

# Emerging biomarkers for the diagnosis and monitoring of urothelial carcinoma

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**Abstract:** Urothelial carcinoma (UC) arises extensively from the renal pelvis, ureter, urinary bladder, and urethra. UC represents a clinical and social challenge because of its incidence, post-treatment recurrence rate, and prognosis. Combinations of urine cytology, cystoscopy, and conventional imaging such as computed tomography are currently used for diagnosis and monitoring modalities of UC. Both the poor diagnostic accuracy of urine cytology and poor cost performance of cystoscopy and conventional imaging modalities emphasize the urgent need for advancement in clinical guidance for UC. Urine- and blood-based biomarkers for detection of UC of the bladder and upper urinary tract represent a considerable research area. Biomarkers can help to improve UC diagnosis with the aim of replacing cystoscopy and other imaging examinations in future and may enable individualizing risk stratification regarding therapy and follow-up. Over the decades, numerous studies have focused on the potential application of biomarkers for UC, including urine, circulating tumor DNA, RNAs, proteins, and extracellular vesicles. Although some biomarkers such as ImmunoCyt/uCyt+, UroVysion, NMP-22, bladder tumor antigen, CxBladder, and Xpert Bladder Cancer are currently available in clinical practice, few biomarkers achieve high sensitivity and specificity. Emerging biomarkers are continuously developed and reported in medical journals. However, there is a significant lack on following external validation using different cohorts. The positive results are needed to be confirmed by more studies with large-scale cohorts and long follow-up periods to prove the true value of novel biomarkers, followed by their adoption in clinical practice. The present paper provides an overview of the evidence based on high-impact studies regarding urine- and blood-based biomarkers and their clinical applications in bladder cancer and upper tract UC.

**Keywords:** urothelial carcinoma, bladder cancer, upper urinary tract cancer, biomarker, diagnosis, surveillance

## Introduction: clinical issues in urothelial carcinoma of the bladder and upper urinary tract

Urothelium is the epithelial lining of renal collecting ducts, calyces, ureters, bladder, and urethra.<sup>1</sup> Urothelial carcinoma (UC), previously referred to as transitional cell carcinoma, is a histopathologic type of cancer that typically arises from the urothelium. Majority of cases presenting UC are bladder cancers (BCa), whereas upper urinary tract urothelial cancer (UTUC) accounts for only 5%–10% of all urothelial malignancies.<sup>2</sup> Primary urethral cancer is an extremely rare lesion, accounting for only <1% of the total incidence of malignancies. UC of the bladder (accounting for 90% of BCa) is the

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most common malignancy involving the urinary tract and the sixth most common cancer in the USA, with an estimated 79,030 cases diagnosed in 2017.<sup>2,3</sup> The incidence of BCa is approximately four times higher in men than in women. Cigarette smoking is a significant risk factor for both BCa and UTUC, with the reported OR of 3.22 and 4–11, respectively.<sup>4,5</sup> Occupational carcinogen exposure,<sup>6</sup> infection with *Schistosoma haematobium*,<sup>7</sup> phenacetin,<sup>4</sup> pioglitazone,<sup>8</sup> and thiazolidinediones<sup>9</sup> have been reported as other risk factors for the incidence of UC.

Approximately 70%–80% of BCa are diagnosed as non-muscle invasive BCa (NMIBC), consisting of Ta (70%), T1 (20%), and primary Tis (10%).<sup>10</sup> The clinical course of treated NMIBC is characterized by a favorable survival prognosis with a high intravesical recurrence rate. NMIBC is a heterogeneous subset with different treatment options, such as intravesical instillation and immediate cystectomy, follow-up schedules, and varying outcomes.<sup>11</sup> Despite transurethral resection of the bladder tumor (TURBT) followed by adjuvant intravesical instillation with chemotherapeutics or bacillus Calmette–Guérin (BCG), up to 70% of cases with NMIBC will experience intravesical recurrences, whereas 10%–30% will progress to life-threatening muscle-invasive BCa (MIBC,  $\geq$ T2).<sup>12,13</sup> Two major scoring systems for prediction of recurrence and progression after TURBT for NMIBC based on clinicopathologic parameters, the EORTC model (3), and the CUETO model (4) have been clinically available for the management of NMIBC.<sup>14,15</sup> According to the risk stratification, patients with NMIBC should be under appropriate intensive surveillance following treatment. Once disease progression is observed, the prognosis significantly declines.<sup>16–18</sup> The incredibly high rates of intravesical recurrence and disease progression require follow-up of treated NMIBC patients with cystoscopy and urine cytology at regular intervals (every 3–6 months during the next several years). Postoperative routine examinations make NMIBC one of the costliest malignancies from diagnosis to death, with an estimated cost of \$187,000 per case in the USA.<sup>19</sup> In addition, its total annual expenditure was estimated at \$4 billion in 2010, which is expected to increase up to \$5 billion by 2020.<sup>19,20</sup> The current gold standard method for detection, diagnosis, and monitoring of BCa is still a combination of flexible cystoscopy and urine cytology. As to the medical cost, cystoscopy coupled with urine cytology is expensive, about \$150 in Japan (with reference to the exchange rate against the US dollar value as of March 2016).<sup>11</sup>

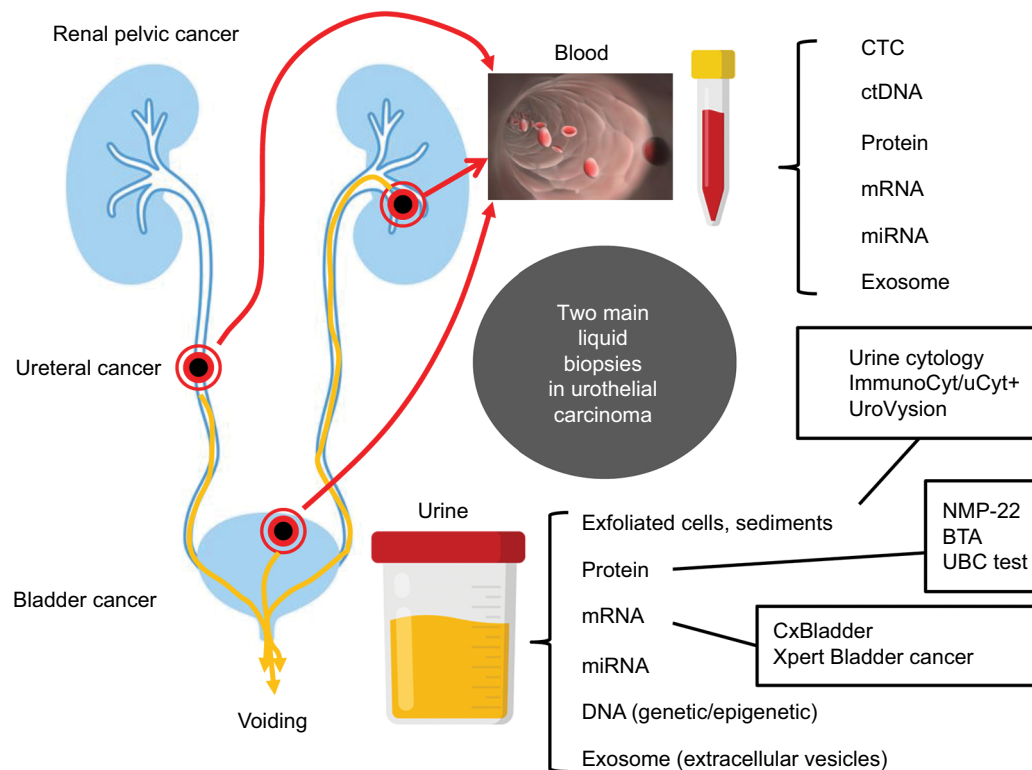
UTUC is a rare and heterogeneous carcinoma. Among UTUCs, pelvic cancer is four times as large as ureteral

cancer.<sup>21</sup> UCs involving the urinary tract have been supposed to have a similar carcinogenic mechanism and pathogenesis. However, recent studies have suggested that there are several differences, between UTUC and UC of the bladder such as tumor behavior and molecular mechanisms underlying tumor development and progression.<sup>22,23</sup> The estimated annual incidence of UTUC is ~2 cases per 100,000 person-year and has slowly risen over the past three decades.<sup>24,25</sup> Despite the improvements in diagnosis, surgery, and systemic chemotherapy, the prognosis of patients with UTUC has not improved over the past two decades.<sup>26</sup> Radical nephroureterectomy (RNU) with bladder-cuff removal is the gold standard treatment for localized UTUC. However, 60% of UTUCs are invasive at diagnosis, and oncologic outcomes are unacceptable for patients with locally advanced-stage and/or lymph node involvement.<sup>27</sup> The clinical issue of UTUC includes heterogeneous clinical courses: intravesical recurrence, extra-urinary tract recurrence, and distant metastasis. The mortality after diagnosis and treatment is significantly associated with tumor stage (pathologic Ta-1, T2, T3, and T4 stages, representing 5-year survival rates of 92.1%–97.8%, 74.7%–84.1%, 54.0%–56.3%, and 0%–12.2%, respectively).<sup>28–30</sup> Therefore, early detection of primary and recurrent UTUC is mandatory for improvement of clinical outcome. Several biomarkers with tissue-, blood-, and urine-based biomaterials have been investigated to date. Here, we review promising blood- and urine-based biomarkers, especially genomic biomarkers, for diagnosis and monitoring of UTUC.

The definition of “liquid biopsy” is the sampling and analysis of biologic fluids such as urine, blood, pleural liquid, ascites, cerebrospinal fluid, and saliva. The target of molecular analysis includes circulating tumor cells (CTCs), circulating cell-free tumor DNA, proteins, mRNAs, miRNAs, long noncoding RNAs, and vesicles (Figure 1). These biomarkers in liquid biopsy hold extensive potential because they can help in diagnosis and monitoring of disease stage, recurrence, and treatment response without invasive intervention. Numerous studies have investigated the diagnostic impact of various urine- or blood-based biomarkers such as genomic assays in urothelial malignancies. The present paper provides an overview of the evidence based on high-impact studies regarding the urine- and blood-bound biomarkers and their clinical applications in BCa and UTUC.

## Diagnostic and post-TURBT monitoring markers in BCa

Representative urine-based tests to diagnosis and post-TURBT monitoring for BCa are listed in Table 2.



**Figure 1** Main liquid biopsies in urothelial carcinoma of the bladder and upper urinary tract carcinoma

**Notes:** Several materials are available as liquid biopsy samples. In urothelial carcinoma, two main liquid biopsies are widely used for initial detection of primary tumors and monitoring systems after treatment. Urine sample is mostly used for non-muscle disease, whereas serum and plasma allow the follow-up and prediction of treatment response predominantly in advanced diseases. The commercially available assays that are approved by the US Food and Drug Administration and/or Japanese health insurance are indicated by rectangles.

**Abbreviations:** BTA, bladder tumor antigen; CTC, circulating tumor cells; UBC, urinary bladder cancer.

## Urine cytology and its derivatives

Cytology remains only one gold standard urine-based test in the clinical management of UC, with excellent specificity but unsatisfactory sensitivity for diagnosis and monitoring of NMIBC (Table 1).<sup>31–33</sup> The sensitivity significantly depends on tumor grade, size, and stage, attaining a decent sensitivity of about 60% for high-grade tumors and carcinoma in situ.<sup>34</sup> For screening recurrence of NMIBC, urine cytology has a high sensitivity for high-grade tumors but an especially low sensitivity in patients with low-grade tumors (16%–53%).<sup>32,33</sup> Another problem faced by urine cytology is that its results depend on the expertise of the cytopathologist. ImmunoCyt/uCyt+ tests based on detection of three BCa antigens (M344, LDQ10, and 19A11) in urinary exfoliated cells improve the predictive values of cytology, with 73% sensitivity, but its specificity decreased to 66%.<sup>35</sup>

UroVysion bladder cancer kit is a multitarget fluorescence in situ hybridization (FISH) assay that detects aneuploidy for chromosomes 3, 7, and 17, and focal loss of 9p21 from exfoliated urothelial cells in urine.<sup>36</sup> A meta-analysis from 14 studies demonstrated a diagnostic accuracy of 72% sensitivity

and 83% specificity of the UroVysion kit (Table 1). Several studies demonstrated that the UroVysion has higher sensitivity than urine cytology.<sup>37,38</sup> Positive UroVysion has proven to be a significant predictor of recurrence and progression in patients under NMIBC surveillance with suspicious cytology but negative cystoscopy.<sup>39</sup> In addition, positive UroVysion at the end of intravesical treatment with BCG is a risk factor for progression to MIBC.<sup>40,41</sup> Thus, surveillance with the UroVysion test can provide prognostic information for treated NMIBC patients. However, most previous studies have demonstrated that UroVysion has lower specificity than urine cytology.<sup>37,38</sup>

Over the last two decades, 5-aminolevulinic acid (5-ALA) or hexaminolevulinic acid-induced fluorescence cystoscopy has been established with the aim of detecting flat and/or small lesions that are hardly visible under conventional cystoscopy, leading to the decreased rate of post-TURBT residual tumor and recurrence.<sup>42</sup> Since 2014, we have investigated the feasibility and usefulness of urine-based tests taking advantage of 5-ALA-derived fluorescence.<sup>43,44</sup> Detection modalities are based on fluorescence microscopic cytology, fluorescence spectrophotometry, and flow cytometry. Although analyses

**Table 1** Urine-based tests to diagnosis and post-TURBT monitoring for bladder cancer (BCa)

Name of assay	Biomaterial	Marker description	Assay	Application purpose	Diagnostic accuracy (%)		Approval		Reference
					Sensitivity	Specificity	FDA	Japanese health insurance	
Urine cytology	Sediment	Morphology	Staining and microscopic exam	Diagnosis	38	98	Yes	Yes	31
ImmunoCyt/uCyt+	Sediment	Tumor-associated cellular antigens (M344, LDQ10, 19A11)	Immunofluorescence	Monitoring	16-53	94-100	Yes	Yes	32, 33
UroVysion	Sediment	Aneuploidy for chromosomes 3, 7, and 17 and loss of the 9p21 locus	Multitarget FISH	Monitoring	73	66	Yes	No	35
5-ALA-mediated fluorescent urine cytology	Sediment	Intracellular accumulation of protoporphyrin IX	Fluorescence detection devices	Diagnosis	72	83	Yes	No	36
NMP-22	Protein	NuMA	Sandwich ELISA	Surveillance	50-92	72-93	Yes	Yes	37, 38
NMP-22 BladderChek	Protein	NuMA	Dipstick immunoassay (POC)	Diagnosis and monitoring	40	99	Yes	Yes	45
BTA TRAK	Protein	hCFHrp	Sandwich ELISA	Monitoring	68	79	Yes	No	46
BTA stat	Protein	hCFHrp	Dipstick immunoassay (POC)	Monitoring	57-83	60-92	Yes	No	47, 48
UBC test	Protein	Cytokeratin 8/18 fragment	Sandwich ELISA	Monitoring	58	72-95	Yes	No	47
CxBladder Detect	mRNA	IGFBP5, MDK, HOXA13, CDK1, and CXCR2	RT-PCR	Diagnosis	64	80	No	Yes	49
CxBladder Monitor	mRNA	IGFBP5, MDK, HOXA13, CDK1, and CXCR2	RT-PCR	Monitoring	82	85	Yes	No	61
Xpert Bladder Cancer Detection	mRNA	ABL1, CRH, IGF2, UPK1B, and ANXA10	RT-PCR	Diagnosis	91	96 (NPV)	Yes	No	62
Xpert Bladder Cancer Monitor	mRNA	ABL1, CRH, IGF2, UPK1B, and ANXA10	RT-PCR	Monitoring	77	85	Yes	No	64
FGFR3 gene mutation	DNA	FGFR3	PCR	Diagnosis	84	91	Yes	No	65
				Monitoring	46% (Ta tumor) 53% (low-grade tumor)	NA	No	No	59
				Monitoring	78	100	No	No	53

**Abbreviations:** 5-ALA, 5-aminolevulinic acid; BTA, bladder tumor antigen; FDA, US Food and Drug Administration; FISH, fluorescence in situ hybridization; NPV, negative predictive value; POC, point-of-care; RT-PCR, reverse transcription-PCR; TURBT, transurethral resection of bladder tumor; UBC, urine bladder cancer.

using spectrophotometry and flow cytometry enable quantitative measurement of accumulated protoporphyrin IX (PPIX) leading to increased objectivity, they require cumbersome manual procedures. We have been developing an automated detection system for 5-ALA-derived intracellular fluorescence.

### Protein markers

Two of the most extensively studied proteins as BCa urinary biomarkers are NMP-22 and BTA. There are currently two commercialized detection assays: a quantitative sandwich ELISA test (NMP-22 Test and BTA TRAK) and a qualitative point-of-care test (NMP-22 BladderChek and BTA stat). The diagnostic performance of NMP-22 exceeds conventional urine cytology in detection with regard to sensitivity (68% vs 44%), which was predominantly due to an improved detection rate of low-grade tumors.<sup>46</sup>

In contrast, diagnostic performance of the BTA test has been evaluated by several clinical studies, showing a sensitivity of 57%–83% and a specificity of 60%–92%.<sup>47,48</sup>

Urine bladder cancer (UBC) test is a quantitative immunoassay that detects soluble fragments of cytokeratins 8 and 18, which are derived from dead UC cells in urine supernatant. The UBC test enables the discrimination of patients with BCa from noncancer individuals with a sensitivity of 64% and specificity of 80%.<sup>49</sup>

A recent report demonstrated that the protein level of two subunits of collagens (4A1 and 13A1) in urine supernatant was increased in patients with BCa compared to healthy controls. The sensitivity and specificity of the combination of both collagens (COL4A1 + COL13A1) for BCa detection were 72.1% and 65.6%, respectively.<sup>50</sup> In addition, high urinary COL4A1 + COL13A1 was found to be an independent risk factor for intravesical recurrence after TURBT. Urinary collagens could be potential diagnostic and prognostic biomarkers for BCa.<sup>50</sup> In 2014, Rosser et al reported the potential of a urine-based biomarker panel consisting of ten proteins (ANG, APOE, CA9, IL8, matrix metalloproteinase 9 [MMP9], MMP10, SDC1, SERPINA1, SERPINE1, and VEGFA) to detect intravesical recurrence (79% sensitivity and 88% specificity).<sup>51</sup> In 2016, the same group described a comparable study with a similar protein panel (including PAI-1 and A1AT instead of SERPINA1 and SERPINE1) that enabled detection of BCa patients from patients with benign disease and healthy controls (85% sensitivity and 81% specificity).<sup>52</sup>

### Genetic detection markers

Various studies have demonstrated that DNA mutations derived from urothelial cells in urine samples could detect and

predict the recurrence of NMIBC.<sup>53,54</sup> While overexpression of FGFR3 protein has been observed in 29% of muscle MIBC and 49% of metastatic MIBC, *FGFR3* mutation has been rarely observed in metastatic MIBC (6%–9%).<sup>55,56</sup> In contrast, FGFR mutations are highly associated with low-grade NMIBC (66.2% of Ta tumors and 37.9% of T1 tumors).<sup>57</sup> *FGFR3* mutations detected in urine sediments by highly sensitive PCR assay were found to be a useful detection markers of recurrence in NMIBC and its sensitivity and specificity were 78% and 100%, respectively.<sup>53</sup> Zuiverloon et al revealed that *FGFR3* mutation-positive urine was associated with 3.8-fold ( $P < 0.001$ ) higher risk of development recurrence during surveillance in NMIBC.<sup>58</sup> CertNDx™ Bladder Cancer Assay (Predictive Biosciences, Lexington, MA, USA) is a commercial urine-based *FGFR3* genetic test (not approved by the US Food and Drug Administration [FDA]). CertNDx™ was based on a study by Fernandez et al,<sup>59</sup> in which several coauthors were employees of Predictive Biosciences. When results of *FGFR3* mutation analysis were combined with the results of other molecular tests including MMP2 and DNA methylation in Twist 1/Nid2, the diagnostic accuracy for detecting cancer recurrence was a 92% sensitivity and 51% specificity.

Roperch et al demonstrated that DNA hypermethylation of CpG island marker (*HS3ST2*, *SEPTIN9*, and *SLIT2*) combined with positive *FGFR3* mutation improved the diagnostic accuracy of recurrence as compared to *FGFR3* mutation assay alone, especially for Ta/low-grade tumors (96.4/93.6% vs 58.3/54.6%).<sup>60</sup> A similar approach reported by Kandimalla et al revealed that DNA methylation CpG island markers (*OTX1*, *ONECUT2*, and *OSR1*) combined with *FGFR3* mutations could improve the sensitivity of cytology alone and *FGFR3* mutation alone for detection of recurrence of NMIBC.<sup>54</sup> The DNA-based urine biomarker could increase the detection rate of recurrence and reduce the frequency of follow-up cystoscopy.

### mRNA detection markers

Several mRNA-based urine biomarkers have been developed and have improved the accuracy of BCa diagnosis. The Cxbladder test measures five mRNA targets (*IGFBP5*, *MDK*, *HOXA13*, *CDK1*, and *CXCR2*) by quantitative real-time PCR.<sup>61</sup> The former four biomarkers are associated with growth and propagation of the tumor tissue, whereas *CXCR2* is an inflammation biomarker. Although the Cxbladder Monitor could improve the sensitivity compared to cytology (81.8% vs 56.1%), the sensitivity was slightly low (85.1% vs 94.5%).<sup>61</sup> In an external validation study including 1,036 urine samples from 803 patients undergoing surveillance,



the sensitivity was 91% and negative predictive value (NPV) was 96%.<sup>62</sup> In addition, diagnostic accuracy (sensitivity and NPV) was not affected by past history of BCG treatment.<sup>63</sup>

The Xpert BC Monitor measures five target mRNAs (*ABLI*, *CRH*, *IGF2*, *UPK1B*, and *ANXA10*) using quantitative real-time PCR.<sup>64,65</sup> These markers are related to proliferation and survival (*IGF2*), neuroendocrine stress response and inflammation (*CRH*), cell growth and signal transduction (*ANXA10*), and epigenetic dysregulation in BCa (*UPK1B*). Recently, the first prospective study was conducted to evaluate the efficiency of the Xpert BC Monitor.<sup>64,65</sup> In this study including 155 urine samples obtained from 140 patients with history of NMIBC, the sensitivity (84%) and specificity (91%) of the Xpert BC Monitor were significantly higher than bladder washing cytology (33% and 76%). The sensitivity of the Xpert BC Monitor was superior to that of cytology in low-grade (77%) and Ta tumors (82%). The sensitivity of the Xpert BC Monitor (91%) was not inferior to that of cytology (94%). This mRNA-based urinary test could increase the accuracy of diagnosis of recurrence in patients with NMIBC even for low-grade tumors and reduce invasive surveillance. Further prospective validation studies and more cost effectiveness are required for widespread use of this promising test.

### Circulating biomarkers

The existence of tumor-derived epithelial cells in peripheral blood obtained from cancer patients is associated with distant metastasis. The CellSearch system is a widely used technique that was approved by the FDA for CTC detection in patients with metastatic breast, colorectal, and prostate cancer.<sup>66</sup> However, only a few studies analyzing CTCs using the CellSearch system have been reported in patients with BCa. As to NMIBC, CTCs were detected in 18% (8/44) patients and presence of CTCs was associated with shorter time to first recurrence.<sup>67</sup> Importantly, CTC-positive NMIBC patients showed significantly worse overall, progression-free, and cancer-specific survival.<sup>67</sup> A meta-analysis of patients with BCa demonstrated that overall sensitivity of CTC detection was 35.1% and specificity was 89.4%.<sup>68</sup> CTCs may be useful for detection of residual tumors after surgery, monitoring subsequent recurrence and metastasis, or decision-making for perioperative chemotherapy. Given that there is low overall sensitivity, CTC should not be used for initial screening test for BCa.

## Diagnostic and post-RNU monitoring markers in UTUC

Representative urine-based tests to diagnosis and post-RNU monitoring for UTUC are listed in Table 2.

### Urine cytology and its derivatives

Urine cytology is a gold standard diagnostic tool for UTUC as well as BCa. Voided urine cytology provides high specificity but low sensitivity.<sup>69,70</sup> Contrarily, cytology of urine or lavage fluid obtained from upper tract showed high sensitivity and low specificity compared with that using voided urine.<sup>70,71</sup> With regard to ImmunoCyt/uCyt+ test for UTUC, the sensitivity of voided urine is 50% for cytology, 75% for ImmunoCyt/uCyt+, and 87% for both methods combined. In addition, the sensitivity of urine obtained from the upper tract is 82% for cytology, 91% for ImmunoCyt/uCyt+, and 100% for both methods combined.<sup>69</sup> The potential of UroVysion test is reported to diagnose UTUC. The overall sensitivity of FISH analysis is superior to that of cytology (77% vs 36%), and the specificities of FISH and cytology are 94.7% and 100%, respectively.<sup>72</sup>

### Protein detection markers

NMP-22 test was evaluated as a detection tool of upper urinary tract recurrence in patients having a history of NMIBC, resulting in disappointing accuracy even in high-grade tumors.<sup>73</sup> The overall sensitivity and specificity of urine obtained from the upper tract are 82% and 89% for BTA test, 11% and 54% for voided urine cytology, and 48% and 33% for the diagnosis of UTUC.<sup>74</sup>

### mRNA/miRNA detection markers

miRNAs/miRs in serum have the potential to diagnose UTUC and predict prognosis of patients with UTUC. Ten miRNAs (miR-664a-3p, miR-431-5p, miR-423-5p, miR-191-5p, miR-33b-3p, miR-26a-5p, miR-22-3p, miR-16-5p, let-7b-5p, and let-7c) were reported for the first time to have the potential to distinguish UTUC from controls (areas under the curve [AUC] > 0.8).<sup>75</sup> Furthermore, miRNA-141 could be used as a diagnostic biomarker for UTUC (AUC = 0.73), and miRNA-151b was significantly associated with tumor progression and cancer-specific survival (HR = 0.33,  $P < 0.001$  and HR = 0.25,  $P < 0.001$ , respectively).<sup>76,77</sup>

### Genetic/epigenetic risk factors and detection markers

Cyclooxygenase 2 (COX2) and caveolin-1 (CAV1) genotyping assays have showed that polymorphic genotypes (COX2; G-765C and intron 5, CAV1; rs3807987 and rs7804372) were significantly different between patients with UTUC and healthy controls. COX2 G-765C/intron 5 carrying GG/AT variants have a significantly increased risk of UTUC (OR = 4.83, 95% CI = 1.79–13.06), whereas

**Table 2** Urine-based tests to diagnosis and post-RNU monitoring for upper tract urothelial carcinoma

Name of assay	Biomaterial	Marker description	Assay	Application purpose	Diagnostic accuracy (%)		Reference
					Sensitivity	Specificity	
Urine cytology	Sediment (voiding urine)	Morphology	Staining and microscopical exam	Diagnosis	50–59	83–100	69, 71
				Monitoring the intravesical recurrence	50	100	70
	Sediment (ureteral catheterization)	Morphology	Staining and microscopical exam	Diagnosis	75	67	71
ImmunoCyt/uCyt+	Sediment (voiding urine)	Tumor-associated cellular antigens (M344, LDQ10, I9A11)	Immunofluorescence	Diagnosis	75	95	69
	Sediment (ureteral catheterization)	Tumor-associated cellular antigens (M344, LDQ10, I9A11)	Immunofluorescence	Diagnosis	91	100	69
UroVysion	Sediment (voiding urine)	Aneuploidy for chromosomes 3, 7, and 17 and loss of the 9p21 locus	Multitarget FISH	Diagnosis	77	95	72
NMP-22	Protein (voiding urine)	NuMA	Sandwich ELISA	Diagnosis	44	98	73
BTA stat	Protein (voiding urine)	hCFHrp	Dipstick immunoassay (POC)	Diagnosis	82	89	74
NA	DNA	<i>CDH1</i> , <i>HSPA2</i> , <i>RASSF1A</i> , <i>TMEFF2</i> , <i>VIM</i> , and <i>GDF15</i> promoter methylations	Quantitative methylation-specific PCR	Diagnosis	84	91	81
NA	DNA	<i>TERT</i> promoter mutations	Sanger sequencing	Diagnosis	60	97	82
			Competitive allele-specific TaqMan PCR	Diagnosis	90	92	82
NA	DNA	<i>GDF15</i> , <i>TMEFF2</i> , and <i>VIM</i> promoter methylations	Quantitative methylation-specific PCR	Diagnosis	91	100	83

**Abbreviations:** BTA, bladder tumor antigen; FISH, fluorescence in situ hybridization; NA, not available; POC, point-of-care; RNU, radical nephroureterectomy; TERT, telomerase reverse transcriptase.

those carrying CG/TT variants have a decreased risk (OR =0.26, 95% CI =0.14–0.49) than those carrying GG/TT haplotype. *CAV1* rs3807987 and rs7804372 were significantly different between patients with UTUC and healthy controls ( $P=0.0188$  and  $0.0090$ , respectively). Haplotype analysis showed that *CAV1* rs3807987/rs7804372 haplotypes carrying GG/TT, AG/TT, and AA/TT variants have a significantly high risk of UTUC compared with the GG/AT and GG/AA haplotypes (OR =1.61, 1.50 and 2.67, 95% CI =1.05–2.47, 1.18–1.90, and 1.37–5.18, respectively).<sup>78,79</sup> In addition, the cell cycle regulator, cyclin D1 (*CCND1*) G870A and *CCND1* C1722G polymorphisms were related with development and prediction of UTUC.<sup>80,81</sup> With regard to genomic biomarkers of urine, detection of promoter mutation in telomerase reverse transcriptase (*TERT*) could distinguish patients with UTUC from healthy controls and *TERT* promoter mutations were significantly correlated

with distant metastasis in patients with UTUC.<sup>82</sup> As epigenetic biomarkers, *GDF15*, *TMEFF2*, and *VIM* promoter methylations were also investigated as diagnostic biomarkers for UTUC. The detection of *GDF15*, *TMEFF2*, and *VIM* promoter methylations in urine at the same time can lead to accurate diagnosis of patients with UTUC (sensitivity 91% and specificity 100%) and patients with low level of *VIM* promoter methylation were at a high risk of cancer-specific mortality.<sup>83</sup> The presence of *CDH1*, *HSPA2*, *RASSF1A*, *TMEFF2*, *VIM*, and *GDF15* promoter methylations simultaneously identified the cause of gross hematuria as UTUC (sensitivity 82% and specificity 68%).<sup>84</sup>

Previous studies demonstrated that a subset of UTUC tumors harbors *FGFR3* mutation and this mutation is associated with favorable clinical outcome as well as BCa.<sup>85,86</sup> A case report by Silverberg indicates that the *FGFR3* urine assay, which was originally developed to diagnose BCa

(CertNDx™ Bladder Cancer Assay), could be a useful detection tool for UTUC.<sup>87</sup>

### Circulating biomarkers

In a study regarding comprehensive genomic profiling of circulating tumor DNA, 75 blood samples of metastatic UTUC were evaluated by cell-free circulating DNA next-generation sequencing, and genetic alterations, including single-nucleotide variants, indels, fusions, and copy number amplifications, were identified in 71 patients (95%). Among these, *TP53* (51%), *PIK3CA* (23%), *ARID1A* (20%), *TERT* (17%), *EGFR* (14%), *BRCA1* (11%), *ERBB2* (11%), *FGFR3* (11%), *NF1* (11%), and *MET* (10%) were the ten most frequent alterations. In patients with metastatic UTUC, the frequency of genetic alterations significantly differed between circulating tumor DNA next-generation sequencing and historical tumor tissue studies for *TP53* and *FGFR3*. Gene alterations in *TP53* and *FGFR3* were significantly decreased and increased, respectively, in circulating tumor DNA compared to tumor tissue.<sup>88</sup> *FGFR3* and *HRAS* alterations are more common in UTUC, whereas *TP53* and *RBI* alterations are more common in BCa.<sup>89</sup> Accumulation of evidence could lead to a novel convenient method for diagnosis and monitoring of UTUC.

### Limitations of urine-based biomarkers

Miyake et al have shown that BTA stat/BTA TRAK is a surrogate for hematuria and NMP-22 test, which detects nuclear mitotic apparatus-associated protein, identifies status of cellular proliferation.<sup>90,91</sup> The target protein of BTA is complement factor H-related protein, which is abundant in blood. Therefore, positive BTA or NMP-22 test results can be obtained easily in benign urologic conditions such as benign prostatic hyperplasia, stones, endourologic stents, or urinary tract infections.

Fantony et al suggested that no advancement in urine- and blood-based noninvasive testing has occurred in BCa during recent years and no significant change in the current monitoring scheme (cystoscopy and urine cytology) has occurred.<sup>92</sup> In addition, they pointed out the poor performance, marginal clinical utility, and potential harm of the currently available urine-based tests, which make them inadequate for regular clinical use. One of the biggest limitations of both cytology and FISH is spectrum bias. In biostatistics, spectrum bias is defined as the phenomenon in which the performance of a diagnostic test varies in different clinical settings because each setting has a different mix of patients. For example, age, sex, and smoking history can result in a change in diagnostic test accuracy. Urine tests for BCa have different sensitivities

and specificities depending greatly on the patient background, which would halt the wide use of a diagnostic test to real-world populations being screened for BCa. Another concern of urine-based tests for cancer is the potential harm. A positive or equivocal result of urine-based tests accompanied with a negative result of cystoscopy leads to patients' anxiety and pressure for further examinations, such as bladder biopsies, dynamic contrast-enhanced imaging, retrograde pyelography, or ureteroscopy. Some of these procedures require preoperative counseling, medical optimization, and anesthesia. Moreover, these are time-consuming, expensive, and put the patient at risk for iatrogenic complications including hematuria, ureteral injury, and urinary tract infections, sometimes associated with multidrug-resistant bacteria and leading to septic status. A false-positive urine test result can raise significant risks caused by overtesting, overestimation, and overtreatment that should be avoided and prevented.

### Future perspective

This review does not mention about biomarkers on treating UC. A recent publication, "Comprehensive molecular characterization of the muscle-invasive bladder cancer", by Robertson et al refers to the new classification of BCa, specifying how biomarkers can play a role in selecting the patients most likely to respond to treatment with different agents, including immunotherapeutic agents.<sup>93</sup> Our prospective view is whether blood/urine-based biomarkers shown in this review would be applicable to the therapeutic approach, such as predictive markers for drug efficacy/resistance and disease markers reflecting clinical status of the advanced disease.

### Conclusion

This review highlights that there is considerable interest in the use of urinary/blood biomarkers for the clinical management of BCa and UTUC. Unfortunately, many potential biomarkers are still under evaluation. The most frequent indication for these malignancies is hematuria. Urine-based biomarkers apply to the initial screening test of hematuria patients as well as postoperative surveillance of patients with treated NMIBC. Cystoscopy has great sensitivity (<95%) for detecting BCa, and computed tomography urography has sensitivity and specificity of ~95% for detecting UTUC.<sup>31,94</sup>

The requirement for cystoscopy and computed tomography urography represents a significant cost to health care services in diagnosing UC. Traditional noninvasive imaging modalities with high-cost performance do not have the satisfactory diagnostic accuracy to replace cystoscopy for the detection of BCa and computed tomography for the



detection of UTUC. A highly sensitive and specific urinary/blood assay will revolutionize both the screening method for hematuria and surveillance pathway for NMIBC and UTUC. Although emerging biomarkers are continuously developed and reported in medical journals, there is a significant lack on following external validation using different cohorts. Positive results need to be confirmed by more studies with large-scale cohorts and long follow-up periods to prove the true value of novel biomarkers, followed by their adoption in clinical practice.

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## Disclosure

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