Review Article

Biomarkers for non-muscle invasive bladder cancer: Current tests and future promise

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ABSTRACT

The search continues for optimal markers that can be utilized to improve bladder cancer detection and to predict disease recurrence. Although no single marker has yet replaced the need to perform cystoscopy and urine cytology, many tests have been evaluated and are being developed. In the future, these promising markers may be incorporated into standard practice to address the challenge of screening in addition to long-term surveillance of patients who have or are at risk for developing bladder cancer.

Key words: Biomarkers, bladder cancer, transitional cell carcinoma, urothelial carcinoma

INTRODUCTION

Urothelial carcinoma of the bladder (UCB) is the 7th most common cancer worldwide in men and the 17th most common cancer worldwide in women. Approximately 75% of newly diagnosed UBCs are non-muscle invasive (carcinoma *in situ*, Ta and T1). Smoking is the most common risk factor and accounts for approximately half of all UBCs. Occupational exposure to aromatic amines and polycyclic aromatic hydrocarbons are other important risk factors for the development of UBC.[1] Bladder cancer has the highest cost of any malignancy when categorized on a per patient basis. The direct economic cost of non-muscle invasive bladder cancer (NMIBC) is primarily related to the need for lifelong cystoscopic examination as non-muscle invasive tumors are characterized by a high recurrence rate (50–70% within 5 years).[2] Stringent surveillance protocols are followed because of the high likelihood of recurrence and poor prognosis of patients who may subsequently develop muscle-invasive disease.

Development of ideal biomarkers that will enable diagnosis at an earlier stage of disease and accurately monitor for recurrence remains challenging. Urinary markers that could be used in place of or as an adjunct to current screening and surveillance techniques, or markers that could be used to risk-stratify patients and predict progression of disease, would be beneficial to clinicians for determining surveillance regimens and potential therapeutic response. This review examines the clinically available tests and emerging biomarkers in the context of potential application for bladder cancer diagnosis, prognostication and surveillance.

An ideal biomarker for bladder cancer would provide sufficient negative predictive value to allow patients to avoid invasive tests such as cystoscopy or to risk-stratify patients with indolent versus aggressive disease. The Food and Drug Administration (FDA)-approved tests include Bladder Tumor Antigen (BTA) stat®, BTA TRAK®, nuclear matrix protein (NMP22)/BladderChek® and UroVysion™ for diagnosis and surveillance, while ImmunoCyt™/uCyt™ is approved for surveillance [Table 1].

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Table 1: Summary of clinically available bladder cancer urinary biomarkers

Test	Sensitivity (%)	Specificity (%)	Comment
Cytology	25-95	86-97	High specificity, low sensitivity (especially for low-grade lesions). Interobserver variation
High grade	80-90	98-100	
Low grade	4-31	6-100	
Immunocyt	81-90	61-78	High sensitivity (even for low-grade disease), use in conjunction with cytology. Interobserver variation
BTA stat	53-83	67-72	Rapid point-of-care. High false-positive rate
BTA TRAK	66-72	51-75	Specialized laboratory required. High false-positive rate
BladderCheck/NMP22	51-85	77-96	Point-of-care. High specificity and sensitivity. High false-positive rate
Urovysion/FISH	69-75	82-89	High sensitivity and specificity. May identify early subclinical neoplastic changes. Specialized laboratory required. Expensive

BTA=Bladder tumor antigen, NMP=Nuclear matrix protein, FISH=Fluorescence in situ hybridization

URINE CYTOLOGY

Voided urine cytology remains the gold standard for non-invasive testing for bladder cancer, and is the most commonly used urinary marker in clinical practice. The overall sensitivity of voided urine cytology ranges from 25% to 95%.[3-9] Cytology has proven to be very useful for the detection of high-grade and high-stage disease. High-grade lesions and carcinoma *in situ* (CIS) can be detected by voided cytology with a sensitivity of 80–90% and a specificity of 98–100%.[4,10] Morphologic changes associated with high-grade malignant cells include increased size, increased nuclear-to-cytoplasmic ratio, nuclear pleomorphism, coarse and irregular chromatin and frequent mitotic figures. These characteristics are associated with a higher risk for bladder cancer, even in the presence of a negative cystoscopic examination.[11] Despite its effectiveness in detecting high-grade lesions, cytology has the propensity to miss low-grade disease. In a review by Renshaw and colleagues, the sensitivity for detecting low-grade lesions ranged from 0% to 100% and the specificity ranged from 6% to 100%.[12] Low-grade malignant cells may only appear slightly different from dysplastic or normal cells and can pose a challenge for cytopathologic interpretation. Conditions that can cause inflammatory changes in the bladder, such as recent intravesical therapy, radiation treatment and infection, may result in false-positive readings up to 12% of the time.[13] Moreover, the definition of a positive cytology reading can be highly variable.[7,8]

Cytology is also relatively expensive and time-consuming, costing approximately $100 per test and taking over 24 h for the results to become available.[13] The positive predictive value of atypical, suspicious and malignant reports has been reported to be 12%, 39% and 67%, respectively.[14] Urine cytology is highly specific but has intermediate sensitivity, indicating that it has a role in adjunct diagnosis but not in screening for primary bladder cancer. High tumor grade is associated with significantly higher sensitivity compared with low and intermediate grades combined.[14]

ImmunoCyt/uCyt+ assay

In 1997, Fradet and Lockhart developed the ImmunoCyt test to augment urine cytology by using an immunocytofluorescent technique that consisted of antibodies (M344 and LDQ10) labeled with fluorescein, which have been shown to react with a mucin glycoprotein, and another antibody (19A211) that reacted with a glycosylated form of carcinoembryonic antigen. These antigens are expressed by tumor cells found in the majority of bladder cancer patients and occasionally on tumor cells of some patients with prostate cancer. The antigens can be detected in tumor cells exfoliated in the urine and are not expressed in the normal genitourinary tissues, with the exception of a few umbrella cells in a small percentage of patients.[15-16]

ImmunoCyt/uCyt+ is performed under microscopy by a trained cytopathologist. A relatively large number of exfoliated cells are necessary to perform an accurate test. A cytology slide must contain a minimum of 500 cells for a negative score to be valid, while the presence of one fluorescent cell is considered positive. Sensitivity of urinary cytology could be increased from 50% to 90% (range, 81–90%) when incorporating the ImmunoCyt/uCyt+ test, but the specificities of the combined assays were less than that achieved by cytology alone (range, 61–78%).[17,20,22,24] Studies also suggest that ImmunoCyt/uCyt+ has a superior sensitivity to cytology for early pathological stage (Ta–T1) and low-grade tumors, and can significantly improve the detection of CIS.[18,19,21] Comploj *et al*. evaluated 7422 cytology and ImmunoCyt/uCyt+ tests. The overall sensitivity was 35% for cytology, 68% for ImmunoCyt and 73% for the two tests combined. The overall specificity was 98% for cytology, 72% for ImmunoCyt/uCyt+ and 72% for the two tests combined. Cytology and ImmunoCyt/uCyt+ together had an overall sensitivity of 73%, with 59% for grade 1, 77% for grade 2 and 90% for grade 3 tumors (1973 World Health Organization grading classification).[23]

ImmunoCyt/uCyt+ is less affected by hematuria and inflammatory conditions because it is a cellular assay, but

the test is subjective and depends on specimen stability and handling as well as interobserver variation.[17] These limitations restrict the ImmunoCyt/uCyt+ test to being recommended as an adjunct to cytology, and it is only approved for the surveillance of patients with a history of bladder cancer.

BTA assays

Two forms of the BTA test (Polymedco Inc., Cortlandt Manor, NY, USA) capable of detecting human complement factor H-related protein (cFH) are available and FDA approved for diagnosis and follow-up of bladder cancer. The BTA stat test is a qualitative dipstick, point-of-care immunoassay[25] while the BTA TRAK is a quantitative enzyme-linked immunosorbent assay (ELISA).[27] The reported sensitivity is 53–83% for BTA stat and 66–72% for BTA TRAK. The specificity is 67–72% and 51–75% for BTA stat and BTA TRAK, respectively.[26-29,33]

While sensitivities can be better for BTA compared with cytology, the disadvantage of these assays is that false-positive results can occur with hematuria, highly concentrated urine, cystitis and previous treatment with BCG. A number of reports have noted that there is often a high correlation between BTA data and hematuria levels, and the presence of hematuria in subjects without malignant disease can result in false-positive BTA assay tests.[30-31] Rather than detecting a bladder tumor antigen, urinary BTA assays may be measuring serum cFH introduced by bleeding,[32] a common presenting factor in bladder cancer patients.[25] BTA stat and BTA TRAK tests cannot replace cystoscopy and have limited utility as an adjunct for surveillance in patients with NMIBC.[34]

NMP22

NMP22 (BladderCheck) is an immunoassay for the detection of nuclear matrix protein 22 in urine. It is a marker of urothelial cell death and is elevated in the urine of patients with bladder cancer. The recommended NMP22 cut-off for diagnosis of urothelial carcinoma by the manufacturer is 10 u/mL; however, different cut-offs (3.6–12 u/mL) have been used. A level of 6.4 units/mL demonstrated sensitivity for urothelial carcinoma (UC) as much as twice that of cytology.[35] NMP22 is a point-of-care assay, making the test an attractive adjunct for cystoscopy. Rather than detecting a specific tumor antigen, urinary NMP22 assays measure the cellularity or amount of cell turnover that may be introduced into the urine by a variety of conditions, including surface shedding from bladder tumors. Thus, false-positive results may be encountered in hematuria and benign inflammatory conditions.[36,41]

NMP22 demonstrated a sensitivity of 51–85% and a specificity of 77–96% in detecting bladder cancer.[37,39-40] Patients with positive NMP22 and negative cystoscopy have a 9.57-times greater risk of recurrence during 1 year of follow-up compared with patients with a negative test result.[39] Hwang *et al*. reported a lower overall sensitivity for NMP22 of 32% compared with 38% for cytology. The sensitivity of NMP22 for low-grade tumors was higher than that of cytology, and when NMP22 was combined with cytology, the sensitivity for detecting bladder cancer increased.[38]

Fluorescence in situ hybridization (FISH)

The first report of a novel FISH probe set for bladder cancer detection was published in 2000.[42] This assay is referred to as UroVysion (Abbott Molecular Inc., Des Plaines, IL, USA). It is a molecular genetic technique used for detecting aneuploidy of chromosomes 3, 7 and 17 and loss of the 9p21 locus in exfoliated urothelial cells. Suggested criteria for a positive assay include finding five or more urinary cells with gains of two or more chromosomes, ≥10 cells with gain of a single chromosome (e.g. trisomy 7) or homozygous deletion of 9p21 in >20% of epithelial cells.

An overall sensitivity of 84.2% and specificity of 91.8% in detecting urothelial carcinoma was initially reported. A meta-analysis showed a pooled sensitivity and specificity of 72% (69–75%) and 83% (82–85%), respectively.[43] In a large study by Dimashkieh *et al*., the overall sensitivity, specificity, positive predictive value and negative predictive value in detecting UC were 61.9%, 89.7%, 53.9% and 92.4%, respectively. The performance was better in high-grade UC than in low-grade UC, with sensitivities of 75.5% and 40.8%, respectively.[44] The accumulation of copy number variations (CNVs, represented as DNA loss or gain) during the development of bladder cancer can begin 3 years before diagnosis, but the extent required for a positive UroVysion™ test is usually achieved no earlier than 1 year before diagnosis.[45] FISH-based molecular grading has been shown to increase the accuracy of prognostic models to predict both recurrence and progression.[46]

It may be challenging to distinguish inflammatory and reactive changes from recurrent tumor with cystoscopy and urine cytology in patients who have recently been treated with BCG. A positive UroVysion test following BCG treatment is associated with failure of therapy, and patients with superficial bladder cancer who had positive UroVysion at the end of BCG treatment were at a higher risk for progression to muscle-invasive disease. Patients with a positive FISH test should be closely monitored even if cytology and cystoscopy are negative because of the risk of disease recurrence.[47-49,56]

UroVysion has been evaluated as a reflex test when equivocal or atypical cytology has been reported.[50] A negative FISH test result under these situations likely correlates with benign cytological changes. For patients with atypical cytology and negative cystoscopy, 3-year recurrence free survival with negative and positive FISH results were 67% and 34%,

respectively.[51-53] The test appears to have a high specificity among patients who have a variety of benign genitourinary conditions, including microhematuria, BPH, infection and inflammation.[54] In addition to UC, adenocarcinoma, squamous carcinoma, small cell carcinoma of the bladder and renal cell carcinoma have been found to be associated with positive FISH results in urinary specimens.[55]

In summary, UroVysion™ seems to have a high specificity for the detection of bladder cancer and the ability to detect bladder tumor recurrence prior to clinical symptoms. Thus, it may be used as a confirmatory test for either cytology or uCyt+™ testing. One major limitation of the FISH assay is the lack of consensus regarding criteria used to evaluate abnormal cells. Additionally, the test has relatively low sensitivity in the detection of low-grade bladder tumors and may not improve sensitivity as an adjunct to cytomorphologic analysis.

Other protein markers

Many proteins are expressed in the urine of patients with bladder cancer and have potential application as diagnostic or prognostic tumor markers. These include blood group antigens, tumor-associated antigens, proliferating antigens, oncogenes, peptide growth factors and their receptors, cell adhesion molecules, tumor angiogenesis and angiogenesis inhibitors and cell cycle regulatory proteins.[57]

Cytokeratins: UBC tests and Cyfra 21.1

Cytokeratins are intracellular proteins in the intracytoplasmic cytoskeleton of epithelial cells. These proteins are released in urine following cell death. UBC-Rapid and UBC-ELISA tests are immunological assays (IDL Biotech, Borlange, Sweden) that detect cytokeratin 8 and 18 fragments in urine. The UBC-Rapid test is a qualitative point-of-care assay, UBC-ELISA is a quantitative assay requiring trained personnel to perform the ELISA and UBC rapid quantitative is a new point-of-care assay.[58-59] In a recent study, performance of UBC rapid determined visually and quantitatively and UBC-ELISA showed sensitivities of 53%, 61% and 50%, respectively, and specificities of 82%, 69% and 69%, respectively.[59] Prior studies revealed a high variability of sensitivities (36–78%) and specificities (63–97%) for the UBC rapid test (evaluated visually).[33,60-61] The UBC ELISA demonstrated a sensitivity and specificity of 40–70% and 63–75%, respectively.[61-62] High false-positive rates and the inability to detect low-grade tumors have limited the utility of these tests for bladder tumor surveillance.[63]

Cyfra 21.1 is an ELISA-based assay that detects fragments of CK19 in urine with monoclonal antibodies. Inflammatory bladder conditions can cause false-positive results.[64] The reported sensitivity and specificity of Cyfra 21.1 was 61–85% and 75–91%, respectively.[65,66]

BLCA-1 and BLCA-4

BLCA-1 and BLCA-4 are nuclear transcription factors expressed early in the development of urothelial carcinoma. These proteins are associated with tumor cell proliferation, survival and angiogenesis. BLCA-4 is expressed in tumor and adjacent benign areas of the bladder, but not in bladders without malignancy. Its expression does not appear to be affected by tumor grade or by various benign urologic disorders such as urinary tract infection, catheterization or cystitis, but may be elevated in patients with spinal cord injuries.[67] It may also be a biomarker of field changes.[68] The ELISA assay for detection of BLCA-4 in urine has a sensitivity of 89–96% and specificity of 90–100%.[69-73] In a study by Myers-Irvin *et al*., BLCA-1 demonstrated 80% sensitivity and 87% specificity.[69] These markers may help to identify individuals with earlier stages of bladder cancer, but still need further refinement and validation.[67]

INVESTIGATIONAL BIOMARKERS

Advances in proteomic technologies and urinary proteome profiling studies for bladder cancer have identified many biomarker candidates for UC, including alfa-defensin, apolipoprotein A-1 (APOA1) and alfa 1-antitrypsin (A1AT).[74-76] Alfa-defensin was able to detect bladder cancer with better sensitivity and specificity than commercial tests.[75] APOA1 was significantly elevated in urine samples from bladder cancer patients and has a high diagnostic potential. A1AT achieved a sensitivity of 74% and a specificity of 80% for bladder cancer detection.[77-78] A panel consisting of an eight-protein biomarker combination achieved 92% sensitivity and 97% specificity for the detection of bladder cancer.[79] These multiplex biomarker panels are undergoing further validation.

DNA biomarkers

Understanding bladder cancer genomics may provide opportunities for discovery of new biomarkers for diagnosis as well as possible therapeutic targets. Genomic changes in bladder cancer are complex and vary with different histological types.[80] Low-grade, papillary, non-invasive tumors are generally characterized by constitutive activation of the receptor tyrosine kinase-Ras pathway, such as activating mutations in the HRAS and fibroblast growth factor receptor 3 (FGFR3) genes. In contrast, high-grade invasive tumors are characterized by alterations in the tumor suppressor protein p53 (TP53) and retinoblastoma 1 (RB1) pathways. Recent genomic platforms have revealed that urothelial cancer is much more complex and heterogeneous, and many new significantly mutated genes have been reported.

Amplified focal regions include genes such as E2F3, CCND1, MDM2, ERBB2, CCNE1, MYC and FGFR3, and deleted regions contain genes such as CDKN2A, RB1 and

CREBBP.[81,82] The most common focal deletion contained CDKN2A and was observed in approximately 50% of bladder cancer samples. Three clusters have been identified based on somatic mutations and focal copy number alterations (CNAs): Cluster A was enriched in focal somatic CNAs in several genes and mutations in MLL2. Cluster B was characterized by deletion of CDKN2A and mutations in FGFR3 and papillary morphology. Cluster C showed TP53 mutations as well as enrichment with RB1 mutations and amplifications of E2F3 and CCNE1.[82] Kompier *et al*. monitored multiple mutations in five genes including FGFR3 in bladder tumors.[83] Mutations in individual genes were not overly prevalent (11–63%), but mutations in one or more target genes were found in 88% of primary tumors and 88% of recurrent tumors. Mutational analyses have been applied successfully to voided urine sediments, and tumor-specific mutational screening assays may have utility for diagnosis and surveillance of bladder cancer patients.[84,85]

DNA methylation

Epigenetic alterations, such as DNA methylation, are frequently observed in tumors.[86] DNA methylation occurs at cytosines located at CpG dinucleotides. CpG islands are enriched for these base pairs and typically overlap with gene regulatory regions. DNA methylation is associated with downregulation of tumor suppressor genes and may contribute to the development of bladder cancer.[87] In the past decade, several methylation markers have been identified that could aid in the detection of bladder tumors based on urinary assays. Chung *et al*. identified a panel of eight genes (A2BP1, NPTX2, SOX11, PENK, NKX6-2, DBC1, MYO3A and CA10) that were highly methylated in bladder cancer, with methylation frequencies ranging from 62% to 92%.[88] Reinert *et al*. found that POU4F2 and HOXA9 genes had a high frequency of methylation (92%) in bladder cancer tissues and were unmethylated in the normal urothelium.[89]

There is also potential utility of using gene methylation as a prognostic marker. Methylation of CDH1, FHIT, LAMC2, RASSF1A, TIMP3, SFRP1, SOX9, PMF1 and RUNX3 genes is associated with poor survival in muscle-invasive bladder cancer.[90,93] Methylation of RASSF1A and HOXB2 were associated with bladder tumor stage, grade and tumor progression.[91,92] Several studies have examined methylation markers in the urine of patients with bladder cancer. Methylation of VAX1, KCNV1, TAL1, PPOX1 and CFTR in 212 urine samples from patients (157 with primary tumors and 55 with recurrent tumors) and 190 samples from healthy controls was found to detect both primary and recurrent disease, with a sensitivity of 89% and a specificity of 88%.[94] Zuiverloon *et al*. studied the use of APC, TERT and EDNRB methylation in a urinary test for the detection of recurrent bladder cancer. A sensitivity of 63% was reported with a specificity of 58%.[95] In conclusion, methylation markers for bladder cancer diagnosis are still at an early stage compared with other FDA-approved markers and need further validation. Most methylation markers show higher sensitivity compared with cytology, but potentially at the cost of a lower specificity.[96]

RNA markers

Non-invasive detection of RNA tumor markers in urine samples represents another attractive diagnostic option. RNA isolation procedures, stability of polymerase chain reaction (PCR) amplicons as well as the amount and particularly the quality of RNA are factors that may influence the outcome of gene expression studies. Development of one general standard operating protocol is important in order to compare gene expression data across studies.[97]

Survivin

Survivin is an anti-apoptotic protein. The urinary levels of survivin are elevated in bladder cancer, and this protein has shown promise as a biomarker for UC.[98] A reverse transcription (RT)-PCR assay called BioDot has been used to detect survivin. Urinary levels of survivin have been shown to have high sensitivity (64–83%) and specificity (88–93%) for the detection of UC.[99-101] In a large, prospective study that examined survivin performance for bladder cancer screening, the marker demonstrated good negative predictive value (99%) and specificity (98%), but a low positive predictive value and sensitivity. Survivin was not influenced by confounders such as inflammation and hematuria, and the test was associated with a relatively low number of false-positive results.[102]

Telomerase

Telomerase is a RT responsible for adding tandem repeat sequences (TTAGGG) to the ends of chromosomes. Telomerase activity is increased in many cancers, and urinary telomerase activity has been shown to be an accurate marker for the detection of bladder tumors, with a specificity in the range of 87–100%.[103] Different assays for detecting telomerase activity have variable sensitivities because of the low stability of telomerase in urine. In a prospective, case–control series of 218 men, the sensitivity of telomerase with the telomeric repeat amplification protocol assay was 90% and the specificity was 88%.[63] The clinical application of this biomarker has been limited due to lack of standardization.

Other potential markers

Measurement of urinary hTERT, SENP1, PPP1CA and MCM5 mRNAs has been used to identify bladder cancer recurrence. All of these mRNA markers have been shown to be more sensitive than cytology. The combination of each marker with cytology resulted in increased detection rates.[104] Nicotinamide N-methyltransferase (NNMT) has been reported to be highly expressed in bladder cancer. Urinary NNMT expression levels have been suggested as a tool for early diagnosis.[105] Holyoake *et al*. developed a quantitative RT-PCR urine-based assay for bladder cancer detection. Overexpressed genes identified in bladder

cancer tissues (CDC2, MDK, IGFBP5 and HOXA13) were selected for this assay. Measurement of the combination of mRNA markers detected UC at a sensitivity of 85% and a specificity of 80% across all stages, with the best performance obtained with stage T1 disease.[106] In a cohort of 485 patients presenting with hematuria, an assay derived from this biomarker panel, the uRNA test, achieved a higher sensitivity (62%) compared with NMP22 and cytology, with a specificity of 85%.[107] Further validation studies are needed before these markers can be incorporated into routine clinical practice.

MicroRNA (miRNA)

miRNAs are endogenous, non-coding RNA molecules approximately 22 nucleotides in length that regulate gene expression by inhibition of translation or by degradation of mRNA.[108,115] miRNAs play an important role in normal development, cell growth, differentiation and apoptosis.[109] Dysregulated miRNAs can ultimately lead to aberrant expression of genes that may predispose normal cells to malignant transformation.[119]

Downregulation of miRNAs, including those that target the FGFR3 pathway, such as miR-145, miR-101, miR-100 and miR-99a, may be associated with increased expression of FGFR3 in the absence of FGFR3 mutation and has been observed in low-grade NMIBC.[110,111] In contrast, increased expression of miRNAs is observed in high-grade muscle-invasive bladder cancer compared with adjacent normal bladder urothelium, including miRNAs predicted to target p53, such as miR-21 and miR373.[111]

Ratert *et al*. screened 723 miRNAs for potential diagnostic and prognostic value in bladder cancer and found seven upregulated miRNAs (miR-20a, miR-106b, miR-130b, miR-141, miR-200a, miR-200a and miR-205) and eight downregulated miRNAs (miR-100, miR-125b, miR-130a, miR-139-5p, miR-145, miR-199a-3p, miR-214 and miR-222).[112] Analyses demonstrated a robust ability for miRNAs to distinguish between normal tissue and bladder cancer tissue, highlighting the prognostic potential for miR-141 in muscle-invasive bladder cancer. The combination of four miRNAs (miR-130b, miR-141, miR-199-3p and miR-205) resulted in correct classification of 100% of tissue samples. Rosenberg *et al*. found that miR-29c was significantly decreased in NMIBC that progressed and showed potential utility for risk stratification of patients with T1 disease into those with high vs. low risk of progression.[113] Only two of 36 cases with high expression of miR-29c progressed, only one of which was in the first 5 years; while 50% of patients with low expression progressed with a median progression-free survival of 35 months.

The RNA ratio of miR-126 to miR-182 in urine samples was found to detect bladder cancer with a sensitivity of 72% and specificity of 82%.[120] The sensitivity and specificity of miR-145 levels in urine to distinguish bladder cancer patients from non-cancer controls was 80% and 60%, respectively. Furthermore, miR-200a has been shown to be an independent predictor of NMIBC recurrence, with lower levels associated with a higher risk of recurrence.[117] Mengual *et al*. used a 6-miRNA diagnosis model to accurately diagnose UC with a sensitivity and specificity of 83% and 87%, respectively.[114] Another panel of miRNAs including miR-135b, miR-15b and miR-1224-3p could detect bladder cancer with 94% sensitivity and 51% specificity.[121] A diagnostic test based on three miRNAs (miR-200c, miR-141 and miR-30b) demonstrated sensitivity of 100% and specificity of 96% for the identification of invasive cancers and was able to identify invasive bladder tumors misclassified in pathologic evaluation of bladder biopsy specimens.[118] Although urinary miRNAs show promise as potential biomarkers for bladder cancer, a major challenge is that most miRNAs are down-regulated, making it difficult to use reduced miRNA levels as a diagnostic tool.[116] The clinical value and application of miRNA signatures requires external validation in larger trials as well as comparison against current standards of care.

CONCLUSIONS

The search for the ideal bladder cancer marker(s) remains challenging. A multitude of tests for bladder cancer detection and surveillance have been evaluated, but most assays are currently not routinely used in clinical practice. Identification of novel genomic alterations and advances in molecular techniques may result in the development of a new generation of molecules that could be used in clinical practice. The use of any single biomarker may prove to be inadequate for bladder cancer testing. Instead, a combination of biomarkers or panels that include the use of exfoliated cells, DNAs, RNAs, proteins and/or metabolites may yield the best approach for bladder cancer detection, surveillance and prognostication.[122-124]

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Conflicts of interest

There are no conflicts of interest.

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