found to have an overall sensitivity of 72% and an overall specificity of 83%. However, a sensitivity for low-grade cancer was found to be as low as 41%.[25] Although hematuria and inflammation do not typically alter the test's reliability, false positives are detected by the presence of umbrella cells, chromosome tetraploidy, or heteroploidy, which may be due to human polyomavirus infection.[24]

Although Urovysion appears to be superior to cytology, there have been mixed results. In a study conducted by Lavery et al, voided urine sample sensitivities of Urovysion were compared to urine cytology; urine cytology outperformed Urovysion in both low-grade and high-grade tumor cells.[26] The authors asserted that a possible explanation for these results was that all urine samples were taken after bladder washout.[26] This resulted in a higher yield of exfoliated cells and allowed optimal evaluation of abnormal cells in urine cytology, which in other studies was limited. Conversely, a study by Dimashkieh et al found Urovysion sensitivity was superior (61.9%) to urine cytology (29.1%), with the caveat of Urovysion generating more false positives.[27] Other studies have suggested that the combination of FISH technology with cytology has improved its overall sensitivity.[28] With the addition of FISH, the number of unwanted biopsies could be reduced. A positive FISH was found to predict recurrence and progression in patients who had a negative cystoscopy with abnormal cytology.[29] More studies are warranted to further establish guidelines and validate FISH as a tool for bladder cancer screening.

ImmunoCyt

ImmunoCyt utilizes immunofluorescence with three monoclonal antibodies to detect antigens M344, LDQ10, and 19A11.[30] These antigens are highly expressed on cancerous urothelial cells detected in voided urine.[31] ImmunoCyt is FDA-approved for bladder cancer surveillance. Multiple studies have shown it to improve the sensitivity of urine cytology; however, the test lacks the specificity. Specifically, sensitivities and specificities range from 68% to 85% and 72% to 82%, respectively.[19]

In a meta-analysis of seven articles, ImmunoCyt had a higher pooled sensitivity of 72.5% compared to urine cytology at 56.6%.[32] However, ImmunoCyt lacked the specificity with 65.7% compared to 90.6% for cytology.[32] It is less affected by hematuria and inflammation compared to other assays, but can be influenced by the presence of UTIs, urolithiasis, BPH.[31] Additionally, the technology has high interobserver variability and requires cytopathologists for appropriate and accurate implementation.[33] Thus, its use in clinical practice has been limited.

Xpert Bladder Cancer Detection Assay

Xpert Bladder Cancer Detection Assay (Xpert BC) works by quantitating mRNA targets expressed on bladder cancer cells, more specifically ABL1, ANXA10, UPK1B, CRH and IGF2.[34] Urine samples are identified as either positive or negative based on a linear regression model that considers the concentration of the markers in a urine sample of patients. Largely expressed quantities in the urine sample are considered a positive test, whereas less expressed quantities would be negative.[34]

In a large study comparing Xpert BC Monitor to cytology and Urovysion using pre-cystoscopy voided urine samples, Xpert BC was found to have the highest overall sensitivity at 78% compared to 44% (urine cytology) and 59% (Urovysion).[35] Although its sensitivity is superior, it lacks specificity. It was found to have an overall specificity of 84% compared to 97% for cytology and 88% for Urovysion.[35] When compared to low-grade tumors, Xpert BC was found to outperform cytology in sensitivity at 77% compared to 13%.[36] In a recent study conducted by Cancel-Tassin et al, 500 patient-voided urine samples with a previous NMIBC diagnosis and a positive cystoscopy or CT urogram were analyzed by Xpert BC and cytology.[37] Although specificity was found to be higher for cytology (73% vs 98%), the Xpert BC Monitor had a NPV of 99.7% for exclusion of aggressive tumors.[37] In a recent meta-analysis, Laukhtina et al evaluated studies utilizing Xpert BC for diagnosis of recurrence during NMIBC follow-up. Overall, ten studies had a pooled sensitivity, specificity, and NPV of 72%, 76%, and 92% respectively, although there was significant heterogeneity among the studies. In a subgroup analysis, Xpert BC demonstrated similar diagnostic detection for high-grade recurrence to those in the overall population.[38]

CxBladder

Similar to Xpert BC, CxBladder is another novel test that measures the expression of five mRNAs, CDK1, CXCR2, HOXA12, IGFBP4, and MDK in voided urine.[29] There are multiple assays available for bladder detection and surveillance. The CxBladder Detect is performed alongside cystoscopy and utilizes genomes to determine the detection of bladder cancer. CxBladder Triage is similar but incorporates age, gender, and smoking history to exclude bladder cancer for low-risk patients presenting with hematuria. CxBladder Monitor can be used for surveillance to prevent recurrence of NMIBC.[9]

O'Sullivan et al analyzed voided urine samples of patients with hematuria after cystoscopy.[39] The samples were analyzed by multiple non-invasive assays including cytology, NMP22 Bladderchek and CxBladder. CxBladder was found to have 90% sensitivity and 91% specificity, while cytology had a sensitivity of 56% and specificity of 94%.[39] A larger study conducted by Lotan et al found similar results; urine samples were collected from patients undergoing clinical surveillance for bladder cancer. CxBladder was found to outperform both NMP22 Bladderchek and cytology.[40] The CxBladder had sensitivity of 91% compared to 22% by cytology. NPV was much higher (96%) compared to cytology (87%).[40] A surveillance study with 309 patients conducted by Koya et al found that the addition of CxBladder Monitor reduced the number of annual cystoscopies by 39%.[41] This greatly reduced patient anxiety and discomfort but kept the same detection rates.[41] Thus, studies have shown the potential of CxBladder's use in clinical practice for bladder cancer diagnosis and screening.

Adxbladder

The ADXBLADDER is an ELISA test, which uses antibodies to detect minichromosome maintenance protein 5 (MMP5) found in voided urine.[42] MCM5 proteins are important in initiating DNA replication and have been found to be highly expressed in proliferating and cancerous cells.[42] In the urothelium, it was found that normal epithelium has a low or absent expression of MMP5. The amount of MMP5 found in voided urine has been found to be correlated to the grade of the cancer, with more MMP5 indicating higher grade.[42]

The ADXBLADDER test is inexpensive, quick, and convenient, proving itself to be potentially useful in a clinical setting. However, it is relatively new and little research has been performed evaluating its efficacy. There has been broad variability among multiple studies, with its sensitivity ranging from 45% to 73% and specificity from 62% to 88%.[38,43–45] The sensitivity and specificity for detection of high-grade tumor was found to be 71% and 76%, respectively, in a meta-analysis of 3 studies.[38] ADXBLADDER may be a good addition to the non-invasive tests used with cytology in order to increase the sensitivity, however since the specificity is still lower than that of cytology, it would not be recommended to be used alone.

Epicheck

DNA methylation plays a role in regulating gene expression without changing the DNA code. Several studies have highlighted the presence of methylated loci in the context of bladder cancer, indicating its potential application as a diagnostic and prognostic biomarker.[46,47] The Bladder EpiCheck test was recently developed for the surveillance of bladder cancer recurrence. The analysis is based on the detection of the DNA methylation status of 15 genomic loci, which are strongly associated with bladder cancer in specimens of voided urine.[48] The test provides a value between 0 and 100; this value, also known as the EpiScore, is based on the methylation patterns present, where a positive score (>60) indicates that methylation patterns match NMIBC.[48]

Laukhtina et al performed a meta-analysis on five studies using Epicheck for recurrence and found a pooled sensitivity, specificity and NPV of 74%, 84%, and 94%, respectively. For high-grade tumors, the NPV was the same; however, the sensitivity was 80% and specificity was 78%.[38] In a validation study conducted by Wasserstrom et al, Epicheck was found to have a higher sensitivity of 90%.[49] However, the sensitivity varied on staging and grading of tumors, with a higher sensitivity found in higher stages and higher grades. The overall specificity was 83% and the NPV was 97%.[49] Compared to urine cytology, Epicheck showed a greater sensitivity, 90% vs 38%, in both low- and high-grade tumors, but a lower specificity, 83% vs 96%.[49]

In a blinded prospective multicenter study conducted by Witjes et al, Bladder Epicheck and urine cytology were performed on urine samples collected from patients undergoing standard cystoscopy in an outpatient urology clinic. The study concluded that when excluding low-grade Ta tumors, the Epicheck test had a sensitivity of 91.7%, NPV of 99.3%, and a high specificity of 88.0%.[50] In a recent blinded clinical prospective trial, Cochetti et al assessed the diagnostic performance of Epicheck for surveillance of high-risk bladder cancer compared to photodynamic diagnosis (PDD)-guided cystoscopy.[51] Compared to urine cytology, Bladder Epicheck had a higher sensitivity (100% vs 88.9%) but a lower specificity (90.9% vs 100%). PDD-guided cystoscopy had the lowest sensitivity and specificity (61% vs 41% respectively). Bladder Epicheck had the highest area under the curve (AUC) compared to both PDD-guided cystoscopy and cytology (0.95 vs 0.51 vs 0.94). The authors suggested a potential algorithm to combine Epicheck with urine cytology and found that it would have predicted the correct diagnosis 90% of the time and would subsequently reduce the number of cystoscopies.[51] Thus, Bladder Epicheck has the potential to be used in clinical practice as a tool to rule-out recurrence and therefore potentially avoid unnecessary cystoscopies.

Uromonitor and AssureMDx

Alterations in specific genes are known to drive cancerous growth. Uromonitor is a urine-based assay that detects hotspot gene mutations in telomerase reverse transcriptase (TERT) and FGFR3. TERT maintains telomere integrity; mutations have been upregulated in many forms of bladder cancer and may play an important role in carcinogenesis. Specifically, there is molecular evidence suggesting the involvement of hTR, HTERT, and CSK2 gene expression in bladder cancer carcinogenesis.[52] Many FGFR3 mutations have been found in bladder cancers, with a higher number in NMIBC.[53] Sieverink et al compared Uromonitor-V2 to cytology and found a superior sensitivity (93% vs 26%) but slightly lower specificity (85% to 91%). PPV (79% vs 63%) and NPV (95% vs 68%) were both higher in Uromonitor-V2 than cytology.[54] The high NPV suggests that the Uromonitor may be beneficial in detecting recurrence in NMIBC patients. In a network meta-analysis, Uromonitor was found to be significantly higher than other urinary biomarker tests for detection of NMIBC recurrence. It was superior in sensitivity, PPV, and NPV; however, cytology remained superior in specificity.[38] Another commercially available test similar to Uromonitor, AssureMDx, detects the TERT, FGFR3, HRAS with methylation analysis of OTX1, ONECUT2, and TWIST1. When combined with age, AssureMDx has a sensitivity of 97% and specificity of 93% in a multicenter cohort study.[55] Further evaluation of the Uromonitor and AssureMDx is warranted to evaluate its use in clinical practice.

Future Directions

Single-Cell Sequencing Enabled Hexokinase 2 Assay

Single-cell RNA sequencing (scRNA-seq) is a novel tool used to analyze targeted cells and to study the tumor micro-environment. Conventional RNA sequencing works by analyzing the transcriptomic information collected in bulk from a mixture of different cells from the same cancer tissue.[56] However, the information extracted from bulk RNA sequencing collects from other regularly expressed cells such as fibroblasts, endothelial and immune cells. scRNA-seq allows the analysis of a select population of cancerous cells, thereby providing more tumor-specific data.[56] A promising use of this innovative technology is its incorporation into bladder cancer screening as it allows for the identification of specific transcription-related information to cancerous cells.

The Hexokinase 2 (HK2) assay is an example of the potential of this technology. A recent study by Wang et al analyzed the potential of scRNA-seq on exfoliated urothelial cells from a voided urine sample.[57] HK2 was chosen as a biomarker for high-throughput screening of cells in urine and detecting exfoliated tumor cells showing elevated glycolysis, a tumor-specific property. The sensitivity, specificity, PPV, and NPV of the assay was 90%, 88%, 83% and 93%, respectively.[57]

UBC Assay

There are other biomarkers under investigation for the detection of bladder cancer. Cytokeratins, proteins that make up the cytoskeleton of epithelial cells, are released into urine after cell death. Of these proteins, cytokeratins 8, 18, 19 and 20

have been found to have a positive association with bladder cancer.[58] One test, the Urinary Bladder Cancer (UBC) ELISA assay, was developed to detect the presence of the cytokeratin fragments 8 and 17 in voided urine.[59] This test was developed as a POC assay to rapidly identify cancerous cells. Sensitivity for CIS was found to be 87%, 30% for low-grade tumors, and 72% for high-grade tumors with the specificity 91%.[59]

miRNA

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally through interactions with complementary target sites on mRNAs.[60] They can either induce mRNA degradation or impair its translation. Therefore, altered miRNA expression can lead to carcinogenesis, progression, and metastases.[60–62] Various studies have discovered miRNAs that are unique to bladder cancer. Puerta-Gil et al analyzed bladder cancer urinary samples for expression of miR-143, miR-222, and miR-452 to discover how their expression correlated with pathogenesis. miR-222 and miR-452 were found to significantly increase with tumor grade, tumor size, and presence of CIS, indicating their potential role in bladder cancer diagnostics. miR-222 along with miR-143 expression correlated with clinical recurrence and progression. In addition, miR-452 expression was highly correlated with lymph node metastasis.[60] Urquidi et al investigated a miRNA biomarker panel using voided urine samples from 85 subjects with known bladder cancer; in their study, they identified a 25-target diagnostic signature that could predict bladder cancer with an estimated sensitivity of 87% and specificity of 100%.[63] Despite its potential utility in bladder cancer diagnosis, further investigation is warranted to validate miRNA's role as a non-invasive bladder cancer biomarker and its use in the clinical setting.

Extracellular Vesicles

Novel agents investigated for cancer detection include exosomes, microvesicles and extracellular vesicles, which are secreted by cells and may play a role in tumor progression. They are composed of a small lipid bilayer, which allows tumor components to avoid degradation by the circulation and provides protection from the extracellular space.[64]

Bladder tumors secrete specific exosomes that may be elevated in NMIBC, allowing them to potentially serve as non-invasive biomarkers for NMIBC through ELISA detection. The level of circulating exosomes correlates with tumor progression. The exosomes allow the cancer cells to interact with the surrounding tumor microenvironment and thus affect angiogenesis, invasion, immune response, and metastasis.[64] Several exosomal miRNAs detected in urine of bladder cancer patients were found to be correlated with disease. They are hypothesized to act as cancer messengers and have shown potential promise as bladder cancer biomarkers.[65] Elsharkawi et al evaluated ELISA detection of CD9 on exosomes in urine and serum and found that specificity was 100% in serum and 83.3% in urine, whereas sensitivity was 82.4% in serum and 92.6% in urine.[64] Further investigation of tumor exosomes and microvesicles is needed to determine their effectiveness in NMIBC detection.

Bladder Cancer Detection Device

Various bladder cancer detection devices have been explored to improve diagnostics. One such device, the Microfluidic Urinary Photo-Specific Diagnostic of bladder cancer (MicroUPSD), relies on an immunoaffinity microfluidic platform to detect bladder epithelial cells. MacGregor et al covalently immobilized bioactive anti-epithelial cell adhesion molecule (EpCAM) antibodies to microchannels to selectively capture bladder cancer cells that have a greater expression of EpCAM in excreted urine.[66] They evaluated centrifuged and settled urine samples with the device and compared it to standard cytology and cystoscopy. The device was found to have a 100% sensitivity in both centrifuged and settled urine samples, compared to 20% in cytology. The specificity for the settled urine was 80% compared to 100% in cytology.[66] The group found a specificity higher for settled urine compared to centrifuged urine, indicating the device could potentially be used as a POC assay outside of pathology laboratories. A potential drawback to this device is that EpCAM is not bladder cancer specific and thus can lead to false positives from other cancers such as prostate cancer.[66]

Artificial Intelligence

With recent technological advancements, methods utilizing artificial intelligence (AI) have been proposed to aid in bladder cancer detection. Algorithms have been created to detect subtle changes in the bladder based on cystoscopic images and videos. Six studies created their own specific AI algorithms and were found to have a pooled sensitivity of 89.7% and specificity of 96.1% for detection of bladder cancer.[67] Seemingly, the ideal scenario includes incorporating this technology along with real-time cystoscopy in order to optimize the detection of bladder cancer and to potentially reduce the need for subsequent cystoscopies.[67] In addition, incorporation of AI may assist physicians-in-training in detection of bladder cancer. Wu et al found that their AI Algorithm was superior to expert urologists in the detection of complex lesions such as CIS and very small lesions.[68] Further prospective studies are needed to determine incorporation of AI in clinical practice and its use in different patient populations.

Expert Opinion

With the increasing incidence of bladder cancer and recent technological advancements in its detection, urinary biomarkers hold great promise for future utilization in clinical practice. Compared to the standard screening method of cystoscopy, urinary biomarkers may greatly reduce the burden and stress placed on patients. Laukhtina et al calculated the number of cystoscopies avoided (true negatives and false negatives) and risk of missing recurrences by avoiding cystoscopy (true negative) for detection of NMIBC recurrence using urinary biomarkers; they found that urinary biomarkers could avoid 500–740 cystoscopies and would miss recurrence in 10–78 patients per 1000 patients.[38] Furthermore, in addition to reducing mental stress for the patient, urinary biomarker utilization may decrease the financial burden imposed on patients during bladder cancer diagnosis. WLC alone costs around US$210, WLC with biopsy costs approximately US$370, and cytology costs $100 USD.[69–71] Common urinary biomarkers are currently available ranging from US$25 to US$80.[71,72] However, novel tests are less cost-effective, with Bladder Epicheck ranging from US$168 to US$476 and Urovysion estimated to cost approximately $800 USD.[71,73] Many tests are not yet mass produced and may have a lower cost once used more robustly. However, it may be difficult to determine the true financial burden as great variability exists among countries with respect to product cost.

Most urinary biomarkers show superiority to urine cytology and have the potential to be used in clinical practice for the diagnosis and surveillance of NMIBC. Based on the specific patient presentation, a unique biomarker kit or panel combination may be indicated, allowing for physicians to provide more patient-centered care. Due to limited studies, it remains to be seen whether urinary biomarkers could be incorporated into screening for initial diagnosis of bladder cancer. Urinary biomarkers are on the horizon for detection of high-grade tumors, but further research is warranted to assess their detection of low-grade and CIS tumors. Unfortunately, there are few prospective studies investigating urinary biomarkers in low-grade NMIBC tumors and the available cross-sectional studies seem to overestimate the sensitivity of biomarkers.[74]

Cell-based biomarkers such as ImmunoCyt and Urovysion appear to have higher sensitivities and specificities in low-grade NMIBC compared to urine cytology and markers analyzing soluble tumor-associated antigens.[74] Due to these findings, a prospective multi-center randomized study, UroFollow, was initiated to investigate whether non-invasive biomarkers could be used for follow-up for patients with low and intermediate grade NMIBC over a 3-year period.[74] Currently, though urinary biomarkers may not yet be ready to replace cystoscopy, they show promise in conjugation with current practices for monitoring bladder cancer recurrence.

Conclusion

Bladder cancer remains a complex clinical entity to diagnose, monitor and treat. Specifically, the ability to diagnose bladder cancer in a reliable, reproducible way represents a particularly challenging subject. Recently, there has been interest in incorporating non-invasive means of bladder cancer detection and surveillance, as well as in improving surveillance of low-grade lesions with modalities that enhance tumor detection. This review highlights novel means of detecting bladder cancer. With the adoption of non-invasive methodologies, patients may avoid the potential physical, mental, and financial burden of repeated cystoscopy procedures. Therefore, non-invasive methodologies may not only benefit patients but also health care systems and physicians.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

Dr Alex Sankin is an advisor for Ambu Inc. The authors report no other conflicts of interest in this work.

References

1. Saginala K, Barsouk A, Aluru JS, et al. Epidemiology of bladder cancer. *Med Sci*. 2020;8(1):15. doi:10.3390/medsci8010015
2. Wong MCS, Fung FDH, Leung C, et al. The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. *Sci Rep*. 2018;8(1):1129. doi:10.1038/s41598-018-19199-z
3. van Rhijn BW, Burger M, Lotan Y, et al. Recurrence and progression of disease in non-muscle-invasive bladder cancer: from epidemiology to treatment strategy. *Eur Urol*. 2009;56(3):430–442. doi:10.1016/j.eururo.2009.06.028
4. American Cancer Society. Cancer facts & figures 2021. Atlanta, Ga: American Cancer Society; 2021. Available from: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/cancer-facts-figures-2021.html. Accessed October 27, 2021.
5. Ferlay J, Soerjomataram I, Ervik M, et al. *GLOBOCAN 2012 V.1.0, Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012*. Lyon: International Agency for Research on Cancer; 2013.
6. Cheung G, Sahai A, Billia M, et al. Recent advances in the diagnosis and treatment of bladder cancer. *BMC Med*. 2013;11(1). doi:10.1186/1741-7015-11-13
7. Fankhauser CD, Mostafid H. Prevention of bladder cancer incidence and recurrence: nutrition and lifestyle. *Curr Opin Urol*. 2018;28(1):88–92. doi:10.1097/MOU.0000000000000452
8. Woldu SL, Bagrodia A, Lotan Y. Guideline of guidelines: non-muscle-invasive bladder cancer. *BJU Int*. 2017;119(3):371–380. doi:10.1111/bju.13760
9. Truta A, Popon TA, Saraci G, et al. Novel non invasive diagnostic strategies in bladder cancer. *Clujul Med*. 2016;89(2):187–192. doi:10.15386/cjmed-534
10. Smith AB, Jaeger B, Pinheiro LC, et al. Impact of bladder cancer on health-related quality of life. *BJU Int*. 2018;121(4):549–557. doi:10.1111/bju.14047
11. Anastasiadis A, de Reijke TM. Best practice in the treatment of nonmuscle invasive bladder cancer. *Ther Adv Urol*. 2012;4(1):13–32. doi:10.1177/1756287211431976
12. Poli G, Cochetti G, Boni A, et al. Characterization of inflammasome-related genes in urine sediments of patients receiving intravesical BCG therapy. *Urol Oncol*. 2017;35(12):674.e19–674.e24. doi:10.1016/j.urolonc.2017.08.004
13. Soubra A, Risk MC. Diagnostics techniques in nonmuscle invasive bladder cancer. *Indian J Urol*. 2015;31(4):283–288. doi:10.4103/0970-1591.166449
14. Amin MB, Greene FL, Edge SB, et al. The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population- based to a more "personalized" approach to cancer staging. *CA Cancer J Clin*. 2017;67(2):93–99. doi:10.3322/caac.21388
15. Schroeck FR, Smith N, Shelton JB. Implementing risk-aligned bladder cancer surveillance care. *Urol Oncol*. 2018;36(5):257–264. doi:10.1016/j.urolonc.2017.12.016
16. Ng K, Stenzl A, Sharma A, Vasdev N. Urinary biomarkers in bladder cancer: a review of the current landscape and future directions. *Urol Oncol*. 2021;39(1):41–51. doi:10.1016/j.urolonc.2020.08.016
17. Yafi FA, Brimo F, Steinberg J, et al. Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. *Urol Oncol*. 2015;33(2):66.e25–31. doi:10.1016/j.urolonc.2014.06.008
18. Henning GM, Barashi NS, Smith ZL. Advances in biomarkers for detection, surveillance, and prognosis of bladder cancer. *Clin Genitourin Cancer*. 2021;19(3):194–198. doi:10.1016/j.clgc.2020.12.003
19. 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(12):922–931. doi:10.7326/M15-0997
20. Smith ZL, Guzzo TJ. Urinary markers for bladder cancer. *F1000 Prime Rep*. 2013;5. doi:10.12703/P5-21
21. Raitanen M-P. The role of BTA stat Test in follow-up of patients with bladder cancer: results from FinnBladder studies. *World J Urol*. 2008;26:45–50. doi:10.1007/s00345-007-0230-3
22. 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(5–6):E347–E352. doi:10.5489/cuaj.1668
23. Thomas L, Leyh H, Marberger M, et al. Multicenter trial of the quantitative BTA TRAK assay in the detection of bladder cancer. *Clin Chem*. 1999;45(4):472–477. doi:10.1093/clinchem/45.4.472
24. Nagai T, Naiki T, Etani T, et al. UroVysion fluorescence in situ hybridization in urothelial carcinoma: a narrative review and future perspectives. *Transl Androl Urol*. 2021;10(4):1908–1917. doi:10.21037/tau-20-1207
25. Hajdinjak T. UroVysion FISH test for detecting urothelial cancers: meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. *Urol Oncol*. 2008;26(6):646–651. doi:10.1016/j.urolonc.2007.06.002
26. Lavery HJ, Zaharieva B, McFaddin A, et al. A prospective comparison of UroVysion FISH and urine cytology in bladder cancer detection. *BMC Cancer*. 2017;17(1):247. doi:10.1186/s12885-017-3227-3
27. Dimashkieh H, Wolff DJ, Smith TM, et al. Evaluation of urovysion and cytology for bladder cancer detection: a study of 1835 paired urine samples with clinical and histologic correlation. *Cancer Cytopathol*. 2013;121(10):591–597. doi:10.1002/cncy.21327

28. Moonen PM, Merkx GF, Peelen P, et al. UroVysion compared with cytology and quantitative cytology in the surveillance of non-muscle-invasive bladder cancer. *Eur Urol*. 2007;51(5):1275–80; discussion 1280. doi:10.1016/j.eururo.2006.10.044
29. Kim PH, Sukhu R, Cordon BH, et al. Reflex fluorescence in situ hybridization assay for suspicious urinary cytology in patients with bladder cancer with negative surveillance cystoscopy. *BJU Int*. 2014;114:354–359.
30. Fradet Y, Lockhard C. Performance characteristics of a new monoclonal antibody test for bladder cancer: immunoCyt trademark. *Can J Urol*. 1997;4:400–405.
31. Bhat A, Ritch CR. Urinary biomarkers in bladder cancer: where do we stand? *Curr Opin Urol*. 2019;29(3):203–209. doi:10.1097/MOU.0000000000000605
32. He H, Han C, Hao L, Zang G. ImmunoCyt test compared to cytology in the diagnosis of bladder cancer: a meta-analysis. *Oncol Lett*. 2016;12(1):83–88. doi:10.3892/ol.2016.4556
33. Oeyen E, Hoekx L, De Wachter S, et al. Bladder cancer diagnosis and follow-up: the current status and possible role of extracellular vesicles. *Int J Mol Sci*. 2019;20(4):821. doi:10.3390/ijms20040821
34. Wallace E, Higuchi R, Satya M, et al. Development of a 90-minute integrated noninvasive urinary assay for bladder cancer detection. *J Urol*. 2018;199:655–662. doi:10.1016/jjuro.2017.09.141
35. Valenberg FJ, Hiar AM, Wallace E, et al. Validation of an mRNA-based urine test for the detection of bladder cancer in patients with haematuria. *Eur Urol Oncol*. 2021;4(1):93–101. doi:10.1016/j.euo.2020.09.001
36. Pichler R, Fritz J, Tulchiner G, et al. Increased accuracy of a novel mRNA-based urine test for bladder cancer surveillance. *BJU Int*. 2018;121(1):29–37. doi:10.1111/bju.14019
37. Cancel-Tassin G, Roupret M, Pinar U, et al. Assessment of Xpert bladder cancer monitor test performance for the detection of recurrence during non-muscle invasive bladder cancer follow-up. *World J Urol*. 2021;39(9):3329–3335. doi:10.1007/s00345-021-03629-1
38. Laukhtina E, Shim SR, Mori K, et al. Diagnostic accuracy of novel urinary biomarker tests in non-muscle-invasive bladder cancer: a systematic review and network meta-analysis. *Eur Urol Oncol*. 2021;4(6):927–942. doi:10.1016/j.euo.2021.10.003
39. O'Sullivan P, Sharples K, Dalphin M, et al. A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. *J Urol*. 2012;188(3):741–747. doi:10.1016/j.juro.2012.05.003
40. Lotan Y, O'Sullivan P, Raman JD, et al. Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. *Urol Oncol*. 2017;35(8):531.e15–531.e22. doi:10.1016/j.urolonc.2017.03.008
41. Koya M, Osborne S, Chemaslé C, et al. An evaluation of the real world use and clinical utility of the Cxbladder Monitor assay in the follow-up of patients previously treated for bladder cancer. *BMC Urol*. 2020;20(1). doi:10.1186/s12894-020-0583-0
42. Wolfs JRE, Hermans TJN, Koldewijn EL, van de Kerkhof D. Novel urinary biomarkers ADXBLADDER and bladder EpiCheck for diagnostics of bladder cancer: a review. *Urol Oncol*. 2021;39(3):161–170. doi:10.1016/j.urolonc.2020.11.014
43. Roupret M, Gontero P, McCracken SRC, et al. Diagnostic accuracy of MCM5 for the detection of recurrence in nonmuscle invasive bladder cancer followup: a blinded, prospective cohort, multicenter European study. *J Urol*. 2020;204(4):685–690. doi:10.1097/JU.0000000000001084
44. Dudderidge T, Stockley J, Nabi G, et al. A novel, non-invasive test enabling bladder cancer detection in urine sediment of patients presenting with haematuria-a prospective multicentre performance evaluation of ADXBLADDER. *Eur Urol Oncol*. 2020;3(1):42–46. doi:10.1016/j.euo.2019.06.006
45. Anastasi E, Maggi M, Tartaglione S, et al. Predictive value of MCM5 (ADXBLADDER) analysis in urine of men evaluated for the initial diagnosis of bladder cancer: a comparative prospective study. *Diagn Cytopathol*. 2020;48:1034–1040. doi:10.1002/dc.24530
46. Beukers W, Kandimalla R, Masius R, et al. Stratification based on methylation of TBX2 and TBX3 into three molecular grades predicts progression in patients with pTa-bladder cancer. *Mod Pathol*. 2015;28:515–522. doi:10.1038/modpathol.2014.145
47. Friedrich MG, Toma MI, Chun JK, et al. DNA-Methylierung in der Urindiagnostik und als Prognosemarker beim Urothelkarzinom der Harnblase [DNA methylation on urinalysis and as a prognostic marker in urothelial cancer of the bladder]. *Urologe A*. 2007;46(7):761–768. German. doi:10.1007/s00120-007-1360-3
48. Mancini M, Righetto M, Zumerle S, et al. The bladder EpiCheck test as a non-invasive tool based on the identification of DNA methylation in bladder cancer cells in the urine: a review of published evidence. *Int J Mol Sci*. 2020;21(18):6542. doi:10.3390/ijms21186542
49. Wasserstrom A, Frumkin D, Dotan Z, et al. MP13-15 molecular urine cytology – bladder epicheck is a novel molecular diagnostic tool for monitoring of bladder cancer patients. *J Urol*. 2016;195:e140. doi:10.1016/j.juro.2016.02.2496
50. Witjes JA, Morote J, Cornel EB, et al. Performance of the bladder EpiCheck™ methylation test for patients under surveillance for non-muscle-invasive bladder cancer: results of a multicenter, prospective, blinded clinical trial. *Eur Urol Oncol*. 2018;1(4):307–313. doi:10.1016/j.euo.2018.06.011
51. Cochetti G, Rossi de Vermandois JA, Maula V, et al. Diagnostic performance of the Bladder EpiCheck methylation test and photodynamic diagnosis-guided cystoscopy in the surveillance of high-risk non-muscle invasive bladder cancer: a single centre, prospective, blinded clinical trial. *Urol Oncol*. 2021;S1078-1439(21)00483-X. doi:10.1016/j.urolonc.2021.11.001
52. Mezzasoma L, Antognelli C, Del Buono C, et al. Expression and biological-clinical significance of hTR, hTERT and CKS2 in washing fluids of patients with bladder cancer. *BMC Urol*. 2010;10:17. doi:10.1186/1471-2490-10-17
53. Descotes F, Kara N, Decaussin-Petrucci M, et al. Non-invasive prediction of recurrence in bladder cancer by detecting somatic TERT promoter mutations in urine. *Br J Cancer*. 2017;117:583–587. doi:10.1038/bjc.2017.210
54. Sieverink CA, Batista RPM, Prazeres HJM, et al. Clinical validation of a urine test (Uromonitor-V2®) for the surveillance of non-muscle-invasive bladder cancer patients. *Diagnostics*. 2020;10(10):745. doi:10.3390/diagnostics10100745
55. van Kessel KE, Van Neste L, Lurkin I, et al. Evaluation of an epigenetic profile for the detection of bladder cancer in patients with hematuria. *J Urol*. 2016;195(3):601–607. doi:10.1016/j.juro.2015.08.085
56. Slovin S, Carissimo A, Panariello F, et al. Single-cell RNA sequencing analysis: a step-by-step overview. *Methods Mol Biol*. 2021;2284:343–365. doi:10.1007/978-1-0716-1307-8_19
57. Wang Z, Chen J, Yang L, et al. Single-cell sequencing-enabled hexokinase 2 assay for noninvasive bladder cancer diagnosis and screening by detecting rare malignant cells in urine. *Anal Chem*. 2020;92(24):16284–16292. doi:10.1021/acs.analchem.0c04282
58. Southgate J, Harnden P, Trejdosiewicz LK. Cytokeratin expression patterns in normal and malignant urothelium: a review of the biological and diagnostic implications. *Histol Histopathol*. 1999;14:657–664. doi:10.14670/HH-14.657

59. Ecke TH, Weiß S, Stephan C, et al. UBC ® Rapid Test for detection of carcinoma in situ for bladder cancer. *Tumor Biol*. 2017;39:101042831770162. doi:10.1177/1010428317701624
60. Puerta-Gil P, García-Baquero R, Jia AY, et al. miR-143, miR-222, and miR-452 are useful as tumor stratification and noninvasive diagnostic biomarkers for bladder cancer. *Am J Pathol*. 2012;180(5):1808–1815. doi:10.1016/j.ajpath.2012.01.034
61. Gottardo F, Liu CG, Ferracin M, et al. Micro-RNA profiling in kidney and bladder cancers. *Urol Oncol*. 2007;25:387–392. doi:10.1016/j.urolonc.2007.01.019
62. Yang H, Dinney CP, Ye Y, et al. Evaluation of genetic variants in microRNA-related genes and risk of bladder cancer. *Cancer Res*. 2008;68:2530–2537. doi:10.1158/0008-5472.CAN-07-5991
63. Urquidi V, Netherton M, Gomes-Giacoia E, et al. A microRNA biomarker panel for the non-invasive detection of bladder cancer. *Oncotarget*. 2016;7(52):86290–86299. doi:10.18632/oncotarget.13382
64. Elsharkawi F, Elsabah M, Shabayek M, Khaled H. Urine and serum exosomes as novel biomarkers in detection of bladder cancer. *Asian Pac J Cancer Prev*. 2019;20(7):2219–2224. doi:10.31557/APJCP.2019.20.7.2219
65. Poli G, Egidi MG, Cochetti G, et al. Relationship between cellular and exosomal miRNAs targeting NOD-like receptors in bladder cancer: preliminary results. *Minerva Urol Nefrol*. 2020;72(2):207–213. doi:10.23736/S0393-2249.19.03297-1
66. MacGregor M, Shirazi HS, Chan KM, et al. Cancer cell detection device for the diagnosis of bladder cancer from urine. *Biosens Bioelectron*. 2021;171:112699. doi:10.1016/j.bios.2020.112699
67. Chan E, Pradere B, Teoh J, Chuna Y. The use of artificial intelligence for the diagnosis of bladder cancer: a review and perspectives. *Curr Opin Urol*. 2021;31(4):397–403. doi:10.1097/MOU.0000000000000900
68. Wu S, Chen X, Pan J, et al. An artificial intelligence system for the detection of bladder cancer via cystoscopy: a multicenter diagnostic study. *J Natl Cancer Inst*. 2022;114(2):220–227. doi:10.1093/jnci/djab179
69. Halpern JA, Chughtai B, Ghomrawi H. Cost-effectiveness of common diagnostic approaches for evaluation of asymptomatic microscopic hematuria. *JAMA Intern Med*. 2017;177(6):800–807. doi:10.1001/jamainternmed.2017.0739
70. Zhu CZ, Ting HN, Ng KH, Ong TA. A review on the accuracy of bladder cancer detection methods. *J Cancer*. 2019;10(17):4038–4044. doi:10.7150/jca.28989
71. Zuiverloon T, de Jong F, Theodorescu D. Clinical decision making in surveillance of non–muscle-invasive bladder cancer: the evolving roles of urinary cytology and molecular markers. Cancer Network; 2017. Available from: https://www.cancernetwork.com/view/clinical-decision-making-surveillance-nonmuscle-invasive-bladder-cancer-evolving-roles-urinary. Accessed December 10, 2021.
72. NICE. ADXBLADDER for detecting bladder cancer; 2019. Available from: https://www.nice.org.uk/advice/mib180/chapter/The-technology. Accessed December 10, 2021.
73. Lotan Y, Gakis G, Manfredi M, et al. Alternating cystoscopy with bladder EpiCheck in the surveillance of low-grade intermediate-risk NMIBC: a cost comparison model. *Bladder Cancer*. 2021;7(3):307–315. doi:10.3233/BLC-211528
74. Benderska-Söder N, Hovanec J, Pesch B, et al. Toward noninvasive follow-up of low-risk bladder cancer - rationale and concept of the UroFollow trial. *Urol Oncol*. 2020;38(12):886–895. doi:10.1016/j.urolonc.2020.01.006