Urine-Based Cell-Free DNA Tests in Urothelial Cancer: Additional Value for Clinical Decision-Making?

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Liquid biopsy analyses encompass a wide range of materials and analytes derived from tumors. Although blood tests are commonly used for liquid biopsy analysis, other fluids like urine, saliva, stool, and cerebrospinal fluid may also be used and provide novel insights into specific cancer types[1]. Analysis of tumor-specific cell-free DNA (cfDNA) in blood has emerged as a strong biomarker, particularly for the detection of minimal residual disease and metastatic relapse in various cancers, including bladder cancer[2]. Ongoing clinical intervention trials are currently ongoing—such as IMVIGOR011 (NCT04660344) and TOMBOLA (NCT04138628)—to determine the clinical benefit of blood-based tests. For patients with bladder cancer, urine analysis has been performed for decades for the diagnosis and detection of recurrence, particularly in the non-muscle invasive bladder cancer (NMIBC) setting. Urine studies conducted in NMIBC patients are typically based on urine pellets, as these pellets harbor the cancer cells shed directly into the urine. However, despite the superior performance of biomarker tests over cytology, none have been recommended as replacements or supports for cystoscopy.

Historically, most research in this area has focused mainly on cancer type–specific biomarkers, which often exhibit low sensitivity and specificity for clinical decision-making. Additionally, the highly accurate detection of a few molecules of patient-specific alterations requires use of novel technologies and analysis protocols developed over the past decade. Studies applying sensitive methods like next-generation sequencing (NGS) and droplet digital PCR (ddPCR) for urine analysis have demonstrated promising results for recurrence detection in the early-stage setting. Dudley et al. used a 311 kb custom panel (460 gene regions) sequencing method (uCAPP-Seq) to analyze urine tumor DNA in 118 patients with NMIBC. They detected urine tumor DNA in 91% of patients who experienced recurrence in the surveillance setting, with a positive lead time in 92% of cases[3]. In another study, Ward et al. applied deep-targeted sequencing of mutations in 23 genes and demonstrated a sensitivity of 87% and specificity of 85% in 165 hematuria patients diagnosed with bladder cancer. Moreover, in the surveillance setting of NMIBC patients, the test showed a sensitivity of 86% and specificity of 63%[4]. While patient-specific tests for NMIBC surveillance may further increase sensitivity, they can significantly increase costs because of the increased workload associated with designing patient-specific assays. The reported lower specificity of these tests may also result from an increased sensitivity compared to cystoscopy, as evidenced by the increased risk for disease relapse often associated with "false-positive" tests in this setting. New randomized clinical trials are needed that incorporate sensitive technologies to determine whether cystoscopies can be omitted in certain cases during disease surveillance. In this setting, it is also important to acknowledge that the gold standard (cystoscopy) is not 100% sensitive[5].

Renal clearance of plasma cfDNA into the urine[6] is another important aspect to consider. This means that the cfDNA detected in cell-free urine (supernatant) harbors biological signals from both tumor cells in the bladder and from DNA cleared from plasma. However, in the nonmetastatic setting, the DNA fractions originating from plasma may be small compared to contributions from apoptotic cells in the bladder. Previous studies on cfDNA have shown that mutated DNA is present in urine at elevated levels years before the diagnosis of disease progression and metastasis[7,8]. In muscle-invasive bladder cancer (MIBC), urine samples have shown promise in detecting residual disease prior to radical cystectomy, with mutated cfDNA levels correlating to clinical outcomes[8,9].

Key Words

Bladder cancer, urine, biomarker, cfDNA

Competing Interests

None declared.

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Abbreviations

cfDNA cell-free DNA
NAC neoadjuvant chemotherapy
NMIBC non-muscle invasive bladder cancer

In a recent study conducted by my team, we performed paired urine and plasma analysis on 92 patients before, during, and after neoadjuvant chemotherapy (NAC) to assess the value of urine-based analyses in NAC response and outcome, as well as to evaluate the synergistic effects of urine and plasma analysis[10]. Urine and plasma samples were prospectively collected for cfDNA analysis using standardized protocols to optimize the analysis. We found that detection of mutated cfDNA in urine and plasma prior to cystectomy was significantly associated with lower response rates and poor survival. Furthermore, the dynamics of mutated cfDNA in urine and plasma during NAC was also associated with response and outcome. A combined analysis showed that patients with non-detectable mutated cfDNA in urine or plasma exhibited very good outcomes compared to patients with detectable cfDNA in both sample types. This suggests that a combination of measurements for residual disease in the bladder (urine) and systemic disease (plasma) may hold clinical relevance; however, larger studies are needed. It should be noted that although standardized protocols for urine procurement were applied, significant variation in urine-based results were observed, with sensitivities ranging from 70% to 80% in samples with known residual tumors. This underscores one of the main challenges with urine-based analysis: the concentration of biological signals may fluctuate significantly and may thus be difficult to control. More controlled methods for standardizing this should be evaluated. Ultimately, when validated in larger studies, the combined approach of urine and plasma analysis may guide treatment decisions regarding neoadjuvant therapy response, continuation of treatment, and the potential application of bladder-sparing approaches.

Overall, urine-based analyses hold potential benefits for bladder cancer patients—both in early-stage disease for disease surveillance and in advanced stages for monitoring treatment efficacy and guiding treatment decisions. However, as highlighted in this commentary, several limitations must be addressed in new studies before these tests can become clinically useful.

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