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TOPIC PAPER

Diagnostic biomarkers in non-muscle invasive bladder cancer

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Abstract
Successful treatment of non-muscle invasive bladder cancer (NMIBC) relies heavily on our ability to accurately detect disease typically in the presence of hematuria as well as to detect the early recurrent tumors in patients with a history of NMIBC. Unfortunately, the current biomarker landscape for NMIBC is a work in progress. Cystoscopy continues to be the gold standard, but can still miss 10% of tumors. Therefore, physicians frequently use additional tools to aid in the diagnosis of bladder cancer, such as urinary cytology. The urinary cytology is a good option for high-grade disease; however, it is limited by low sensitivity in detecting low-grade disease, as well as variable interpretation among cytopathologists. Thus, the limitations of cystoscopy and urinary cytology have brought to light the need for more robust diagnostic assays. In this non-systematic review, we discuss the performance, potential advantages or disadvantages of these tests, and the future direction of biomarkers in NMIBC.

Keywords Bladder cancer · Urine · Diagnosis · Biomarkers

Introduction

Successful treatment of non-muscle invasive bladder cancer (NMIBC) relies heavily on our ability to accurately detect disease, typically in the presence of hematuria, in addition to the early detection of recurrent tumors in patients with a history of NMIBC. The current accepted standard for bladder cancer (BCa) detection is cystoscopy, which has withstood the test of time, and continues to be the gold standard. Cystoscopy has a sensitivity of 85–90%, and can miss 10% of papillary tumors [1]. For this reason, physicians frequently use ancillary tests to aid in the diagnosis of BCa, including urine-based assays. One example is urinary cytology, which is a good option for high-grade disease; however, it performs poorly in low-grade disease, and there is variable interpretation among cytopathologists. Furthermore, patients undergoing tumor surveillance require repeat cystoscopy, which may still miss tumors requiring adaptation of newer cystoscopic techniques with fluorescent light or narrow band imaging [2, 3]. These concerns have led to the development of urine- and blood-based biomarkers for NMIBC to offset some of the issues with the current standard, albeit with mixed results. These tests are wide ranging and include the measurement of soluble proteins in the urine such as bladder tumor-associated antigen (BTA), proteins detected on fixed urothelial cells (ImmunoCyt), chromosomal abnormalities detected by fluorescence in situ hybridization (UroVysion), or genetic-based blood tests that detect DNA or RNA abnormalities. Despite the current availability of these markers, however, their modest performance, limited added diagnostic value, increased cost, and lack of an accurately defined role in the course BCa diagnosis and treatment timeline have limited widespread use [4, 5]. As such, there is still a continued need for the further development and validation of biomarkers for this disease space [6]. In this review, we discuss the performance, potential advantages or disadvantages of these tests, and the future direction of biomarkers in NMIBC.

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Methods

We performed a non-systematic review of the literature using PubMed search for keywords "biomarkers bladder cancer," "urothelial biomarkers," and "urinary biomarkers bladder cancer." The results were reviewed by the authors, and studies were eliminated by title and abstract review. We subsequently identified the most relevant studies, which included original studies, and systematic reviews discussing urinary and blood-based markers. We also identified the other studies by reviewing the reference list of key articles.

Current markers

Urine cytology

Urine cytology was first introduced into practice in 1945 by Papanicolaou and Marshall and continues to be the gold-standard urine-based test for the detection of BCa, primarily in conjunction with cystoscopy [7]. In a recent meta-analysis, the pooled sensitivity and specificity was reported as 0.37 (95% CI 0.35–0.39) and 0.95 (95% CI 0.94–0.95), respectively [8]. Other reports indicate a sensitivity of 84% in high-grade tumors, but low sensitivity in low-grade tumors (16%) [9]. Cytology is listed in American Urologic Association (AUA) guidelines in the evaluation of patients with hematuria; however, only approximately 10% of hematuria patients undergo cytologic evaluation, suggesting that underutilization of cytology may be linked to its limited sensitivity [10]. The primary advantages of this test are its ease of use and high-specificity (mainly high-grade tumors). Furthermore, as a pathologic test, it is not affected by biochemical changes and clinical confounders such as hematuria. The disadvantages are variable interpretation among cytopathologists, however, with continued effort to standardize the approach to diagnosis and nomenclature among interpreters may, perhaps, decrease this variability [11]. Furthermore, specimens frequently have low cellular yield, and may be affected by urinary tract infections, stones, or previous intravesical therapies.

FDA approved biomarkers

BTA The BTA test (Polymedco Inc., Cortlandt Manor, NY, USA) is currently FDA approved and is available as an immunochromatographic, qualitative point-of-care assay (BTA stat), which uses monoclonal antibodies to identify human complement factor H-related protein. BTA TRAK is a quantitative ELISA test that measures the human complement factor H (cFH)-related protein. Factor H is a protein that acts as a cofactor for factor I-mediated cleavage of C3b in the in the alternative pathway of complement inhibition and complement mediated killing [12]. The ease of use and the availability of a point-of-care assay make it an attractive marker. The performance of these tests in diagnosing BCa has been shown to have a sensitivity ranging between 57 and 83% and a specificity of 60 and 92%, which can be severely affected by other urologic conditions such as urinary tract infections, stones, benign prostatic hypertrophy, and indwelling catheters or stents. The main reason is that factor H is present in high quantities in the blood; therefore, any condition that may cause hematuria will yield to significant false-positive results [13]. Another consideration is that the source of the BTA may not be directly from bladder tumor tissue. This is mainly due to the fact that large soluble glycoproteins of the factor H family are produced and secreted into the serum by Kupffer cells, hepatocytes, vascular endothelial cells, and platelets [14]. Therefore, due to the significant false-positive results and highly variable sensitivity, the routine use of this test is not recommended.

Nuclear matrix protein 22 NMP22 plays a role in cancer cell proliferation due to its involvement in DNA replication, transcription, and splicing, which are important for mitosis and cell division, and is found in the urine as result of shedding from cancer cells that have undergone apoptosis [15]. Urinary NMP22 can be detected by an FDA approved point-of-care test (BladderChek), which is an immunochromatographic assay, as well as quantitative immunoabsorbent ELISA (Matritech Inc., Newton, MA, USA). The former test is currently approved for diagnosis, and the latter for diagnosis as well as surveillance. These tests perform relatively well, are easy to use, low cost, and easily interpretable. Overall, the sensitivity of both tests ranges between 49 and 91% and a specificity of 40 and 88% [16]. The studies evaluating this test have shown that this assay has a better sensitivity compared with cytology, especially in detecting low-grade tumors, but has inferior specificity. In a study by Grossman et al. evaluating 1300 patients at risk for BCa who underwent the BladderChek NMP22 test, they found a sensitivity of 56%, compared with 16% for cytology, with a specificity of 86% compared with 99% for cytology [17]. In addition, when incorporating BladderChek with cystoscopy and cytology, the predictive accuracy of BCa was 80% [18]. As in BTA tests, false-positive results do occur in patients with urologic conditions that increase the rate of cellular turnover and apoptosis such as age, smoking, infection, stones, or instrumentation or even the presence of leukocytes or nucleated cells [19]. This test may be useful in the surveillance setting to help decide on a delayed vs. immediate cystoscopy in patients with negative cytology and a history of BCa [20].

ImmunoCyt/uCyt+ The ImmunoCyt/uCyt+ test (Scimedx Corp, Denville, NJ, USA) is used primarily in the surveillance setting and as an adjunct to cytology. The assay functions as an immunocytological test that detects the fluorescence of monoclonal antibodies targeting high-molecular-weight form of carcinoembryonic antigen as well as bladder tumor cell-associated mucins [21]. This assay requires a cytopathologist examination of a large number of exfoliated cells and subsequently scores the findings with ≥ 1 red or green cells are observed. This lends itself to interobserver variability, as well as handling and processing variability which can be a major drawback. However, it performs reasonably well with reported overall sensitivity and specificity of 50–100% and 69–79%, respectively [9]. A recent study showed that ImmunoCyt/uCyt+ may also be used in the diagnostic setting, suggesting that when included in a predictive model, it achieved a predictive accuracy of 91% for the diagnosis of BCa [22]. In a study analyzing over 7000 ImmunoCyt and cytology tests, the authors report a combined sensitivity of 73% and 73% sensitivity, essentially showing an improved sensitivity for both tests while better specificity for cytology alone (98%) [23].

UroVysion fluorescence in situ hybridization (FISH) assay UroVysion (Abbott Molecular Inc., Des Plaines, IL, USA) uses the classical FISH technology to detect aneuploidy in chromosomes 3, 7, and 17, and the loss of the 9p21 locus in urothelial cells. This test is currently approved for diagnosis or surveillance of BCa and is meant to be an adjunct to cystoscopy and cytology. The interpretation of the test requires specialized equipment and personnel; however, studies have shown superior sensitivity compared with cytology alone with sensitivity ranging between 69 and 75% and a specificity of 82 and 85% [24]. When combining morphologic features to FISH-detected aneuploidy, there are reports of automated systems achieving upwards of 100% sensitivity. In one study, 243 patients with suspicious cytology and negative cystoscopy were evaluated and found that a positive FISH was significantly associated with recurrence (HR 2.35, 95% CI 1.42–3.90, $p = 0.001$) [25]. Interestingly, some studies have found that although there may be false-positive rates associated with this test, there was a significant number of those patients who subsequently developed BCa recurrence, indicating that the underlying biology of some BCa is driven by these chromosomal aberrations, and perhaps, these patients are to be followed more closely [26]. In a study by Siedeman et al, they found that, on multivariable analysis, T-stage and FISH result were independent predictors of progression ($p < 0.05$), suggesting a role for this test in patients with negative cystoscopy and abnormal cytology/FISH findings [27]. In addition, FISH was able to predict recurrences that occurred sooner compared with FISH negative patients ($p = 0.03$). The primary disadvantage as mentioned is the requirement of specialized personnel and equipment, lack of consensus on the definition of a positive result, and the fact that there are patients with BCa that do not have the chromosomal aberrations detected by FISH, which limits its widespread use. Perhaps, the greatest utility of FISH is in patients with equivocal cytology and/or cystoscopy as it can be used to aid in treatment decisions, which has been recommended by the AUA guidelines [5]. In a study by Lotan et al., the use of reflexive FISH testing in patients with equivocal cystoscopy predicted high-grade disease [28]. Similar studies confirm these results reporting an NPV of 100% [29].

Non-FDA approved commercially available assays

CxBladder

The CxBladder test (Pacific Edge Ltd.) is a multiplex assay that detects specific mRNA in voided urine (IGFBP5, HOXA13, MDK, CDK1, and CXCR2) in combination with clinical data such as age, gender, and smoking. In one study evaluating 803 undergoing surveillance, CxBladder had a superior sensitivity compared with cytology (91% vs. 22%), as well as a superior NPV (97% vs. 87%) [30]. In another recent large trial in the surveillance setting evaluating 763 patients who had a previous diagnosis of BCa, the test performed relatively well with internal validation showing a sensitivity of 95% (95% CI 0.88–0.98) for recurrent disease in intermediate-risk patients compared with 86% sensitivity (95% CI 0.77–0.92) in low-risk disease (low-grade Ta) patients [31]. The test is commercially available in the US, Australia, New Zealand, and Singapore for monitoring patients with a history of BCa to aid in decision on delayed vs. immediate cystoscopy.

Urine bladder cancer test (UBC)

UBC (IDL Biotech) is available as a urine-based ELISA immunoassay (UCB IRMA) and a point-of-care test (UCB Rapid) measuring cytokeratin fragments implicated in tumor invasion. A number of studies have been conducted with the findings that are highly variable with sensitivities ranging from 21 to 71% and specificity ranging from 54 to 89% [32]. When performed in combination with urinary cytology, sensitivity was reported to be 77% overall, 50% for low-grade tumors, and 100% for high-grade tumors. More studies are needed prior to recommending the use of this test [33].

Assure MDx

In a recent validation study, Kessel et al. evaluated 200 patients with hematuria and no prior history of BCa using a multiplex urine-based assay assessing the mutations in

FGFR3, TERT and HRAS, and methylation of OTX1, ONECUT2, and TWIST1 [34]. In a multivariable model including mutation and methylation status as well as age, the test showed good discriminability of the assay [93% sensitivity and 86% specificity, AUC of 0.96 (95% CI 0.92–0.99) and NPV of 99%]. Furthermore, when looking at high-grade tumors only, the AUC was 1.00 (vs. 0.93 for low-grade) and similarly for T1 vs. Ta (0.99 vs 0.93). The authors conclude that these results suggest a 77% decreased need for diagnostic cystoscopy, and that use of this test in lieu cystoscopy in the setting of hematuria may be justified.

Oncuria

Oncuria (Nonagen Bioscience Corporation) is a multiplex urine-based marker that detects 10 proteins (IL8, MMP9, MMP10, ANG, APOE, SDC1, A1AT, PAI1, CA9, and VEGFA) via electrochemiluminescence detection and patterned arrays [35]. In a cohort of 288 subjects comprising of BCa (182), benign (96), and healthy volunteers (41), the 10-protein assay achieved an AUC of 0.89 (95% CI 0.85–0.93), and an overall diagnostic sensitivity specificity of 85% and 81%. Furthermore, in a prediction model, the assay predicted 99% of the BCa cases. A recent meta-analysis with 1173 patients showed that the combination of the ten biomarkers demonstrated a higher log odds ratio (log OR 3.46, 95% CI 2.60–4.31) than did any single biomarker irrespective of histological grade or disease stage of tumors [36].

Guideline recommendations

The ideal setting in which to use these validated markers, as well as which particular test to use has not been well elucidated. In a study by Bell et al. comparing the prognostic value of cytology, ImmunoCyt, BTA Stat, hemoglobin dipstick, and NMP22, the authors demonstrated that only cytology was associated with worse PFS on the univariate analysis (HR 2.67; $p = 0.017$), while NMP22 was associated with decreased RFS (HR 0.41, $p < 0.01$) and PFS (HR 0.32, $p = 0.02$) on the multivariable analysis [37]. The authors explain that perhaps NMP22 abnormality is indicative of benign disease. In the surveillance setting with patients without the evidence of recurrence undergoing cytology, Urovysion, immunocytuCyt+, and NMP22, the authors found that all positive tests were associated with increased rates of recurrence; however, in patients with negative cytology, a positive NMP22 was associated with worse recurrence (HR 4.2, $p = 0.001$) [38]. In addition, in patients with negative cytology and negative NMP22, only 13.5% recurred and 5.4% progressed at 2-year follow-up. The results from these studies, perhaps, highlight the difficulty in pinpointing the comparability of the tests and the exact setting in which to use them. As such, major guideline recommendations generally do not advocate routine use of these markers. The American Urological Association guidelines' expert opinion has advised that these markers may be used in the setting of post-BCG surveillance or to help with equivocal cytology [5]. Similarly, The National Comprehensive Cancer Network recommends the consideration of NMP22 or Urovysion testing in the surveillance setting [39]. Finally, the European Association of Urology guidelines do not recommend the routine use of biomarkers [40].

Investigational biomarkers

Single biomarkers

Apolipoproteins

In a BCa discovery biomarker series, ApoA-I was found to have increased expression in BCa [41]. The function of this lipoprotein is mainly to mediate the reverse transport of cholesterol from peripheral cells to the liver for excretion. Furthermore, this protein constitutes approximately 70% of the apolipoprotein content of high-density lipoprotein (HDL) [41]. In a study by Li et al. using a large number of samples, they showed a sensitivity and specificity of 89% and 85%, respectively [42]. In a study by Kumar et al., the combination of five different biomarkers (ApoA-I Coronin-1A, Smenogelin-2, Gamma-synuclein [SNCG] and DJ-1/PARK7) was used to assess detection of BCa using ELISA and Western blot [43]. For low-stage disease (Ta and T1) using ELISA method, it showed a sensitivity of 79% and a specificity of 100%. Using Western blot method, the sensitivity was higher at 94% with a specificity of 97%. Although the results are encouraging, further studies and external validation are required before clinical application.

BLCA

BLCA is a nuclear matrix protein family that has been associated with BCa, namely BLCA-1 and BLCA-4 [44]. Functional studies suggest that BLCA-1 has role in cancer via angiogenesis, while BLCA-4's role remains unclear other than its presence in BCa tissue and not in normal tissue [45]. In a small study by Meyers-Irvin et al., the authors demonstrated that, with the use of immunoblot and ELISA, the BLCA-1 has an 80% sensitivity and 87% specificity [46]. In recent meta-analysis of published studies on BLCA-4 show promising results with a sensitivity 93% (95% CI 0.90–0.95), and specificity of 97% (95% CI 0.95–0.98) [47].

Cyfra 21-1

CYFRA 21-1 is a soluble cytokeratin 19 fragment that was originally described in metastatic urothelial carcinoma and its presence correlated with clinical outcomes [48]. Further studies showed an association of serum levels with tumor stage and grade [49]. The diagnostic use of this marker was subsequently described to detect urine levels. In a recent meta-analysis of urine-based studies, CYFRA 21-1 demonstrated a combined sensitivity of 82% (95% CI 0.70–0.90) and a specificity of 80% (95% CI 0.73–0.86) [50]. However, prior studies have demonstrated poor performance of urine-based detection in the surveillance setting of NMIBC—limiting its utility [51].

Survivin

Survivin is a is a 12-amino acid protein and acts as an inhibitor of apoptosis protein thereby regulating cell division and survival and has been shown to have diagnostic value in BCa [52]. The presence of Survivin seems to be limited to tumors and is absent in normal tissue, and is, therefore, an attractive biomarker. Both the Survivin protein and RNA can be detected in the urine using a variety of methods that include q-PCR, Bio-Dot, ELISA, and chemiluminescence immunoassay. In a meta-analysis evaluating all the studies looking at the different detection methods found some promising results for Surviving demonstrating a sensitivity of 79% (95% CI 73–84%), and a specificity of 87% (95% CI 79–92%) with a corresponding AUC of 0.89 (95% CI 0.86–0.91) [53] With regard to detection of RNA, the combined sensitivity and specificity was found to be 84% (95% CI 79–88%) and 94% (95% CI 89–97%), respectively, with a corresponding AUC of 0.94 (95% CI 0.92–0.96).

Panel/multiplex biomarkers

As is evident from the above data, detecting BCa using single diagnostic biomarkers still remains a challenge. Accordingly, investigation into the development of accurate assays for the non-invasive detection of BCa is an active field. Though sensitivity and specificity of these biomarkers are encouraging, translating them into clinical practice may be premature or problematic, since these studies (a) have a small sample size, (b) have not been validated or c) have already met with limited success in the clinic. Furthermore, as with the currently available single biomarker clinical tests described above, these 'novel' single biomarkers are limited by the fact that not all BCa, or even all cases in one category of lesions (e.g., high stage or high grade) will harbor any single molecular change. Thus, the concept that the presence or absence of one molecular biomarker will aid clinical evaluation has not proved to be the case. The emergence of high-throughput technologies has greatly enabled DNA, RNA, protein, and metabolite biomarker discoveries.

DNA signatures

Genomic alterations are prevalent in NMIBC and detection of those alterations can be a potential biomarker for disease recurrence and progression [54]. There are a number of platforms for detecting DNA in the urine, tissue, and blood, and assessing specific genomic alterations has been evaluated with varying results. In a study looking at detection of microsatellite DNA markers and loss of heterozygosity (LOH) in urine samples, the authors have found that microsatellite markers had an AUC 0.82 (95% CI 0.68–0.96). However, when including IFNA, MBP, ACTBP2, D9S162, and of RASSF1A, and WIF1 in a marker panel, they achieved a better diagnostic performance (AUC 0.92, 95% CI 0.77–0.98) [55]. In another study evaluating a panel of 5 genes (FGFR3, HRAS, KRAS, NRAS and PIK3CA), Kompier et al. found a mutation in one or more of the panel genes in 88% of the tissue in both primary and recurrent BCa [56]. When combining *FGFR3* mutation with methylation of *HS3ST2*, *SEPTIN9* and *SLIT2* yielded a sensitivity and specificity of 94% and 76%, respectively [57]. Using a comparative genomic hybridization-based test called BCA1 in a small cohort, Larre et al. reported a sensitivity of 95% and a specificity of 86% with similar ability to detect grade as well [58]. Other strategies include the detection of DNA methylation in urine samples, which has shown some promise. A study of methylation of *DAPK, RARb, E-cadherin,* and *p16* genes in urine reported a sensitivity of 91% and a specificity of 76% [59]. A similar study evaluating methylation in *APC, RASSF1A, and p14ARF* reported an 87% sensitivity and 100% specificity [60]. Hoque et al. reported that 69% of patients had at least one promoter methylation in a 9-gene set, and in a combined logistic regression prediction model achieved a sensitivity of 82% (95% CI 75–87%) and specificity of 96% (95% CI 90–99%) [61]. A PCR-based assay detecting methylation status of *TWIST1* and *NID2* reported a sensitivity and specificity of 79% and 63%, respectively, as well as an AUC of 0.73 [62]. There are a number of other iterations that use other gene panels or varying combinations with the promising results [14].

Next-generation sequencing (NGS) has allowed for unique discoveries across varying malignancies and was recently applied to NMIBC, although, at the discovery stage, studies suggest that frequent alterations may set the stage for the next phase of biomarker development using this technology. Pietzak et al. reported their results and found significant alterations in telomerase reverse transcriptase (TERT), tumor protein 53 (TP53), Erb-B2 receptor tyrosine kinase 2 (ERBB2), and chromatin remodeling genes such as lysine demethylase 6A (KDM6A) and AT-rich interaction domain

1A (ARID1A) [63]. Similarly, another study demonstrated similar results using cytology specimens, suggesting a possible application to urine samples [64]. Other emerging strategies include detection of circulating tumor cells, which has shown some mixed results in the early studies [65].

RNA signatures

RNA detection in many of its forms, including messenger RNA (mRNA) or microRNA (miRNA), which are posttranscriptional regulators of protein expression, have recently been implicated in genitourinary malignancies [66]. In a discovery cohort of 47 samples profiling 157 miRNAs, the authors found the expression ratio of miR-126 to miR-182 to be sensitive and specific (72% and 82%, respectively) [67]. Others assessing a different panel of three miRNAs found high sensitivity of 94%, but lower specificity of 51% [68]. A commercial assay uRNA Assay (Pacific Edge Ltd., Dunedin, New Zealand) in patients with hematuria showed a sensitivity of 62% at a pre-specified specificity of 85% [69]. In another study, using real-time RT-PCR detecting a 12-gene expression signature was able to detect BCa with 98% sensitivity and 99% specificity [70]. Xpert bladder cancer monitor, which measures the mRNA levels of five genes (ABL1, CRH, IGF2, UPK1B, ANXA10) via RT-PCR, has also showed encouraging results in an early study in patients undergoing surveillance [71]. The overall sensitivity was 0.84 and NPV was 0.93, both significantly better than cytology ($p \leq 0.001$). There are a number of other formats using a similar strategy with the similar findings that are also encouraging, especially as we develop multiple signatures with higher predictive ability in BCa. For example, in a study of the predictability of a BCa using a 14-gene signature, the test was able to detect BCa with 90% sensitivity and a 100% specificity [72].

Future directions

The current use of biomarkers is limited and not widespread. One of the main challenges is using the current biomarkers in the appropriate disease setting that will yield most useful results. In addition, will the knowledge of the biomarker results change management as it does for positive cytology and negative cystoscopy, for example. As next-generation sequencing, technology improves and decreases in cost; the combination of DNA and RNA sequencing with panels of urinary metabolites in a multiplexed fashion may be the next approach. The future success of biomarkers for the diagnosis and surveillance of BCa hinges on a number of important factors. Rational study design to evaluate biomarkers along with the optimal setting in which to use these markers will be crucial [73]. Perhaps, a movement towards personalized assays might be the future of BCa detection [74]. The ultimate goal is to have an accurate assessment that will aid in risk stratification providing a more comprehensive evaluation that allows for better treatment decisions with patients.

Conclusions

The current biomarker landscape for NMIBC is a work in progress. There are many markers available today that are being continually refined for use in this disease space. The current guideline recommendations across major societies do not strongly advocate for their use due to moderate-level evidence. With increasing interest in DNA and RNA alterations in cancer, studies are elucidating the role of detecting multiple specific genomic aberrations in NMIBC as a potential biomarker for progression and recurrence. The validation of these findings will perhaps lead to highly refined and accurate markers affecting our management of NMIBC.

Author contributions IF: data collection and manuscript writing/editing. CJR: protocol/project development and manuscript writing/editing. KC: manuscript writing/editing. HF: protocol/project development and manuscript writing/editing.

Compliance with ethical standards

Ethical approval Charles Rosser is an office of Nonagen Bioscience Corp.

Human and animal rights statement No research involving human participants and/or animals was done.

References

1. Karaoglu I, van der Heijden AG, Witjes JA (2014) The role of urine markers, white light cystoscopy and fluorescence cystoscopy in recurrence, progression and follow-up of non-muscle invasive bladder cancer. World J Urol 32:651–659
2. Mowatt G, N'Dow J, Vale L, Nabi G, Boachie C, Cook JA et al (2011) Photodynamic diagnosis of bladder cancer compared with white light cystoscopy: systematic review and meta-analysis. Int J Technol Assess Health Care 27:3–10
3. Zheng C, Lv Y, Zhong Q, Wang R, Jiang Q (2012) Narrow band imaging diagnosis of bladder cancer: systematic review and meta-analysis. BJU Int 110:E680–E687
4. Soria F, Droller MJ, Lotan Y, Gontero P, D'Andrea D, Gust KM et al (2018) An up-to-date catalog of available urinary biomarkers for the surveillance of non-muscle invasive bladder cancer. World J Urol 8:362
5. Chang SS, Bochner BH, Chou R, Dreicer R, Kamat AM, Lerner SP et al (2017) Treatment of non-metastatic muscle-invasive bladder cancer: AUA/ASCO/ASTRO/SUO Guideline. J Urol 198:552–559
6. Rosser CJ, Urquidi V, Goodison S (2013) Urinary biomarkers of bladder cancer: an update and future perspectives. Biomark Med. 7:779–790

7. Papanicolaou GN, Marshall VF (1945) Urine sediment smears as a diagnostic procedure in cancers of the urinary tract. Science 101:519–520
8. Xie Q, Huang Z, Zhu Z, Zheng X, Liu J, Zhang M et al (2016) Diagnostic value of urine cytology in bladder cancer. A meta-analysis. Anal Quant Cytopathol Histpathol. 38:38–44
9. Yafi FA, Brimo F, Steinberg J, Aprikian AG, Tanguay S, Kassouf W (2015) Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. Urol Oncol. 33(66):e25–e31
10. Elias K, Svatek RS, Gupta S, Ho R, Lotan Y (2010) High-risk patients with hematuria are not evaluated according to guideline recommendations. Cancer 116:2954–2959
11. Barkan GA, Wojcik EM, Nayar R, Savic-Prince S, Quek ML, Kurtycz DF et al (2016) The Paris system for reporting urinary cytology: the quest to develop a standardized terminology. Acta Cytol 60:185–197
12. Cheng ZZ, Corey MJ, Parepalo M, Majno S, Hellwage J, Zipfel PF et al (2005) Complement factor H as a marker for detection of bladder cancer. Clin Chem 51:856–863
13. Miyake M, Goodison S, Rizwani W, Ross S, Bart Grossman H, Rosser CJ (2012) Urinary BTA: indicator of bladder cancer or of hematuria. World J Urol 30:869–873
14. Goodison S, Rosser CJ, Urquidi V (2013) Bladder cancer detection and monitoring: assessment of urine- and blood-based marker tests. Mol Diagn Ther. 17:71–84
15. He D, Zeng C, Brinkley BR (1995) Nuclear matrix proteins as structural and functional components of the mitotic apparatus. Int Rev Cytol 162B:1–74
16. Budman LI, Kassouf W, Steinberg JR (2008) Biomarkers for detection and surveillance of bladder cancer. Can Urol Assoc J. 2:212–221
17. Grossman HB, Messing E, Soloway M, Tomera K, Katz G, Berger Y et al (2005) Detection of bladder cancer using a point-of-care proteomic assay. JAMA 293:810–816
18. Lotan Y, Svatek RS, Krabbe LM, Xylinas E, Klatte T, Shariat SF (2014) Prospective external validation of a bladder cancer detection model. J Urol 192:1343–1348
19. Miyake M, Goodison S, Giacoia EG, Rizwani W, Ross S, Rosser CJ (2012) Influencing factors on the NMP-22 urine assay: an experimental model. BMC Urol. 12:23
20. Shariat SF, Savage C, Chromecki TF, Sun M, Scherr DS, Lee RK et al (2011) Assessing the clinical benefit of nuclear matrix protein 22 in the surveillance of patients with nonmuscle-invasive bladder cancer and negative cytology: a decision-curve analysis. Cancer 117:2892–2897
21. Li HX, Li M, Li CL, Ma JH, Wang MR, Rao J et al (2010) ImmunoCyt and cytokeratin 20 immunocytochemistry as adjunct markers for urine cytologic detection of bladder cancer: a prospective study. Anal Quant Cytol Histol 32:45–52
22. Cha EK, Tirsar LA, Schwentner C, Christos PJ, Mian C, Hennenlotter J et al (2012) Immunocytology is a strong predictor of bladder cancer presence in patients with painless hematuria: a multicentre study. Eur Urol 61:185–192
23. Comploj E, Mian C, Ambrosini-Spaltro A, Dechet C, Palermo S, Trenti E et al (2013) uCyt+/ImmunoCyt and cytology in the detection of urothelial carcinoma: an update on 7422 analyses. Cancer Cytopathol. 121:392–397
24. Hajdinjak T (2008) UroVysion FISH test for detecting urothelial cancers: meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. Urol Oncol. 26:646–651
25. Kim PH, Sukhu R, Cordon BH, Sfakianos JP, Sjoberg DD, Hakimi AA et al (2014) Reflex fluorescence in situ hybridization assay for suspicious urinary cytology in patients with bladder cancer with negative surveillance cystoscopy. BJU Int. 114:354–359
26. Halling KC, King W, Sokolova IA, Meyer RG, Burkhardt HM, Halling AC et al (2000) A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J Urol 164:1768–1775
27. Seideman C, Canter D, Kim P, Cordon B, Weizer A, Oliva I et al (2015) Multicenter evaluation of the role of UroVysion FISH assay in surveillance of patients with bladder cancer: does FISH positivity anticipate recurrence? World J Urol 33:1309–1313
28. Lotan Y, Bensalah K, Ruddell T, Shariat SF, Sagalowsky AI, Ashfaq R (2008) Prospective evaluation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. J Urol 179:2164–2169
29. Schlomer BJ, Ho R, Sagalowsky A, Ashfaq R, Lotan Y (2010) Prospective validation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. J Urol 183:62–67
30. Lotan Y, O'Sullivan P, Raman JD, Shariat SF, Kavalieris L, Frampton C et al (2017) Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. Urol Oncol. 35(531):e15–e22
31. Kavalieris L, O'Sullivan P, Frampton C, Guilford P, Darling D, Jacobson E et al (2017) Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma in a multicenter study. J Urol 197:1419–1426
32. Ritter R, Hennenlotter J, Kuhs U, Hofmann U, Aufderklamm S, Blutbacher P et al (2014) Evaluation of a new quantitative point-of-care test platform for urine-based detection of bladder cancer. Urol Oncol. 32:337–344
33. Pichler R, Tulchiner G, Fritz J, Schaefer G, Horninger W, Heidegger I (2017) Urinary UBC rapid and NMP22 test for bladder cancer surveillance in comparison to urinary cytology: results from a prospective single-center study. Int J Med Sci. 14:811–819
34. van Kessel KE, Beukers W, Lurkin I, Ziel-van der Made A, van der Keur KA, Boormans JL et al (2017) Validation of a DNA methylation-mutation urine assay to select patients with hematuria for cystoscopy. J Urol 197:590–595
35. Goodison S, Ogawa O, Matsui Y, Kobayashi T, Miyake M, Ohnishi S et al (2016) A multiplex urinary immunoassay for bladder cancer detection: analysis of a Japanese cohort. J Transl Med. 14:287
36. Masuda N, Ogawa O, Park M, Liu AY, Goodison S, Dai Y et al (2018) Meta-analysis of a 10-plex urine-based biomarker assay for the detection of bladder cancer. Oncotarget. 9:7101–7111
37. Bell MD, Yafi FA, Brimo F, Steinberg J, Aprikian AG, Tanguay S et al (2016) Prognostic value of urinary cytology and other biomarkers for recurrence and progression in bladder cancer: a prospective study. World J Urol 34:1405–1409
38. Todenhofer T, Hennenlotter J, Guttenberg P, Mohrhardt S, Kuehs U, Esser M et al (2015) Prognostic relevance of positive urine markers in patients with negative cystoscopy during surveillance of bladder cancer. BMC Cancer. 15:155
39. Spiess PE, Agarwal N, Bangs R, Boorjian SA, Buyyounouski MK, Clark PE, Cancer Bladder et al (2017) Version 5.2017, NCCN clinical practice guidelines in oncology. J Natl Compr Cancer Netw 15:1240–1267
40. Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM et al (2017) EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 71:447–461
41. Lei T, Zhao X, Jin S, Meng Q, Zhou H, Zhang M (2013) Discovery of potential bladder cancer biomarkers by comparative urine proteomics and analysis. Clin Genitourin Cancer. 11:56–62
42. Li C, Li H, Zhang T, Li J, Liu L, Chang J (2014) Discovery of Apo-A1 as a potential bladder cancer biomarker by urine proteomics and analysis. Biochem Biophys Res Commun 446:1047–1052

43. Kumar P, Nandi S, Tan TZ, Ler SG, Chia KS, Lim WY et al (2015) Highly sensitive and specific novel biomarkers for the diagnosis of transitional bladder carcinoma. Oncotarget. 6:13539–13549
44. Getzenberg RH, Konety BR, Oeler TA, Quigley MM, Hakam A, Becich MJ et al (1996) Bladder cancer-associated nuclear matrix proteins. Cancer Res 56:1690–1694
45. Santoni M, Catanzariti F, Minardi D, Burattini L, Nabissi M, Muzzonigro G et al (2012) Pathogenic and diagnostic potential of BLCA-1 and BLCA-4 nuclear proteins in urothelial cell carcinoma of human bladder. Adv Urol. 2012:397412
46. Myers-Irvin JM, Landsittel D, Getzenberg RH (2005) Use of the novel marker BLCA-1 for the detection of bladder cancer. J Urol 174:64–68
47. Cai Q, Wu Y, Guo Z, Gong R, Tang Y, Yang K et al (2015) Urine BLCA-4 exerts potential role in detecting patients with bladder cancers: a pooled analysis of individual studies. Oncotarget. 6:37500–37510
48. Andreadis C, Touloupidis S, Galaktidou G, Kortsaris AH, Boutis A, Mouratidou D (2005) Serum CYFRA 21-1 in patients with invasive bladder cancer and its relevance as a tumor marker during chemotherapy. J Urol 174:1771–1775 **(discussion 5–6)**
49. Washino S, Hirai M, Matsuzaki A, Kobayashi Y (2011) Clinical usefulness of CEA, CA19-9, and CYFRA 21-1 as tumor markers for urothelial bladder carcinoma. Urol Int 87:420–428
50. Huang YL, Chen J, Yan W, Zang D, Qin Q, Deng AM (2015) Diagnostic accuracy of cytokeratin-19 fragment (CYFRA 21-1) for bladder cancer: a systematic review and meta-analysis. Tumour Biol 36:3137–3145
51. Fernandez-Gomez J, Rodriguez-Martinez JJ, Barmadah SE, Garcia Rodriguez J, Allende DM, Jalon A et al (2007) Urinary CYFRA 21.1 is not a useful marker for the detection of recurrences in the follow-up of superficial bladder cancer. Eur Urol 51:1267–1274
52. Schultz IJ, Kiemeney LA, Witjes JA, Schalken JA, Willems JL, Swinkels DW et al (2003) Survivin mRNA expression is elevated in malignant urothelial cell carcinomas and predicts time to recurrence. Anticancer Res 23:3327–3331
53. Liang Z, Xin R, Yu Y, Wang R, Wang C, Liu X (2018) Diagnostic value of urinary survivin as a biomarker for bladder cancer: a systematic review and meta-analysis of published studies. World J Urol 36(9):1373–1381. https://doi.org/10.1007/s00345-018-2285-8
54. Leiblich A (2017) Recent developments in the search for urinary biomarkers in bladder cancer. Curr Urol Rep. 18:100
55. Roupret M, Hupertan V, Yates DR, Comperat E, Catto JW, Meuth M et al (2008) A comparison of the performance of microsatellite and methylation urine analysis for predicting the recurrence of urothelial cell carcinoma, and definition of a set of markers by Bayesian network analysis. BJU Int. 101:1448–1453
56. Kompier LC, Lurkin I, van der Aa MN, van Rhijn BW, van der Kwast TH, Zwarthoff EC (2010) FGFR3, HRAS, KRAS, NRAS and PIK3CA mutations in bladder cancer and their potential as biomarkers for surveillance and therapy. PLoS One 5:e13821
57. Roperch JP, Grandchamp B, Desgrandchamps F, Mongiat-Artus P, Ravery V, Ouzaid I et al (2016) Promoter hypermethylation of HS3ST2, SEPTIN9 and SLIT2 combined with FGFR3 mutations as a sensitive/specific urinary assay for diagnosis and surveillance in patients with low or high-risk non-muscle-invasive bladder cancer. BMC Cancer. 16:704
58. Larre S, Camparo P, Comperat E, Gil Diez De Medina S, Roupret M, Traxer O et al (2011) staging, and grading of urothelial carcinomas from urine: performance of BCA-1, a mini-array comparative genomic hybridisation-based test. Eur Urol 59:250–257
59. Chan MW, Chan LW, Tang NL, Tong JH, Lo KW, Lee TL et al (2002) Hypermethylation of multiple genes in tumor tissues and voided urine in urinary bladder cancer patients. Clin Cancer Res 8:464–470
60. Dulaimi E, Uzzo RG, Greenberg RE, Al-Saleem T, Cairns P (2004) Detection of bladder cancer in urine by a tumor suppressor gene hypermethylation panel. Clin Cancer Res 10:1887–1893
61. Hoque MO, Begum S, Topaloglu O, Chatterjee A, Rosenbaum E, Van Criekinge W et al (2006) Quantitation of promoter methylation of multiple genes in urine DNA and bladder cancer detection. J Natl Cancer Inst 98:996–1004
62. Abern MR, Owusu R, Inman BA (2014) Clinical performance and utility of a DNA methylation urine test for bladder cancer. Urol Oncol. 32(51):e21–e26
63. Pietzak EJ, Bagrodia A, Cha EK, Drill EN, Iyer G, Isharwal S et al (2017) Next-generation sequencing of nonmuscle invasive bladder cancer reveals potential biomarkers and rational therapeutic targets. Eur Urol 72:952–959
64. Scott SN, Ostrovnaya I, Lin CM, Bouvier N, Bochner BH, Iyer G et al (2017) Next-generation sequencing of urine specimens: a novel platform for genomic analysis in patients with non-muscle-invasive urothelial carcinoma treated with bacille Calmette-Guerin. Cancer Cytopathol. 125:416–426
65. Khetrapal P, Lee MWL, Tan WS, Dong L, de Winter P, Feber A et al (2018) The role of circulating tumour cells and nucleic acids in blood for the detection of bladder cancer: a systematic review. Cancer Treat Rev 66:56–63
66. Catto JW, Alcaraz A, Bjartell AS, De Vere White R, Evans CP, Fussel S et al (2011) MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol 59:671–681
67. Hanke M, Hoefig K, Merz H, Feller AC, Kausch I, Jocham D et al (2010) A robust methodology to study urine microRNA as tumor marker: microRNA-126 and microRNA-182 are related to urinary bladder cancer. Urol Oncol. 28:655–661
68. Miah S, Dudziec E, Drayton RM, Zlotta AR, Morgan SL, Rosario DJ et al (2012) An evaluation of urinary microRNA reveals a high sensitivity for bladder cancer. Br J Cancer 107:123–128
69. O'Sullivan P, Sharples K, Dalphin M, Davidson P, Gilling P, Cambridge L et al (2012) A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. J Urol 188:741–747
70. Mengual L, Burset M, Ribal MJ, Ars E, Marin-Aguilera M, Fernandez M et al (2010) Gene expression signature in urine for diagnosing and assessing aggressiveness of bladder urothelial carcinoma. Clin Cancer Res 16:2624–2633
71. Pichler R, Fritz J, Tulchiner G, Klinglmair G, Soleiman A, Horninger W et al (2018) Increased accuracy of a novel mRNA-based urine test for bladder cancer surveillance. BJU Int. 121:29–37
72. Urquidi V, Goodison S, Cai Y, Sun Y, Rosser CJ (2012) A candidate molecular biomarker panel for the detection of bladder cancer. Cancer Epidemiol Biomarkers Prev 21:2149–2158
73. Lotan Y, Black PC, Caba L, Chang SS, Cookson MS, Daneshmand S et al (2018) Optimal trial design for studying urinary markers in bladder cancer: a collaborative review. Eur Urol Oncol 1(3):223–230
74. Solomon JPKKA, Hansel DE (2018) Emerging molecular approaches in the analysis of urine in bladder cancer diagnosis. In: Hansel D, Lerner S (eds) precision molecular pathology of bladder cancer. Molecular Pathology Library, Springer, Cham