Urinary Tumor DNA-Based Diagnosis and Surveillance for Nonmuscle-Invasive Bladder Cancer—Current Landscape and Future Directions

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Abstract

Bladder cancer has a significant impact on patients, in terms of both morbidity and financial burden. This is especially true for patients with nonmuscle-invasive bladder cancer, who undergo long-term surveillance via cystoscopy and imaging, resulting in significant costs and risks. To address this issue, urinary tumor DNA analysis, or "urinary liquid biopsy," has emerged as a potential solution to reduce the testing burden and mitigate many of the costs and risks. Over time, urinary tumor DNA analysis has undergone several refinements. However, existing FDA-approved urinary biomarker assays currently lack the sensitivity and specificity to significantly impact clinical decision-making. Subsequent iterations of these technologies have attempted to bridge this gap by improving their diagnostic accuracy, and ultimately, clinical utility. Here, we discuss the current role as well as future directions of urinary tumor DNA analysis for the detection and long-term surveillance of nonmuscle-invasive bladder cancer.

Introduction

Each year, there are over 500 000 new cases of bladder cancer diagnosed worldwide[1]. This places a substantial burden on patients, with estimated annual costs exceeding $100 000 per patient[2–4]. Consequently, the development of efficient and cost-effective methods for early disease detection is of the utmost importance. In current practice, diagnostic workup typically involves a combination of urinalysis, cytology, cystoscopy, and imaging[5]. These tests are also used in the surveillance setting after patients have local treatment with transurethral resection of bladder tumor (TURBT). Cystoscopy, in particular, is associated with increased costs of care and exposes patients to potential discomfort and risks for infection and other complications[6,7]. Compliance with invasive testing can also be an issue for patients, leading to increased rates of progression and recurrence. Additionally, cytology comes with significant limitations in the form of low sensitivity[8]. Previous meta-analyses have demonstrated overall sensitivities ranging from 30% to 40%, with higher-grade disease associated with greater sensitivity. Because of this, surveillance of nonmuscle-invasive bladder cancer (NMIBC) beyond cystoscopy poses great challenges.

Key Words

Bladder cancer, nonmuscle-invasive bladder cancer, NMIBC, liquid biopsy, urine tumor DNA

Competing Interests

None declared.

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There has been growing interest in the application of urinary liquid-biopsy–based assays for the detection and surveillance of NMIBC. Because it is a readily available biofluid, urine has received most of this interest. Urine allows for the potential detection of both genetic material released directly from tumor cells and malignant cells exfoliated into the urine[9]. There are currently several FDA-approved urinary biomarker assays for the detection and surveillance of bladder cancer such as ImmunoCyt, NMP22, UroVysion, BTA, and Cxbladder[10]. However, these tests exhibit low sensitivities and specificities, leading to poor utilization by providers within clinical contexts (**Table 1**).

While the first-generation urine-based assays did not meet the required performance levels for clinical applications, further efforts have focused on developing tests with improved sensitivities and specificities. These tests aim to be non-invasive, reproducible, and capable of not only detecting initial disease but also serving as a marker for treatment response and disease recurrence, providing real-time information on a patient's disease status. Ideally, these tests should predict the presence or absence of both low-grade and more invasive disease. Such improvements would aid greatly in alleviating the morbidity and economic hardship associated with frequent clinic visits, imaging, and cystoscopy surveillance.

Currently, plasma circulating tumor DNA (ctDNA) tests are used in the invasive and metastatic settings, as well as for monitoring response to immunotherapy[11,12]. The application of urinary tumor DNA (utDNA) in a purely non-invasive setting has not yet been established. Therefore, multiple trials are exploring utDNA analysis and attempting to characterize tumor biopsy specimens on a molecular basis to improve prognostication and personalize treatment options.

Here, we will highlight an array of exciting past and ongoing investigations related to utDNA analysis for NMIBC, which we consider to be at the forefront of the field.

Completed Studies/Trials

UroSEEK (Springer et al.)

The first generation of urinary tumor DNA assays faced significant limitations in both sensitivity and specificity, particularly regarding the detection of low-grade tumors. Springer et al. attempted to address this issue through a combinatorial approach that involved the development of UroSEEK[13]. UroSEEK combined 3 separate tests (10 gene multiplex, TERT singleplex, and aneuploidy) to detect mutations in 11 different genes and copy number changes on 39 chromosome arms. UroSEEK positivity was defined as a positive result in any one of these 3 tests, yielding a large increase in the assay's sensitivity.

Patients were divided into 3 cohorts: a detection cohort, a surveillance cohort of those with confirmed bladder cancer, and a surveillance cohort of those with confirmed upper tract urothelial carcinoma. The detection cohort consisted primarily of patients who had presented with microscopic hematuria. It is important to emphasize that hematuria, either microscopic or gross, represents the most common initial presentation for bladder cancer. Therefore, the author's detection group represents a realistic clinical application of their assay within a diagnostic context. Within this group, UroSEEK demonstrated a sensitivity of 83% and specificity of 99.5% for detecting bladder cancer. When combined with cytology, the sensitivity increased to 95%. The authors estimated the cost of this UroSEEK-cytology combination to be one-third of the cost of cystoscopy alone. In the surveillance cohort, UroSEEK was able to detect recurrence with a sensitivity of 68% and specificity of 80%. On average, UroSEEK positivity preceded the clinical diagnosis of recurrence by 7 months.

uCAPP-Seq (Dudley et al.)

After the publication of the initial results from UroSEEK, Dudley et al. attempted to streamline the process further by developing uCAPP-Seq (utDNA CAncer Personalized Profiling by deep Sequencing), a novel high-throughput sequencing–based hybrid capture assay[14]. uCAPP-Seq captures single nucleotide variants (SNVs), insertions and deletions, and copy number alterations in genomic regions known to harbor common driver mutations for bladder cancer. The panel was based on data published from The Cancer Genome Atlas (TCGA)[15]. In total, it assays regions encompassing more than 450 genes and was predicted to identify a median of 7 mutations per bladder cancer patient.

Similarly to Springer et al., Dudley et al. tested uCAPP-Seq in both detection and surveillance cohorts. The researchers employed both tumor-informed and tumor-naive profiling approaches to each group. In the detection cohort, the majority of which exhibited stage pTa (74%) with low-grade disease (54%), their tumor-naive approach achieved a sensitivity of 83% with a specificity of 97%. In comparison, cytology alone had a sensitivity of only 14% and specificity of 100%. As expected, their tumor-informed approach reached an even greater sensitivity of 93% with a specificity of 96%. However, it is important to note that this approach requires prior tumor and germline sequencing, making it less suitable for disease detection.

In their surveillance cohort of patients who had previously received local bladder cancer treatment, their tumor-naive approach successfully detected disease recurrence in 84% of patients compared with only 38% for cytology. When cytology and cystoscopy were combined, only 53% of patients who had recurrence were detected, significantly lower than the 84% detected by uCAPP-Seq positivity alone ($P = 0.0057$).

UroMark (Feber et al.)

Feber et al. developed UroMark, a bisulphite urine sediment sequencing assay, and performed a proof-of-concept investigation[16]. This 150-loci multiplex assay was developed using 86 muscle-invasive bladder cancer (MIBC) patients and 30 healthy controls. It was subsequently validated with an independent cohort consisting of 167 bladder cancer (BC) patients and 274 healthy controls.

The assay itself performed robustly with a sensitivity of 98%, a specificity of 97%, and a negative predictive value (NPV) of 97% for detecting primary BC. Future investigations will focus on accurately diagnosing patients presenting with hematuria within a clinical context.

Xiao et al.

Xiao et al. continue to advance the diagnostic landscape of NMIBC[17]. They devised a study to comprehensively characterize the entire DNA methylation landscape of bladder cancer to determine the relevant biomarkers for the diagnosis of non-invasive bladder cancer.

In this multicenter, prospective cohort study, the researchers collected 304 samples from 224 donors and performed genome-wide bisulphite sequencing for DNA methylation signature discovery. They developed a targeted sequence assay for bladder cancer–specific DNA methylation signatures to distinguish tumor tissue from normal urine or MIBC from NMIBC.

Independent validation was performed by double-blinded targeted sequencing of urine samples to determine the clinical diagnosis and prognostic value of DNA methylation-based classification models. The study measured the concordance between pathology results and urinary tumor DNA (utDNA) methylation, genomic mutations, and other state-of-the-art diagnostic methods. The assay showed positivity in 60 of 60 patients (100%) found to have high-grade bladder cancer on initial TURBT. Additionally, 21 of 57 patients (37%) with a positive DNA methylation signal experienced disease progression. A subset of these patients had negative pathology results on initial TURBT but were later found to harbor bladder cancer on subsequent TURBT. This result further highlights the potential of utDNA for early diagnosis before visible tumors are present.

Strandgaard et al.

Strandgaard et al. retrospectively analyzed 156 NMIBC patients who had received Bacillus Calmette-Guérin (BCG) treatment[18]. In all patients, urine samples were available for analysis both before and after treatment with BCG. The researchers analyzed the samples for the presence of 92 immuno-oncology–related proteins using the Olink Target 96 Immuno-Oncology Assay (Olink; Uppsala, Sweden). They also performed deep-targeted sequencing of tumor-specific mutations in urine cfDNA with 3 different NGS panels (Twist Bioscience; San Francisco, US). The researchers found significantly higher tumor DNA (tDNA) levels in the urine of the post-BCG recurrence group, but no difference was observed in tDNA levels for the pre-BCG samples. Notably, the exhaustion status defined by CD8 T-cells in tumor was found to be significantly associated with tDNA levels in both the pre- and post-BCG urine samples. They also reported that patients with tDNA clearance after BCG treatment has significantly better recurrence-free survival than patients without clearance.

Additionally, the investigators identified several genes related to cell division and immune function that were upregulated in patients who subsequently developed high-grade recurrence after BCG treatment. They were then able to assign scores based on these genes to predict the likelihood for tumor recurrence. These exciting new urine biomarkers allow for real-time assessment of a patient's response to standard-of-care treatment,

TABLE 1.

Reported sensitivity and specificity of commercially available urine-based assays for detecting bladder cancer

	NMP22	BTA	UroVysion	ImmunoCyt	Cxbladder	UroMark
Sensitivity	0.69	0.65	0.63	0.78	0.82	0.98
Specificity	0.77	0.74	0.87	0.78	0.85	0.97
Company	Alere	Polymedco	Vysis	Scimedex	Pacific Edge	UCL Cancer Center

enabling a more personalized approach to patient care.

Ongoing Studies/Trials

Assessment of the Concordance of Genomic Alterations Between Urine and Tissue in High-Risk NMIBC Patients (Trial ID: NCT03563443)

This study from France aims to investigate whether analyzing urine cell-free tumor DNA can predict relapse in high-risk NMIBC. The eligibility criteria will be patients with high-risk BCG-naive NMIBC who are set to undergo treatment with BCG. Urine samples will be collected at various intervals, including before their initial BCG treatment, and at each subsequent instillation. Tumor samples will be collected upon initial TURBT and in the event of relapse. The primary outcome will be the agreement rate between urine cfDNA and tumor tissue mutation profile, focusing on the concordance rate between mutations identified in the tumor. The secondary outcome will be to evaluate the prognostic value of tumor mutational burden (TMB), which will be calculated in the urine cfDNA for each patient.

This study also aims to compare the NMIBC cohort to an MIBC cohort, which will have urine samples collected at each routine clinic visit and have their cfDNA analyzed against their initial diagnosis at TURBT.

Genomic Imprinting Testing for Diagnosis of Bladder Cancer (Trial ID: NCT03563443)

In recent years, increased attention has been devoted toward investigating epigenetic changes such as loss of imprinting (LOI) within cancer cells. These changes have been demonstrated to play key roles in cancer mechanisms such as the development of resistance to chemotherapy and radiotherapy[19]. A novel approach investigating these mechanisms, LisenID, is being developed by LISEN Imprinting Diagnostics company (Wilmington, US). Specifically, this trial aims to broadly investigate tumor LOI patterns to establish a predictive and diagnostic urine-based bladder cancer LOI panel.

Because of their ubiquitous nature, epigenetic changes such as LOI can aid in potentially overcomes issues related to tumor heterogeneity when relying solely on mutational methodologies. Furthermore, data has suggested that LOI often precedes many morphological changes within tumor cells, potentially expanding the window for their detection and enhancing the sensitivity of urine-based testing for NMIBC.

Previous studies integrating mutational and methylation changes have demonstrated significant improvements in disease detection and surveillance[20–22]. For example, a study by Cheng et al. combined copy number variant and methylation analyses to improve the sensitivity of detecting NMIBC to 91.9%[22]. When applied only to low-grade tumors, the sensitivity remained relatively robust at 84.2%, indicating a substantial improvement from the first-generation tests described above.

The Performance of Cancer Risk Genes in the Necessity of Secondary TURBT (Trial ID: NCT05112523)

Patients with a bladder mass undergo standard-of-care TURBT for tissue diagnosis ± postoperative intravesical treatment. If the patient is deemed to have NMIBC, depending on the stage and the grade, repeat TURBT is often recommended, or is considered standard-of-care. However, there are currently some patients who do not need an additional procedure, which would reduce overtreatment, patient morbidity, and healthcare costs. This study seeks to investigate whether a urine biomarker can detect residual bladder tumor lesions and thus predict who will need repeat TURBT before further treatment.

Genetron Uro V1 is a non-invasive urinary liquid-biopsy assay for assessing the risk for uroepithelial tumor using DNA extracted from urinary pellets. The investigators will perform this test on patients' urine samples before patients undergo a secondary TURBT and compare the test results with the final pathologic results. These findings will help to predict the need for secondary TURBT in high-risk NMIBC patients and establish correlations with patient recurrence-free survival and overall survival.

Discussion and Future Directions

Relying solely on pathologic analysis of bladder cancer specimens has been demonstrated to be surprisingly unreliable. In a study conducted by Luchey et al., dedicated genitourinary pathologists re-reviewed 1191 bladder cancer biopsy specimens and found that nearly 30% of them resulted in a change in pathology[23]. This significant level of interobserver discordance can lead to suboptimal patient care by providing treatments that do not reflect the true pathologic stage. Urine-based biomarkers have the potential to supplement clinical workflows by allowing for more precise risk stratification and staging of bladder cancer patients (**Figure 1**, created in BioRender). However, these modalities are not perfect, and there lies a great deal of heterogeneity based on methodology used and thresholds placed on the assays themselves.

Future directions in this field involve integration of promising novel methylation-based approaches with preexisting single nucleotide variant and copy number–based assays. The aim of these combinatorial techniques will be to maximize the sensitivity of detecting NMIBC for both diagnostic and surveillance purposes. This, in turn, will allow patients with negative results to be safely spared from the financial burden and risks associated with routine cystoscopy. By mitigating morbidity and costs associated with excess cystoscopy, streamlined and efficient personalized care can be delivered[2,3].

Despite early upfront costs, tailored genomic approaches have the potential for alleviating long-term financial strain[24]. A study by Gordon et al. estimated average costs of $300 for targeted panels and $3000 for whole genome sequencing[25]. These costs could easily be offset by the reduced need for computed tomography (CT) scans and surveillance cystoscopies through the integration of genomic assays. Specifically, these management strategies have been previously modeled to yield cost reductions of up to 9% in low-, intermediate-, and high-risk bladder cancer patients[26].

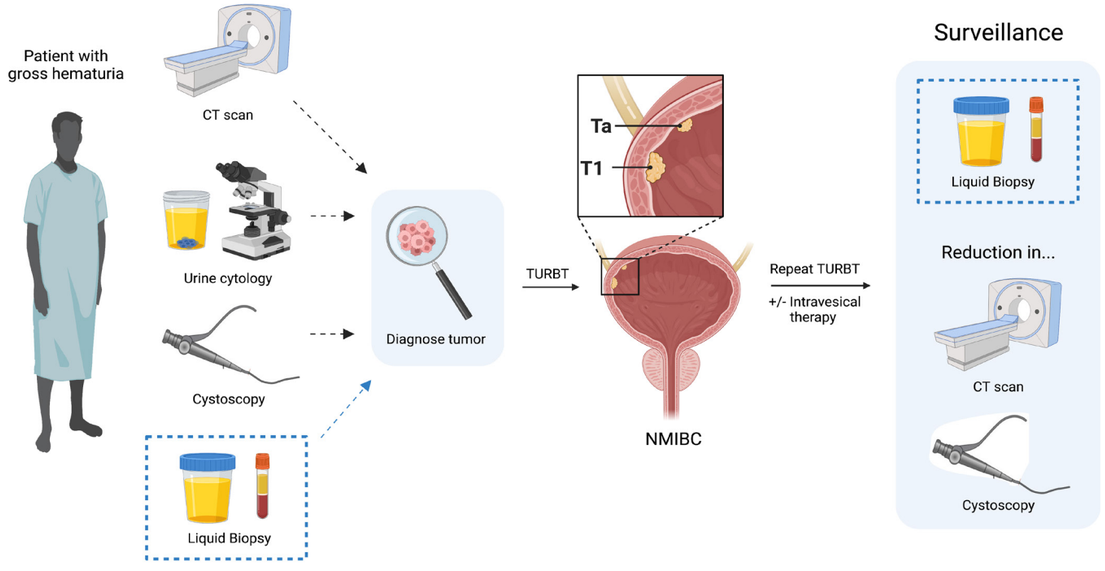
When developing and evaluating these assays, an important consideration is whether urine sediment or supernatant should be used, as tumor DNA can be found in both. The DNA present in the supernatant is known as cell-free (cfDNA) and is thought to contain a higher proportion of tumor DNA compared with sediment[27–29]. However, a consensus has yet to be reached on which fraction is optimal.

Another potential avenue for expansion involves the application of personalized biomarkers derived from a patient's original TURBT sample. This approach could greatly benefit surveillance settings, as demonstrated by studies such as Dudley et al., which showed key improvements in assay sensitivities when using tumor-informed analyses instead of tumor-naive analyses. Further development of tissue registries and investigations of genomic concordance between urine cfDNA and tissue samples will enable urine-based assays to play an expanded role in guiding clinical management and disease prognostication.

Currently, radical cystectomy is a common management strategy for many patients with high-risk, treatment-refractory NMIBC[30,31]. Improvements in urine-based assays can allow for upfront risk stratification and identification of these high-risk patients. This would enable the option of early tumor-informed cystectomy for patients predicted to harbor high-risk NMIBC. The presence of micrometastatic disease represents an additional risk for these patients. While not discussed in this paper, the supplementation of preexisting plasma-based assays could aid in early diagnosis and treatment[32].

FIGURE 1.

A schematic illustrating the potential role for liquid biopsy in both the diagnosis and surveillance of NMIBC. This would allow for reductions in the quantity of CT and cystoscopy required for management. CT: computed tomography; TURBT: transurethral resection of bladder tumor; NMIBC: non-muscle-invasive bladder cancer.



Conclusion

The current landscape of urine tumor-based testing for NMIBC shows promise and tremendous potential for future improvement and growth. Clinical applications, in both diagnostic and surveillance contexts, will enable providers to offer personalized, precision treatments and management strategies. Reducing the risk and financial toxicity associated with unnecessary cystoscopies will result in more efficient delivery of care, benefiting both patients and their providers.

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