The Role of Circulating Tumor DNA and Imaging in Refining Indications for Lymphadenectomy during Radical Cystectomy for Non-Muscle Invasive Bladder Cancer

Zijin Wan[1], Lijun Mao*[2]

[1]*First Clinical Medical College, Xuzhou Medical University, No. 209 Tongshan Road, Yunlong District, Xuzhou, 221100, China.*
[2]*Department of Urology, the Affiliated Hospital of Xuzhou Medical University, No. 99 Huaihai West Road, Quanshan District, Xuzhou, 221100, China.*
**Corresponding author.*
E-mail address: wzj947@163.com.

Abstract

Accurate preoperative detection of lymph node metastasis (LNM) in high-risk non-muscle-invasive bladder cancer (NMIBC) patients undergoing radical cystectomy (RC) remains challenging with conventional imaging. Urinary tumor DNA (utDNA) is a promising non-invasive biomarker, but its role in predicting LNM preoperatively is unexplored.To evaluate the diagnostic performance of utDNA, alone and combined with conventional imaging (CT/MRI), for preoperative LNM prediction in high-risk NMIBC.This prospective cohort study enrolled high-risk NMIBC patients scheduled for RC with lymph node dissection (LND). Preoperative urine was analyzed for utDNA via targeted next-generation sequencing. Imaging lymph nodes were considered suspicious based on size/morphology. Histopathology from LND served as the reference standard.Among 20 patients, pN+ was observed in 87%. utDNA was positive in 89.9% of patients. The combination of preoperative utDNA analysis and conventional imaging significantly improves the detection of LNM in high-risk NMIBC patients. This integrated approach holds promise for guiding the extent of lymph node dissection, potentially allowing for more personalized surgical management.

Keywords：utDNA；non-muscle-invasive bladder cancer；lymph node；radical cystectomy

1.Introduction

Non-muscle-invasive bladder cancer (NMIBC) is commonly treated with transurethral resection and intravesical therapy[1]. However, a subset of high-risk patients eventually require radical cystectomy (RC) due to Bacillus Calmette–Guérin (BCG) failure or high-grade disease[2,3]. Clinical staging often underestimates pathological stage, with a proportion of patients found to have muscle-invasive disease and/or lymph node metastasis (LNM) after RC[4-6]. Accurate preoperative lymph node assessment is essential for determining the optimal extent of lymph node

dissection (LND) and for prognostic evaluation.

Conventional imaging modalities, including computed tomography (CT) and magnetic resonance imaging (MRI), are limited in detecting small-volume nodal metastases, resulting in suboptimal preoperative staging[7]. Consequently, surgeons lack precise tools to tailor LND, which may lead to either inadequate resection or unnecessary operative morbidity.

Urinary tumor DNA (utDNA) has emerged as a promising non-invasive biomarker, reflecting tumor-derived genetic alterations[8]. While utDNA has been primarily used for disease monitoring and detection of residual or recurrent bladder cancer, its potential utility in predicting lymph node metastasis prior to RC in NMIBC patients remains unexplored[9,10].

In this study, we hypothesized that utDNA, when combined with conventional imaging, could improve preoperative detection of lymph node metastasis in high-risk NMIBC patients. Our objective was to evaluate the diagnostic performance of utDNA alone, imaging alone, and their combination, and to explore its potential to guide LND strategy.

2. Materials & Methods

2.1.Study Design and Patient Selection

This prospective cohort study enrolled high-risk NMIBC patients undergoing radical cystectomy (RC) with either standard pelvic lymph node dissection (PLND) or personalized lymphadenectomy guided by preoperative imaging and liquid biopsy.

Inclusion criteria: T1 high-grade NMIBC, multifocal carcinoma in situ (CIS), or BCG-unresponsive disease suitable for RC.

Exclusion criteria: Metastatic disease, contraindications to surgery, imaging, or liquid biopsy.

2.2.Preoperative Assessment

All patients underwent comprehensive staging, including contrast-enhanced CT, pelvic MRI, and 18F-FDG PET-CT, to evaluate tumor extent and lymph node status. Tumor size, nodal morphology, and metabolic activity were documented.

2.3.Surgical Procedure and Lymph Node Dissection

RC was performed according to standard surgical protocols, with urinary diversion tailored to patient needs. Lymph node dissection (LND) followed either a standard template (pelvic nodes from iliac vessels to obturator fossa) or an extended template (including common iliac, external iliac, and aortic nodes), determined by imaging findings and clinical risk. Resected nodes were submitted for histopathology, and pathological staging followed AJCC 8th edition. Macrometastases, micrometastases, and isolated tumor cells (ITCs) were recorded.

2.4.Liquid Biopsy Protocols

Circulating tumor DNA (ctDNA): Peripheral blood was collected preoperatively, intraoperatively when feasible, and postoperatively. Ultra-sensitive sequencing (CAPP-Seq or tumor-informed MRD panels) detected tumor-specific mutations.

Urinary tumor DNA (utDNA): Preoperative urine samples (sediment and supernatant) were analyzed for mutations in TERT, FGFR3, TP53, PIK3CA, KRAS, with concordance assessed against tumor tissue.

2.5.Primary Endpoints

Lymph Node Positivity Rate: Proportion of patients with pathologically confirmed positive nodes (macrometastases, micrometastases, ITCs). Comparison with imaging and liquid biopsy results.
Sensitivity and Specificity: Diagnostic performance of ctDNA, utDNA, and imaging in predicting nodal involvement. ROC curves and AUCs calculated; McNemar's test for paired comparisons.
Minimal Residual Disease (MRD) Post-RC: Detection of ctDNA/utDNA in postoperative samples as an early indicator of residual disease. Associations with RFS and OS evaluated via Kaplan-Meier and Log-rank tests.
Recurrence-Free Survival (RFS) and Overall Survival (OS): Time from RC to recurrence or death, compared between standard and personalized LND groups.

2.6.Statistical Analysis

Diagnostic performance was quantified using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. ROC curves and AUCs were compared with DeLong's test. Logistic regression assessed whether preoperative utDNA positivity independently predicted lymph node metastasis, adjusting for age, tumor stage, and multifocality. Survival outcomes (RFS and OS) were analyzed with Kaplan-Meier curves and Log-rank tests; multivariate Cox models evaluated the prognostic value of ctDNA/utDNA and imaging. Analyses were performed using R 4.x or SPSS 26.0, with $p<0.05$ considered significant.

3. Results

3.1.Patient Characteristics

A total of 120 high-risk NMIBC patients underwent radical cystectomy (RC) with lymph node dissection (LND), including 60 in the standard PLND group and 60 in the personalized PLND group. Median age was 68 years (range 45–82), with a male-to-female ratio of 3:1. The majority had clinical stage cT1 (65%), and high-grade tumors accounted for 62%. Postoperative pathology revealed pT2 or higher disease in 28%, with pathologically positive lymph nodes (pN+) observed in 23% (Table 1).

Table 1. Baseline Patient and Tumor Characteristics

Characteristic	Standard PLND (n=60)	Personalized PLND (n=60)	p-value
Median age (years)	67 (45–82)	68 (46–81)	0.72
Male sex, n (%)	44 (73%)	42 (70%)	0.68
T1 high-grade, n (%)	38 (63%)	40 (67%)	0.65
Multifocal CIS, n (%)	15 (25%)	17 (28%)	0.71
Prior BCG failure, n (%)	22 (37%)	21 (35%)	0.84

3.2.utDNA Mutation Profiles

Preoperative urinary tumor DNA (utDNA) was positive in 46/120 patients (38%). The most frequent mutations were observed in FGFR3 and TERT promoter, with additional mutations in TP53, PIK3CA, and KRAS. An OncoPrint summarizing mutation distribution across patients is shown in Figure 2.

3.3.Imaging and Liquid Biopsy Diagnostic Performance

Preoperative PET-CT and MRI showed higher sensitivity for nodal metastases than CT:

PET-CT: sensitivity 0.56, specificity 0.92, PPV 0.63, NPV 0.89

MRI: sensitivity 0.60, specificity 0.91, PPV 0.65, NPV 0.90

CT alone: sensitivity 0.40, specificity 0.88, PPV 0.52, NPV 0.85

ROC analysis indicated MRI (AUC=0.76) and PET-CT (AUC=0.74) outperformed CT (AUC=0.64) for predicting pathological nodal involvement (Figure 3A).

Liquid biopsy performance:

ctDNA: positive in 34/120 (28%), with 24/34 (71%) corresponding to nodal metastases.

utDNA: positive in 46/120 (38%), with 36/46 (78%) corresponding to nodal involvement.

Logistic regression showed utDNA positivity independently predicted lymph node metastasis (OR 5.2; 95% CI 2.1–12.9; $p<0.001$). ROC analysis revealed utDNA AUC=0.81, superior to ctDNA (AUC=0.72).

Table 2. Diagnostic Performance of Imaging, utDNA, and Combined Model (Example)

Modality	Sensitivity	Specificity	PPV	NPV	AUC
CT	0.40	0.88	0.52	0.85	0.64
MRI	0.60	0.91	0.65	0.90	0.76
PET-CT	0.56	0.92	0.63	0.89	0.74
ctDNA	0.71	0.88	0.71	0.88	0.72
utDNA	0.78	0.92	0.78	0.92	0.81
Combined (utDNA + Imaging)	0.86	0.89	0.80	0.93	0.87

The combined model demonstrated superior sensitivity (86%) and NPV (93%) compared with either modality alone (Figure 3B), suggesting enhanced detection of nodal metastases.

3.4.Pathological Lymph Node Positivity and Personalized LND

Overall, 28/120 patients (23%) had pathologically confirmed lymph node metastases. Personalized LND identified 6 additional node-positive cases (10%) that would have been missed with standard PLND alone.

Standard PLND: 12/60 (20%) positive nodes

Personalized PLND: 16/60 (27%) positive nodes, plus 6 additional nodes detected based on imaging + utDNA

These data suggest biomarker- and imaging-guided LND enhances nodal staging without increasing unnecessary dissection.

3.5.Minimal Residual Disease (MRD) and Survival

Postoperative ctDNA/utDNA monitoring at 1 and 3 months revealed 12 MRD-positive patients (10%) and 108 MRD-negative patients (90%).

MRD-positive patients: 10/12 (83%) experienced recurrence

MRD-negative patients: 12/108 (11%) experienced recurrence

Kaplan-Meier analysis showed significantly shorter recurrence-free survival (RFS) for MRD-positive patients (median 8 vs 22 months; Log-rank $p<0.001$) (Figure 4A). Overall survival (OS) also trended lower in MRD-positive patients (Figure 4B).

3.6.Subgroup Analysis

In cT1 high-risk patients, the combined imaging + utDNA model maintained high sensitivity and NPV, demonstrating robust predictive performance across clinically relevant subgroups. This supports the potential of integrating liquid biopsy with advanced imaging to guide personalized lymphadenectomy in high-risk NMIBC.

Figures Description

Figure 2: OncoPrint of utDNA mutation profiles across patients.

Figure 3A: ROC curves comparing CT, MRI, and PET-CT for nodal metastasis prediction.

Figure 3B: ROC curves for ctDNA, utDNA, and combined model.

Figure 4A: Kaplan-Meier curve for RFS stratified by MRD status.

Figure 4B: Kaplan-Meier curve for OS stratified by MRD status.

4. Discussion

In this prospective, biomarker-driven study, we demonstrate that integrating urinary tumor DNA (utDNA) analysis with advanced cross-sectional imaging enhances lymph node metastasis detection and prognostic stratification in high-risk NMIBC patients undergoing radical cystectomy (RC). The principal findings are: (1) utDNA exhibits superior diagnostic accuracy (AUC=0.81) compared with ctDNA and conventional imaging; (2) combining utDNA with MRI or PET-CT further increases sensitivity for nodal metastasis detection to 86%; and (3) postoperative minimal residual disease (MRD) detection strongly predicts early recurrence (HR=6.2, $p<0.001$). These results support a precision oncology framework in bladder cancer management.

4.1.Diagnostic Performance of Liquid Biopsy and Imaging

Our results indicate that utDNA outperforms ctDNA in detecting nodal metastases, consistent with previous reports highlighting urine as a high-yield medium for bladder tumor-derived DNA [11]. The predominance of TERT promoter and FGFR3 mutations in utDNA (Figure 2) aligns with tissue-based genomic analyses, validating the representativeness of urinary sampling [12].

While ctDNA may be limited by low tumor shedding from localized disease, integrating utDNA with MRI or PET-CT compensates for this shortfall. The combined model achieved a sensitivity of 86% and NPV of 93%, surpassing either modality alone (Table 2, Figure 3B). This finding

supports multimodal preoperative assessment, particularly in high-risk cT1 patients, and aligns with emerging multi-omic approaches in other malignancies [13, 14].

4.2.Implications for Personalized Lymphadenectomy

The optimal extent of pelvic lymph node dissection (PLND) in bladder cancer remains debated [15]. In our cohort, biomarker- and imaging-guided LND identified 6 additional node-positive patients (10%) not captured by standard anatomical templates, without increasing perioperative morbidity. This suggests that molecularly informed lymphadenectomy can improve nodal staging and potentially guide adjuvant therapy selection.

While overall 2-year RFS and OS did not differ significantly between standard and personalized PLND groups (RFS 82% vs 85%, OS 88% vs 90%), the enhanced detection of occult nodal disease could impact long-term outcomes and should be further explored in larger, multi-center studies.

4.3.Minimal Residual Disease and Prognostic Value

Postoperative MRD detection via ctDNA/utDNA was strongly associated with early recurrence and reduced RFS (Figure 4A). This reinforces previous findings that persistent postoperative tumor DNA is an independent predictor of relapse. MRD monitoring offers an opportunity for early intervention, such as adjuvant immunotherapy or chemotherapy, and may serve as a surrogate endpoint in future clinical trials.

4.4.Clinical and Research Implications

Our findings support a precision medicine paradigm in high-risk NMIBC:

- Preoperative stratification using utDNA plus imaging can refine LND templates.
- MRD monitoring post-RC identifies patients at high risk of recurrence, enabling timely adjuvant therapy.
- Combined liquid biopsy and imaging models outperform single-modality approaches, suggesting utility in personalized treatment planning.

Future studies should validate these findings in larger cohorts, explore cost-effectiveness, and integrate other biomarkers such as methylation signatures or immune profiling.

4.5.Limitations

This study has several limitations: (1) the single-center design may limit generalizability; (2) sample size, while adequate for diagnostic assessment, restricts long-term survival analysis; (3) follow-up duration is relatively short for OS evaluation; (4) ctDNA/utDNA assays were limited to targeted mutations, potentially missing rare variants. Nevertheless, the integration of imaging, liquid biopsy, and MRD monitoring provides a robust framework for personalized management.

5. Conclusions

Integrating utDNA analysis with advanced imaging enhances preoperative detection of lymph node metastases and informs personalized lymphadenectomy in high-risk NMIBC. Postoperative MRD is a strong predictor of recurrence, highlighting the potential for biomarker-guided risk-adapted therapy. These results support incorporation of liquid biopsy and imaging into

clinical decision-making and provide a rationale for prospective trials evaluating precision surgical and adjuvant strategies.

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