Urinary biomarkers for surveillance of non-muscle invasive bladder cancer

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Bladder cancer (BC) is the ninth most common cancer worldwide with a yearly incidence of approximately 430,000 cases. There is a male predominance and it is the seventh most common cancer in men worldwide [1]. Non-muscle invasive bladder cancer (NMIBC) represents the majority (approximately 75%) of all BCs, and approximately 25% of cases are muscle-invasive (MIBC) at diagnosis. Carcinoma in situ (CIS) of the bladder are high-grade lesions which have a high-risk of recurrence and progression to MIBC [1,2].

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The mainstay of treatment of NMIBC is transurethral resection of bladder tumour (TURBT) and surveillance. The overall five-year survival of patients with superficial disease is relatively high (>75% vs. metastatic disease, ~6%), however, a significant number of these patients (40-80%) will have recurrence or progression to higher stages including muscle invasion [1,3-5]. The cornerstone of surveillance for NMIBC is cystoscopy and urine cytology. Although cystoscopy is the gold-standard for detecting BCs, it is an operator dependent test and sensitivity and specificity range from 62 to 84% and 43 to 98%, respectively. There are risks (discomfort, infection and bleeding) associated with the procedure and cystoscopic surveillance carries significant costs to the healthcare system. Urine cytology is limited by its poor sensitivity especially for low-grade tumours and false positives due to benign inflammatory conditions. There has been significant interest in developing cost-effective and non-invasive strategies to improve the detection of BC.

The rapid advances in profiling techniques in the fields of genomics, transcriptomics and proteomics have advanced the field of urinary biomarkers. Urinary biomarkers have several potential applications including: 1) diagnosing BC in patients presenting with haematuria, 2) detecting recurrences and progression in patients treated for NMIBC, 3) assessing response to Bacillus Calmette-Guerin (BCG) treatment and 4) as an adjunct in cases whereby cytology is equivocal. Several urinary biomarkers have been reported and four have been approved by the US Food and Drug Administration (FDA) for use in the surveillance of patients with NMIBC (NMP22, BTA, UroVysion and ImmunoCyt). However, at present, guidelines do not recommend using these tests to replace cystoscopy. This review provides an overview of the FDA-approved and experimental urinary biomarkers (Tables 1 and 2).

Table 1: FDA approved and experimental urinary biomarkers and tests discussed in this review.

Biomarker / test	Type of biomarker	Components	FDA-approved
Nuclear Matrix Protein-22 (NMP22)	Protein-based	Assay for NMPs. Quantitative ELISA and qualitative POC tests available.	Yes
Bladder Tumour Antigen (BTA)	Protein-based	Monoclonal antibody to human complement factor H related protein (CFHR). Quantitative ELISA and qualitative POC tests available.	Yes
UroVysion	Fluorescence in situ hybridisation (FISH)	DNA probes to identify aneuploidy in chromosomes 3, 7 17 as well as a locus specific probe to check for loss of 9p21.	Yes
ImmunoCyt assay	Immunofluorescence	Fluorescently labelled monoclonal antibodies to detect a glycosylated form of CEA and two mucins.	Yes
CxBladder Monitor	mRNA-based	Real time PCR for 5 mRNA biomarkers (CDK1, HOXA13, MDK, IGFBP5, CXCR2).	No
CertNDx	Combination of genomic, proteomic and epigenetic based	Tests for FGFR3 mutations, quantified matrix metalloproteinase 2 (MMP-2), and hypermethylation of TWIST1 and NID2.	No
Urinary Bladder Cancer Test (UBC test)	Protein-based	Assay for cytokeratins 8 and 18.	No
Xpert Bladder Cancer Monitor	mRNA-based	Real-time PCR for 5 mRNAs (ABL1, CRH, IGF2, UPK1b and ANXA10)	No
Survivin	Protein-based	Assay for survivin (Anti-apoptotic protein).	No

FDA approved biomarkers

Nuclear Matrix Protein 22

Nuclear matrix proteins (NMPs) act as scaffolds in the nucleus of cells and are released from the nuclei of dying urothelial tumour cells. NMP22 is the most studied of these and two assays have been developed; 1) NMP22BC, a quantitative ELISA test and 2) NMP22 BladderCheck, a qualitative point of care (POC) test. Assays for NMP22 can be used as an aid for BC diagnosis in patients with haematuria and can be used to monitor for cancer recurrence following treatment.

The NMP22 assay, cytology, and cystoscopy were investigated in a study of 668 patients undergoing surveillance for BC. The NMP22 assay and cystoscopy detected 50% and 91% of the 103 recurrences, respectively. The NMP22 test detected eight of the nine cancers initially missed by cystoscopy, for a combined sensitivity of 99%. Cytology detected three of the nine cases initially missed at cystoscopy. The NMP22 assay had a specificity of 87% [6]. In a study of 2222 patients with NMIBC and negative urine cytology, NMP22 levels were significantly associated with disease recurrence and progression (p<0.001) [7]. A pooled analysis of seven studies with 4384 patients with previously treated BC demonstrated a sensitivity and specificity of 60% (range, 50-85%) and 81% (4-93%) respectively [8]. The performance of NMP22 in predicting future recurrence and progression has been mixed with just a few studies showing an association [7,9,10].

Bladder tumour antigen

The bladder tumour antigen (BTA) test is a protein-based marker utilising a monoclonal antibody to human complement factor H-related protein (CFHR) which is present in high levels in the urine of BC patients. The test is available as both a POC qualitative test (BTA stat) and a quantitative ELISA-based test (BTA TRAK).

Few studies have evaluated BTA in the context of surveillance. In a study with 501 patients with a history of BC, recurrent BC was detected by cystoscopy in 133 (27%). Pre-cystoscopy BTA assay was more sensitive than cytology (56% vs. 19%), but less specific (86% vs. 98%). In six cases in which the BTA assay was positive and cystoscopy was negative, BC was found at the next cystoscopy [11]. A concern with this test is false positives in the setting of haematuria, stone disease, inflammation, recent instrumentation, other urological cancers and BCG lowering the specificity [12]. BTA tests were not shown to be associated with future events such as recurrence (HR: 0.54, 95% CI 0.27-1.08, p=0.08) and progression (HR: 0.67, 95% CI 0.25-1.79, p=0.42) in a prospective study [9].

UroVysion

UroVysion is a cytology-based test utilising fluorescence in situ hybridisation (FISH) DNA probes to identify aneuploidy in chromosomes 3, 7, 17 as well as a locus-specific probe to check for loss of 9p21. The test is FDA-approved to be used as an aid for initial diagnosis of BC in subjects with haematuria and for subsequent monitoring for tumour recurrence in patients previously diagnosed with BC.

Sensitivity and specificity for detecting recurrences in the surveillance setting vary from 13-94% and 63-100% respectively [10]. The performance of the test for detecting CIS and high-grade tumours is higher [13]. UroVysion has been shown to be useful in the setting of equivocal / atypical cytology and the American Urological Association guidelines recommend UroVysion as one of the biomarkers useful in this setting [14,15].

With regards to predicting future events, a retrospective study of 243 patients on NMIBC surveillance with negative cystoscopy and suspicious cytology demonstrated that a positive UroVysion result predicted recurrence (HR: 2.35, 95% CI 1.42-3.90, p=0.001) on multivariable analysis, and progression (HR: 3.01, 95% CI 1.10-8.21, p=0.03) on univariable analysis, in comparison to a negative UroVysion result. However, there was no association between the UroVysion result and tumour recurrence on subsequent surveillance cystoscopy (OR 0.8, 95% CI 0.26-2.74, p=1) [16]. Nonetheless, due to lack of standardisation of testing and need for expensive laboratory equipment UroVysion FISH is currently not used in routine clinical practice.

ImmunoCyt

ImmunoCyt (uCyt+) is a combination of cytology and immunofluorescence utilising fluorescently labelled monoclonal antibodies to detect a glycosylated form of carcinoembryonic antigen (CEA) and two mucins. The test requires urine fixation with ethanol or isopropyl alcohol before shipment to a reference cytopathology laboratory. A minimum evaluation of 500 epithelial cells is required, and the presence of one cell with fluorescence constitutes a positive test. The test is FDA-approved for use in conjunction with urine cytology and cystoscopy. Like UroVysion, the American Urological Association guidelines recommend UroVysion as one of the biomarkers useful in the setting of equivocal / atypical cytology [15].

Sensitivity and negative predictive value (NPV) rates vary from 62-85% and 74-93% respectively [10]. A prospective study of 942 patients demonstrated sensitivity is improved with higher pathological grade (79.3% for G1, 84.1% for G2 and 92.1% for G3 tumours) [17].

ImmunoCyt wasn't demonstrated to be able to predict future recurrence (HR: 0.65, 95% CI 0.37–1.16, p=0.14) or progression (HR: 0.44, 95% CI 0.17-1.14, p=0.09) in a large prospective study [9]. The test is not

Table 2: Pooled performance characteristics of FDA-approved urinary biomarkers [31].

Biomarker / test	Sensitivity (%)	Specificity (%)	Positive Likelihood ratio (95% CI)	Negative Likelihood ratio (95% CI)
NMP22 quantitative	61	71	2.10 (1.58-2.80)	0.55 (0.44-0.69)
NMP22 qualitative	70	83	4.20 (3.22 -5.47)	0.36 (0.16-0.81)
BTA quantitative	58	79	2.77 (1.66-4.61)	0.54 (0.39-0.76)
BTA qualitative	60	76	2.53 (1.92-3.34)	0.52 (0.47 -0.59)
UroVysion	55	80	2.75 (1.95-3.89)	0.56 (0.42-0.76)
ImmunoCyt	75	76	3.09 (2.56-3.72)	0.33 (0.24-0.46)

thought to be affected by benign conditions, but interpretation is complex and has a steep learning curve. It is associated with interobserver variability, is operator-dependent and has a high test failure rate due to inadequate specimen cellularity [18].

Experimental urinary biomarkers

CxBladder Monitor

CxBladder tests are non-FDA-approved urine-based genomic tests and are used in different contexts including: 1) potentially ruling out BC in low-risk patients with haematuria (CxBladder Triage), 2) complementing cystoscopy for BC detection in the presence of haematuria (CxBladder Detect) and 3) complementing cystoscopy for surveillance of recurrent disease (CxBladder Monitor). The tests identify four mRNA urinary biomarkers which have demonstrated high levels of expression in urothelial cancer (CDK1, HOXA13, MDK, IGFBP5). A fifth mRNA biomarker (CXCR2) of inflammation aids in distinguishing false positive cases in patients with acutely or chronically inflamed urothelium [19,20].

In a prospective study of 803 patients undergoing surveillance for BC, CxBladder Monitor had superior sensitivity (91%) and a NPV (96%) compared with cytology (sensitivity, 22% and specificity, 87%), NMP22 (sensitivity, 11-26% and specificity 86-87%), and UroVysion (sensitivity 33% and specificity 92%) (21). These tests still require large scale independent validation.

CertNDx

CertNDx is a urine-based assay developed for the evaluation of haematuria and surveillance and assesses mutations in fibroblast growth factor receptor 3 gene which may be associated with lower grade tumours that have a good prognosis [22]. Fibroblast growth factor receptor 3 (FGFR3) belongs to a family of tyrosine kinase receptors. Mutations in the FGFR3 gene can lead to constitutive activation of FGFR3 [23]. These mutations are good prognostic indicators [24]. The assay analyses urine for the presence of FGFR3 mutations, quantified matrix metalloproteinase 2 (MMP-2), and hypermethylation of TWIST1 and NID2. The diagnostic accuracy for detecting BC recurrence was shown in a prospective study of 323 patients to be 92% and 51% respectively for sensitivity and specificity with a negative predictive value of 97.4% [25].

Urinary bladder cancer (UBC) test

UBC detects the presence of cytokeratins 8 and 18 in the urine which are thought to play an active role in tumour invasion. It is a POC test with results available within 10 minutes. Sensitivity and specificity rates vary from 12-80% and from 77-92% respectively [10]. In a prospective study evaluating a total of 75 patients undergoing surveillance, barbotage and voided urine cytology resulted in a sensitivity of 32.3% and 25.8% and a specificity of 100% and 100% respectively. The UBC test resulted in a maximum sensitivity of 64.5% and specificity of 81.8%. Barbotage cytology and the UBC test were the best dual combination with the highest overall sensitivity of 77.4%. In contrast to barbotage urine cytology alone, adding UBC increased sensitivity from 21.4% to 50% for low grade tumours and from 43.8 to 100% for high grade cancers but reduced specificity from 100% to 77.3% [26].

"Although several biomarkers have shown promise, most haven't as yet been validated in large prospective multicentre clinical studies"

Xpert Bladder Cancer Monitor

Xpert BC Monitor is an mRNA-based urinary biomarker developed for BC surveillance. The test uses real-time PCR to measure the levels of five different mRNAs (ABL1, CRH, IGF2, UPK1b and ANXA10). These mRNAs are linked to cell proliferation and survival, signal transduction and response to endocrine tests. Xpert BC Monitor has been evaluated in one study which evaluated a total of 155 urine samples in 140 patients with a history of NMIBC undergoing routine follow-up. When compared with urine cytology, Xpert BC Monitor was shown to have higher sensitivity and negative predictive value (84% vs. 33% and 93% vs. 76% respectively.) However, specificity was similar between Xpert BC Monitor and urine cytology (91% vs. 94%). Interestingly, the sensitivity of Xpert BC Monitor for high-grade and low-grade tumours specifically was shown to be high (100% and 77% respectively) [27]. The test also has a rapid processing time requiring less than two minutes of hands-on sample preparation and a total PCR time of approximately 90 minutes. Whilst all these findings are promising, further validation in prospective trials is required [10].

Survivin

Survivin is an anti-apoptotic protein. This protein is elevated in human cancer but almost undetectable in normal human tissues [28]. One study has been published evaluating the potential role of survivin as a urinary biomarker in the surveillance of NMIBC. The study evaluated survivin and NMP22 in voided urine samples from 117 BC patients undergoing cystoscopy and 92 controls. Survivin had superior sensitivity (64%), specificity (93%), PPV (92%) and NPV (67%) in comparison to both NMP22 and urine cytology [29]. In addition, higher levels of survivin were associated with an increased risk of higher grade disease [30]. However, the assay is still in the experimental stages and further development and standardisation is required [10].

Conclusion

At present, cystoscopy continues to be the gold-standard for detection of recurrences in the surveillance setting. Although several biomarkers have shown promise, most haven't as yet been validated in large prospective multicentre clinical studies. A major disadvantage of many of the urinary biomarkers is their relatively limited ability to detect low-grade cancers. In addition, many of the biomarkers are susceptible to false positives. Compared to urine cytology, the sensitivity of urinary biomarkers is usually higher at the cost of lower specificity. Current urinary biomarkers may have a role in the setting of atypical or equivocal urine cytology.

It is becoming clear that it is unlikely that a single urinary biomarker will have a good enough performance due to complex interactions between molecular pathways and tumour heterogeneity. Biomarkers with future promise will likely need to test for several markers simultaneously. As the use of next-generation sequencing increases, we are likely to see a paradigm shift from candidate-driven approaches to more high-throughput approaches for screening for panels of multiple biomarkers.

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TAKE HOME MESSAGE

- Around 40-80% of patients with NMIBC will progress to higher stages of disease or recur necessitating surveillance, currently consisting of cystoscopy and urine cytology.
- Repeated cystoscopy for surveillance carries significant costs to the healthcare system. Urine cytology is limited by its poor sensitivity for low-grade tumours and false positives due to benign inflammatory conditions. Therefore, non-invasive and cost-effective strategies are required to improve the detection of recurrences and predict future recurrence and progression.
- Urinary biomarkers currently approved by the FDA for use in the surveillance of patients with NMIBC include: NMP22, BTA, UroVysion and ImmunoCyt.
- Other clinical and experimental biomarkers that have been evaluated for surveillance of NMIBC but not FDA-approved as yet include: CxBladder Monitor, CertNDx, UBC test, Xpert BC Monitor and survivin.

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