EVIDENCE AND EMERGING OPTION IN DIAGNOSIS AND MANAGEMENT OF UPPER TRACT UROTHELIAL CARCINOMAS

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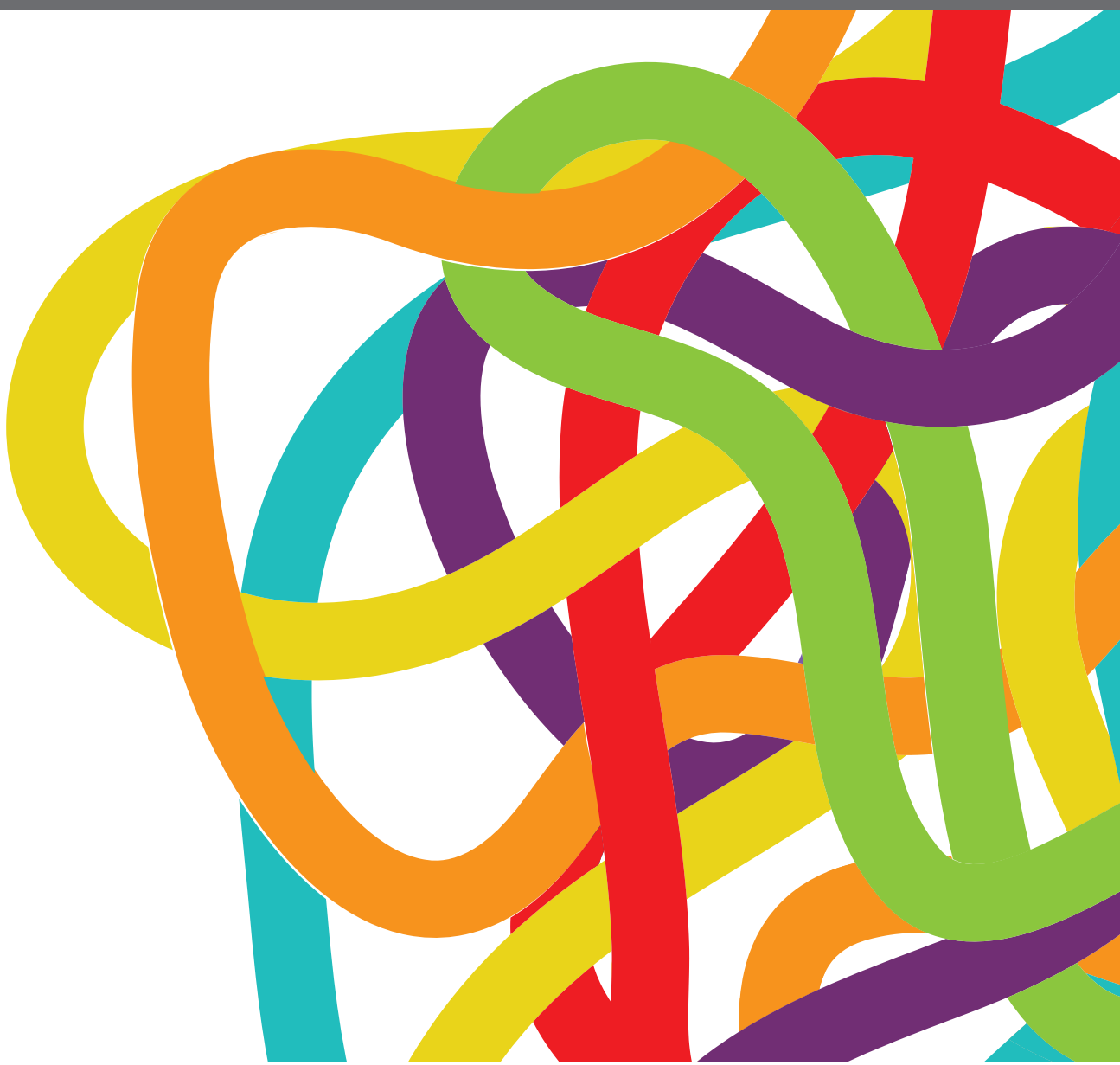

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EVIDENCE AND EMERGING OPTION IN DIAGNOSIS AND MANAGEMENT OF UPPER TRACT UROTHELIAL CARCINOMAS

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Table of Contents

05 ***Editorial: Evidence and Emerging Option in Diagnosis and Management of Upper Tract Urothelial Carcinomas***
Liangren Liu, Yige Bao and Chengfei Liu

08 ***Predictive Value of Preoperative Positive Urine Cytology for Development of Bladder Cancer After Nephroureterectomy in Patients With Upper Urinary Tract Urothelial Carcinoma: A Prognostic Nomogram Based on a Retrospective Multicenter Cohort Study and Systematic Meta-Analysis***
Bo Fan, Yuanbin Huang, Shuang Wen, Qiliang Teng, Xinrui Yang, Man Sun, Tingyu Chen, Yan Huang, Yumei Wang and Zhiyu Liu

31 ***Conditional Intravesical Recurrence-Free Survival Rate After Radical Nephroureterectomy With Bladder Cuff Excision for Upper Tract Urothelial Carcinoma***
Jae Hoon Chung, Wan Song, Minyong Kang, Hwang Gyun Jeon, Byong Chang Jeong, Seong IL Seo, Seong Soo Jeon, Hyun Moo Lee and Hyun Hwan Sung

40 ***Knowledge of and Compliance With Guidelines in the Management of Non-Muscle-Invasive Bladder Cancer: A Survey of Chinese Urologists***
Dan-Qi Wang, Qiao Huang, Xing Huang, Ying-Hui Jin, Yun-Yun Wang, Yue-Xian Shi, Si-Yu Yan, Lu Yang, Bing-Hui Li, Tong-Zu Liu and Xian-Tao Zeng

49 ***Comprehensive Analysis of N6-Methylandenosine-Related Long Non-Coding RNAs Signature in Prognosis and Tumor Microenvironment of Bladder Cancer***
Kang Chen, Shaoming Zhu, Weimin Yu, Yuqi Xia, Ji Xing, Jie Geng and Fan Cheng

64 ***Prospective Clinical Trial of the Oncologic Outcomes and Safety of Extraperitoneal Laparoscopic Extended Retroperitoneal Lymph Node Dissection at Time of Nephroureterectomy for Upper Tract Urothelial Carcinoma***
Jiwei Huang, Hongyang Qian, Yichu Yuan, Xingyun Cai, Yonghui Chen, Jin Zhang, Wen Kong, Xiaorong Wu, Ming Cao, Yiran Huang, Haige Chen and Wei Xue

71 ***The Evolution of Clinicopathological Diagnostic Features of Upper Tract Urothelial Carcinoma in China: A Summary of 2561 Cases in the Last 20 Years***
Chunru Xu, Changwei Yuan, Cuijian Zhang, Dong Fang, Yanfei Yu, Xiang Wang, Zhihua Li, Yan Wang, Qi Tang, Gengyan Xiong, Lei Zhang, Zhisong He, Jian Lin, Liqun Zhou and Xuesong Li

81 ***The Prognostic Impact of Tumor Location in pT3N0M0 Upper Urinary Tract Urothelial Carcinoma: A Retrospective Cohort Study***
Tzu Shuang Chen, Yen Ta Chen, Hung Jen Wang, Po Hui Chiang, Wen Chou Yang, Wei Ching Lee, Yao Chi Chuang, Yuan Tso Cheng, Chih Hsiung Kang, Wei Chia Lee, Chien Hsu Chen, Yuan Chi Shen, Yi Yang Liu, Hui Ying Liu, Yin Lun Chang, Yu Li Su, Chun Chieh Huang and Hao Lun Luo

89 ***Identification of Germline Mutations in Upper Tract Urothelial Carcinoma With Suspected Lynch Syndrome***

Bao Guan, Jie Wang, Xuesong Li, Lin Lin, Dong Fang, Wenwen Kong, Chuangyu Tian, Juan Li, Kunlin Yang, Guanpeng Han, Yucai Wu, Yuhui He, Yiji Peng, Yanfei Yu, Qun He, Shiming He, Yanqing Gong, Liqun Zhou and Qi Tang

97 ***Risk Factors for Unfavorable Pathological Types of Intravesical Recurrence in Patients With Upper Urinary Tract Urothelial Carcinoma Following Radical Nephroureterectomy***

Jun Zhu, Xiaoqing Zhang, Wei Yu, Xuesong Li, Zhisong He, Liqun Zhou, Zhongyuan Zhang and Gengyan Xiong

107 ***Impact of Adjuvant Chemotherapy on Variant Histology of Upper Tract Urothelial Carcinoma: A Propensity Score-Matched Cohort Analysis***

Chi-Wen Lo, Wei-Ming Li, Hung-Lung Ke, Yi-Huei Chang, Hsi-Chin Wu, I-Hsuan Alan Chen, Jen-Tai Lin, Chao-Yuan Huang, Chung-Hsin Chen, Jen-Shu Tseng, Wun-Rong Lin, Yuan-Hong Jiang, Yu-Khun Lee, Chung-You Tsai, Shiu-Dong Chung, Thomas Y. Hsueh, Allen W. Chiu, Yeong-Chin Jou, Ian-Seng Cheong, Yung-Tai Chen, Jih-Sheng Chen, Bing-Juin Chiang, Chih-Chin Yu, Wei Yu Lin, Chia-Chang Wu, Chuan-Shu Chen, Han-Yu Weng and Yao-Chou Tsai

119 ***Is Lymph Node Dissection Necessary During Radical Nephroureterectomy for Clinically Node-Negative Upper Tract Urothelial Carcinoma? A Multi-Institutional Study***

Hsiang-Ying Lee, Chao-Hsiang Chang, Chi-Ping Huang, Chih-Chin Yu, Chi-Wen Lo, Shiu-Dong Chung, Wei-Che Wu, I-Hsuan Alan Chen, Jen-Tai Lin, Yuan-Hong Jiang, Yu-Khun Lee, Thomas Y. Hsueh, Allen W. Chiu, Yung-Tai Chen, Chang-Min Lin, Yao-Chou Tsai, Wei-Chieh Chen, Bing-Juin Chiang, Hsu-Che Huang, Chung-Hsin Chen, Chao-Yuan Huang, Chia-Chang Wu, Wei Yu Lin, Jen-Shu Tseng, Hung-Lung Ke and Hsin-Chih Yeh

129 ***Could Metabolic Syndrome Be a Predictor of Survival Outcomes in Upper Tract Urothelial Carcinoma? A Propensity Score Matching Study in a Large Chinese Center***

Xiang Dai, Fei Wang, Yiqing Du, Caipeng Qin, Shicong Lai, Yuxuan Song, Zixiong Huang, Songchen Han, Xiaopeng Zhang and Tao Xu

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Editorial: Evidence and emerging option in diagnosis and management of upper tract urothelial carcinomas

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KEYWORDS

upper tract urothelial carcinomas (UTUC), diagnosis, management - healthcare, biomarkers, survival data analysis

Editorial on the Research Topic
Evidence and emerging option in diagnosis and management of upper tract urothelial carcinomas

Upper tract urothelial carcinoma (UTUC) is a neglected cancer in urology (1, 2). Due to the relative rarity of UTUC, a great amount of decision-making in UTUC therapeutics comes from evidence based on bladder cancer. However, many discoveries have proven that UTUC has different features compared to bladder cancer (3). Computed tomography urography (CTU), cytology, and ureteroscopy in suspected UTUC show that nearly 60% of UTUC are invasive at the time of diagnosis, and nearly 25% are metastatic (4). There is a general lack of high-level evidence on the early diagnosis and management of UTUC, such as kidney-sparing surgery (KSS), lymph node dissection, neoadjuvant and adjuvant chemotherapy, immune checkpoint inhibition, and systemic therapy for metastatic UTUC. This Research Topic aims at recent advances in the diagnosis and therapy of UTUC, especially new techniques for early diagnosis of UTUC, KSS, lymph node dissection, neoadjuvant and adjuvant chemotherapy, and immune checkpoint inhibition in UTUC.

This is an editorial update in the field of UTUC. The 12 papers published in Frontiers in Oncology-Genitourinary Oncology by internationally renowned researchers cover five major topics:1. New diagnostic techniques for UTUC include confocal laser microscopy and optical coherence tomography (OCT). 2. Radiography techniques and biomarkers for the identification of low-risk UTUC VS aggressive UTUC. 3. Survival data for KSS and lymph node dissection in UTUC. 4. Neoadjuvant and adjuvant chemotherapy and systemic immunotherapies targeting immune checkpoint inhibition in UTUC. 5. Systemic therapy for metastatic UTUC.

The first paper reported by Fan et al. investigated the relationship between preoperative urine cytology and intravesical recurrence (IVR) in patients with UTUC in

in northeast China. They performed a multicenter retrospective cohort study and demonstrated that preoperative positive urine cytology correlated with poor intravesical recurrence-free survival and can serve as a significant independent predictor of IVR. They concluded that preoperative urine cytology is a potential predictor of intravesical recurrence in patients with UTUC after radical nephroureterectomy (RNU) (5). The second paper reported by Chung et al. characterized 1095 patients with UTUC who underwent radical nephroureterectomy with bladder cuff excision (RNUx) and determined the factors affecting IVR. They found that active IVR assessment was required until 36 months after RNU. Regular screening tests, such as urine analysis and cytology, are required for patients with IVRF for >36 months. The third paper is a survey results reported by Wang et al. In this study, they conducted an online survey to analyze the knowledge and compliance of Chinese urologists with the guidelines for non-muscle-invasive bladder cancer (NMIBC) and to identify associated factors. They found that most urologists acknowledged the positive effects of these guidelines. However, compliance with some recommendations of the NMIBC guidelines remains inadequate. The fourth paper published by Chen et al. screened the TCGA dataset to identify N6-methyladenosine (m6A)- related long non-coding RNAs (lncRNAs) in bladder cancer. They constructed an m6A-related lncRNA prognostic signature (m6A-LPS) and found that it was correlated with the immune score and PD-L1 expression. In addition, m6A-LPS may play an important role in regulating tumor microenvironment. The fifth paper published by Xu et al. summarized 2561 cases of UTUC in the last 20 years in China. They found that the clinicopathological diagnostic features of UTUC in the Chinese population have changed significantly over the past 20 years, particularly in terms of patient age, sex, primary site, and multifocality. They found a significant decrease in the incidence of renal pelvic tumors, muscle invasion, and multifocal UTUC in the last 10 years, but the histological grading of the tumors remained unchanged. The sixth paper reported by Guan et al. investigated 108 patients with UTUC and performed universal immunohistochemical staining and whole-exon sequencing to detect the expression of mismatch repair (MMR) proteins and germline mutations. They found that approximately 11% of UTUC cases were suspected to have Lynch syndrome (LS) and 1.4% of cases of LS-related UTUC. The seventh paper published by Huang et al. determined the safety and feasibility of extraperitoneal laparoscopic extended lymph node dissection (LND) in UTUC patients. This prospective study suggests that the procedure provides minimal invasion, rapid recovery, and a lower risk of regional lymph node recurrence. The eighth paper published by Lee et al. further compared the benefits of LND in patients with UTUC without clinical lymph node metastasis during radical nephroureterectomy. They found no significant survival benefits related to LND in these patients. The ninth paper by Dai et al. evaluated the prognostic value of metabolic syndrome (MetS) in patients with UTUC. Their data suggested that MetS was not correlated with survival outcomes in UTUC patients. However, it was correlated with age, history of coronary heart disease, high Charlson comorbidity index, low estimated glomerular filtration rate, and low aspartate/alanine aminotransferase ratio. The tenth paper published by Zhu et al. retrospectively investigated 155 patients diagnosed with bladder cancer following RNU and explored the predictors of unfavorable pathological types of IVR following RNU. They found that operation interval, UTUC T-stage, UTUC grade, surgical approach, and hydronephrosis were independent predictors of unfavorable pathological types of IVR following RNU. The eleventh paper published by Lo et al. reported the advantage of adjuvant chemotherapy for UTUC. Their data suggested that patients who received adjuvant chemotherapy demonstrated significant survival benefits in terms of cancer-specific and disease-free survival. Their findings are consistent with the recent phase 3 trial that adjuvant platinum-based chemotherapy should be considered a new standard of care after nephroureterectomy for patient with UTUC (6). The last paper reported by Chen et al. retrospectively reviewed 302 patients with UTUC who underwent RNU with bladder cuff excision. They found that the tumor location was an independent predictor of local recurrence. Ureter tumors may be associated with worse oncological outcomes, especially local recurrence of UTUC.

In summary, the papers included in this Research Topic provide an emerging update and new avenue on UTUC. We would like to express our sincere gratitude to all authors, editors, and reviewers of these publications, as well as the editorial team at Frontiers for their devotion and assistance in the process of reviewing and publishing this Research Topic.

Author contributions

CL wrote the editorial. LL and YB edited the editorial. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Predictive Value of Preoperative Positive Urine Cytology for Development of Bladder Cancer After Nephroureterectomy in Patients With Upper Urinary Tract Urothelial Carcinoma: A Prognostic Nomogram Based on a Retrospective Multicenter Cohort Study and Systematic Meta-Analysis

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Background: Upper urinary tract urothelial carcinoma (UUT-UC) is a rare and severe urinary malignancy. Several studies have explored the relationship between preoperative urine cytology and intravesical recurrence (IVR) in patients with UUT-UC. However, the results of these studies are controversial or even contradictory, and investigations with UUT-UC patients in northeast China are rare.

Methods: We first estimated the prognostic significance of preoperative urine cytology in the outcomes of intravesical recurrence in 231 UUT-UC patients (training cohort = 142, validation cohort = 89) after radical nephroureterectomy (RNU) by the nomogram model. Subsequently, we quantitatively combined our results with the published data after searching several databases to assess whether preoperative positive urine cytology was associated with poor intravesical recurrence-free survival and a high risk of tumor malignant biological behavior.

Results: Firstly, the multicenter retrospective cohort study demonstrated that preoperative positive urine cytology correlated with poor intravesical recurrence-free survival and can serve as significant independent predictors of IVR by Kaplan–Meier curves and Cox regression analysis. The construction of the nomogram demonstrated that predictive efficacy and accuracy were significantly improved when preoperative urine cytology was combined. Meanwhile, meta-analysis showed that preoperative positive

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urine cytology was associated with a 49% increased risk of IVR. In the subgroup analysis by region, study type, and sample size, the pooled hazard ratios (HRs) were statistically significant for the Japan subgroup (HR 1.32), China subgroup (HR 1.88), cohort study subgroup (HR 1.45), and the single-arm study subgroup (HR 1.63).

Conclusions: Preoperative urine cytology was validated as a potential predictor of intravesical recurrence in patients with UUT-UC after RNU, although these results need to be generalized with caution. Large, prospective trials are required to further confirm its significance in prognosis and tumor malignant biological behavior.

Keywords: upper urinary tract urothelial carcinoma, preoperative urine cytology, intravesical recurrence, cohort study, nomogram, meta-analysis

INTRODUCTION

Upper urinary tract urothelial carcinoma (UUT-UC), which runs from the renal pelvis and calyces to the distal ureter, is widely acknowledged to be a urinary malignancy that originates from the urothelium. UUT-UC accounts for 5%–10% of all urothelial carcinomas, and it is usually considered as a rare urothelial tumor. The average prevalence is 1–2 per 100,000 in the United States (1, 2). UUT-UC is more commonly diagnosed in Asian countries, such as China (including mainland and Taiwan), and can even reach 20–30 cases per 100 persons in high-incidence areas (3). Although the industry-recognized standard treatment for UUT-UC is radical nephroureterectomy (RNU) with bladder cuff excision, several authors have investigated whether subsequent bladder cancer occurrence is highly common after the management of UUT-UC. During follow-up, approximately 22%–50% of UUT-UC patients who undergo RNU have bladder cancer recurrence; additionally, the recurrence rates at 5 years can reach more than 30% (4–7). Recurrence and rapid progression of bladder cancer after UUT-UC significantly increase the psychological anxiety and financial burden of patients and even lower their quality of life (8).

Previously, controversial predictive factors may result in poor prognosis for UUT-UC and intravesical recurrence, such as patient-specific factors (age, sex, diabetes mellitus, or history of bladder cancer), tumor-related factors (location, size, T stage, or architecture), and environmental factors (smoking, long-term use of aristolochic acid, etc.) (8–12). It is worth noting that the 2-year recurrence-free survival rate is less than 56% for stage ≥pT1 cancer, and the 5-year specific survival rate is less than 50% for stage ≥pT2 cancer (13). Therefore, exploring the correlation between various predictors and intravesical recurrence in patients with UUT-UC after RNU plays a crucial role in comprehensive clinical prevention and treatment. Such work depends on many types of diagnostic techniques (14).

As a bladder cancer diagnosis and follow-up method, urine abscission cytology has been used for many years. In 1864, Samders discovered cancer cells in the urine of bladder cancer patients. Since then, urine cytology has become a diagnostic tool for bladder cancer (15, 16). Urine samples are used by researchers to obtain high-quality diagnostic results because of their characteristics. With the advancement of immunology and molecular biology methods, many bladder tumor markers have been found over time. These markers have clinical application value for the early diagnosis and treatment of bladder cancer (17). Cystoscopy and urine cytology have already become standards for the diagnosis and follow-up of bladder cancer. Previous studies have shown that urine cytology correlates with the prognosis of bladder cancer (18–20).

Although studies have focused on urine cytology in the prognosis of intravesical recurrence in UUT-UC patients who underwent RNU (21, 22), the number of studies in China is limited (23, 24). Moreover, evidence is lacking on the current role of this modality. To draw a persuasive conclusion, we first conducted a training cohort (n = 142) and a validation cohort (n = 89). We collected clinical data from UUT-UC patients who underwent RNU to retrospectively evaluate the prognostic value of urine cytology and intravesical recurrence in patients with UUT-UC in China. We then combined related prognostic factors of UUT-UC with preoperative urine cytology and provided a nomogram model for individualized clinical diagnosis, treatment, and follow-up. Additionally, we conducted a meta-analysis to systematically explore the relationship between preoperative urine cytology and intravesical recurrence in patients with UUT-UC. Similarly, we further aimed to adequately determine the impacts of preoperative urine cytology for predicting oncological progression in UUT-UC, including high-grade tumors, muscle invasion, and lymphovascular invasion.

Abbreviations: IVR, intravesical recurrence; UUT-UC, upper urinary tract urothelial carcinoma; RNU, radical nephroureterectomy; IV-RFS, intravesical recurrence-free survival; ROC curve, receiver operating characteristic curve; C-index, concordance index; AJCC, American Joint Commission on Cancer; HR, hazard ratio; RR, risk ratio; CI, confidence interval.

MATERIALS AND METHODS

Histopathological Evaluation

Standardized pathological protocols were adopted to evaluate all the specimens for urine cytology. We collected as much fresh midstream urine as possible in the morning, extracted the

suspension, and smeared it on the slides. Two or three slides were generated for each sample. Hematoxylin and eosin staining was performed for 5 and 3 min, respectively, and then a synthetic resin was used to mount the slides. All specimens were reviewed by urological pathologists. To avoid bias, the samples were blinded.

Study Population

Prior to this study, the information collection and experimental procedures of this study were approved by the institutional review board. In total, from January 2008 to December 2018, medical records of UUT-UC patients who underwent RNU at three medical centers in northeast China, namely, the Second Affiliated Hospital of Dalian Medical University, Affiliated Dalian Friendship Hospital of Dalian Medical University, and Cancer Hospital of China Medical University, were examined for our retrospective study. Ethical approval was granted by the Ethical Review Board of the Second Affiliated Hospital of Dalian Medical University, Cancer Hospital of China Medical University, and the Affiliated Dalian Friendship Hospital of Dalian Medical University. It is worth emphasizing that patients with the following several conditions were excluded from the normative samples: 1) history of prior urothelial carcinoma in the bladder, 2) urothelial carcinoma in both the upper urinary tract and bladder, 3) undergoing attendant cystoprostatectomy, and 4) history of neoadjuvant chemotherapy or radiotherapy. Finally, a total of 231 eligible patients were sequentially included. The training cohort included 142 patients with 83 patients from the Cancer Hospital of China Medical University and 59 patients from the Affiliated Dalian Friendship Hospital of Dalian Medical University. Meanwhile, the validation cohort included 89 patients from the Second Affiliated Hospital of Dalian Medical University (25–27). We extracted the following parameters from the hospital medical records: sex, age, chief complaint, tumor traits (site, stage, grade, laterality, location, and focality), lymph node status, surgery type, and adjuvant chemotherapy. Tumor stage was assessed by the 2002 TNM classification of the American Joint Committee on Cancer, and the 1998 WHO/International Society of Urologic Pathology consensus classification was used to evaluate the grades of tumors.

Preoperative Evaluation of Urine Cytology

According to the judgment of the physician, urine samples for cytological examination were collected one to three times. The specimens with preoperative positive urine cytology could be detected by the following findings: 1) malignancy voided urine cells, 2) suspicious voided urine cells, and 3) both (28, 29). Other cases were all deemed negative samples. Positive cytology indicated malignancy, while negative cytology indicated benign or normal tissue (30). The final judgment was confirmed by two pathologists who were blinded to the clinical outcomes.

Follow-Up and Surveillance Regimen

In the first year after the RNU operation, observations were made every 3 months. From the second year to the fifth year, the frequency was slowed down to every 6 months and once per year thereafter. The measurements of surveillance include serum chemistry studies, routine urine and blood examinations, cystoscopy, and various imaging examinations, such as radiography or CT of the urinary tract. In addition, the physicians responsible for non-surviving patients identified the causes of death and then issued chart reviews or death certificates.

Statistical Analysis

The association between preoperative urine cytology and clinicopathological parameters was appraised using Fisher's exact test and the chi-squared test. To directly reflect the degree of bladder recurrence after RNU, intravesical recurrence-free survival (IV-RFS), which was defined as the death rate caused by recurrence of UUT-UC after RNU from a recurrent lesion located in the bladder, was used to statistically compare the different groups by the log-rank test. Taking various prognostic factors from previous statistics, a univariate analysis was performed to screen out the most correlated factors ($P < 0.05$). Afterward, the prognostic factors selected by the univariate analysis were subjected to multivariate Cox proportional hazard regression models for comparisons and to filter out the significant factors ($P < 0.05$). From the direct Kaplan–Meier survival curves drawn from the outcomes of the log-rank test, we compared the ratio of intravesical recurrence-free survival among all the patients and visually analyzed the survival time of certain cases. The aforementioned procedures were performed in SPSS version 13.0 (SPSS Inc., Chicago, USA). To identify the independent risk factors for moderate/severe preoperative urine cytology, a nomogram plot was programmed with R software (R Foundation for Statistical Computing, Vienna, Austria). Receiver operating characteristic (ROC) curves were plotted to compare the ability to estimate for patient mortality between the nomogram and the criteria of the UUT-UC score set by the American Joint Commission on Cancer (AJCC). In addition, calibration plots were also drawn in order to evaluate the potential clinical value of the nomogram. The concordance index (C-index) was calculated by the means of bootstrapping to estimate the probability that the nomogram prognostic models are consistent with the actual observed results. A *P*-value less than 0.05 indicated statistical significance.

Meta-Analysis

Search Strategy

Up to August 2021, we searched the PubMed, Medline, Embase, Cochrane Library, and Scopus databases with the following retrieved words and medical subject headings, including all spellings ("Urine cytology," "Upper urinary tract," "Renal pelvis," "Ureter," "Urothelial cancer," "Urothelial carcinoma," "Bladder cancer," and "Prognosis," "Recurrence," "Intravesical," and "High grade," "Muscle invasion," "Lymphovascular invasion"). Additionally, we manually searched further eligible bibliographies and company reports to ensure the comprehensiveness of the experimental data from the literature. The publication country and time were not restricted.

Selection Criteria

The following criteria were used to measure whether valuable literature was collected. The inclusion criteria were as follows: (1)

upper urothelial carcinoma was the histologic type, (2) selected samples had corresponding data on the preoperative urine cytology, and (3) relationship was analyzed between preoperative urine cytology and IV-RFS, tumor grade, pathological stages, and lymphovascular invasion. The exclusion criteria were as follows: (1) studies that lacked original data, including reviews, letters to the editor, commentaries, and case reports; (2) studies containing data from earlier studies; (3) articles written by the same authors as another chosen article; (4) studies without original data or no record of intravesical recurrence-free survival, tumor grade, pathological stages, or lymphovascular invasion; and (5) studies with invalid data for calculating the hazard ratio (HR) and its standard error.

Data Extraction

According to the aforementioned criteria, two investigators (BF and YuH) undertook a standard process to improve reliability and minimize bias. A third reviewer (YW) examined the extracted results and eliminated any discrepancies between the independent search results. If a study appeared appropriate, then the full text was reviewed. If the relevant studies met the inclusion criteria, then they were included in our meta-analysis. We simultaneously recorded the primary information of eligible articles, including the author, publication year, region, study type, recruitment period, number of patients, baseline characteristics (mean age, sex, etc.), evaluation of positive urine cytology, etc.

Heterogeneity Evaluation

The I^2 and Q test were used to quantitatively delineate heterogeneity and measure the percentage of volatility. If the heterogeneity P-value was <0.05, a random-effects model rather than the fixed-effect model was employed. If I^2 was >50%, then heterogeneity was observed among the studies.

Sensitivity Analysis

We further adopted a leave-one-out approach to remove individual studies sequentially. If the P-value tested by the Q-test was greater than 0.05, the fixed-effects model was used after removing the heterogeneous studies. A random-effects model was used if heterogeneity was detected after removing any of the studies.

Statistical Analysis

The dichotomous outcomes were compared by assessment of hazard ratios (HRs)/risk ratios (RRs) with 95% CIs. The HRs and 95% CIs were used to demonstrate the statistical outcomes in the prognosis group, while the RRs and 95% CIs were applied in groups of malignant tumor biological behaviors. Overall and subgroup meta-analyses by Begg's test and Egger's test were visually reflected by forest and funnel plots to represent the estimated pooled effects to assess the publication bias. In the forest plots, if the diamond crossed the vertical median line, statistical significance was confirmed. Meta-analyses and forest plots were performed using Stata 12.0 (Stata Corporation, College Station, TX, USA).

Quality Assessment

The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach was used to assess the confidence of evidence for each outcome. Two independent researchers (BF and YuH) downgraded the studies which had five limitations, including risk of bias, inconsistency, indirectness, imprecision, and publication bias (31, 32). Overall quality was classified as "very low," "low," "moderate," or "high" based on the overall judgment. The assessment was performed in using GRADE Pro version 3.6 software.

RESULTS

Cohort Study

Relationship Between the Examination of Urine Cytology and thr Clinicpathological Characteristics of UUT-UC Patients

The malignant grades of preoperative urine cytology were detected by HE staining, as shown in **Figure 1**. According to the outcomes of preoperative urine cytology, 231 patients who met our inclusion criteria were divided into a negative group and a positive group. To ascertain the clinical relationship between the outcomes of urine cytology and intravesical recurrence after radical nephroureterectomy in UUT-UC patients, we conducted the training (n = 142) and validation cohorts (n = 89). The main clinicopathological characteristics in the training and validation cohorts are listed in **Tables 1** and **2**, respectively. The related descriptive clinical and pathological factors included gender, age, smoking, alcohol use, family history of bladder cancer, tumor side, tumor location, tumor focality, pathological stage, histological grade, lymph node status, distant metastasis, and type of surgery. Preoperative positive urine cytology was reported in 68 (47.9%) of 142 patients in the training cohort and 37 (41.6%) of 89 patients in the validation cohort. The correlation between preoperative urine cytology and clinicopathological characteristics was then investigated. We discovered that pathological stage was the only variable positively correlated with preoperative positive urine cytology (P = 0.001) in the training cohort, and the association between smoking and preoperative urine cytology (P = 0.047) was found in the validation cohort.

Predictors and Intravesical Recurrence-Free Survival in UUT-UC Patients

The Kaplan–Meier method was applied to determine the relationships between different prognostic factors and recurrence in the bladder. The survival curves showed that preoperative positive urine cytology (P < 0.001), higher histological grade (P = 0.013), and positive lymph node status (P = 0.002) were associated with poor IV-RFS in the training cohort (**Figures 2A–C**). The patients with preoperative positive urine cytology (P < 0.001), higher histological grade (P = 0.010), and muscle invasion (P = 0.010) had lower intravesical recurrence-free survival in the validation cohort (**Figures 2D–F**).

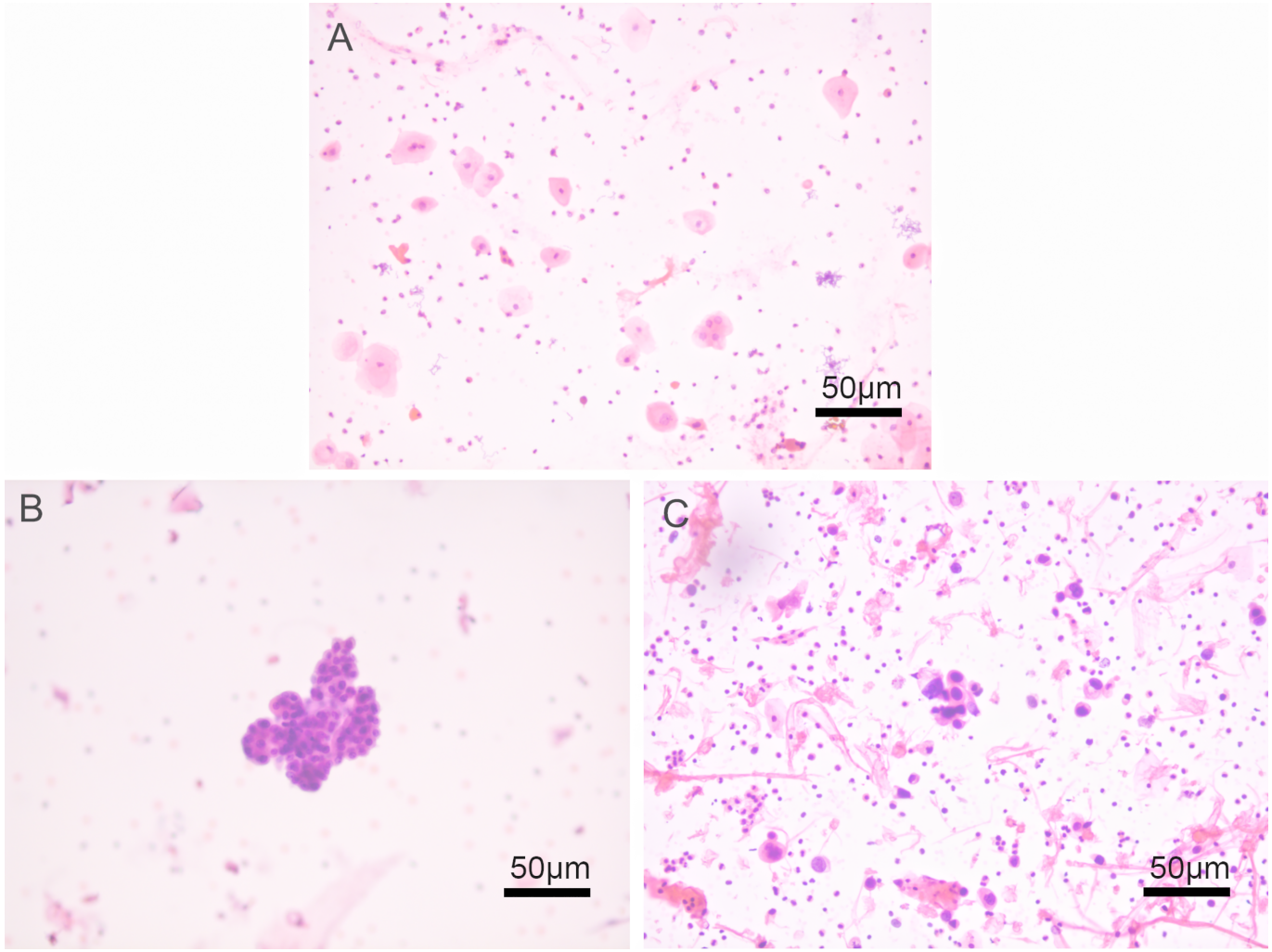

FIGURE 1 | Histological patterns of preoperative urine cytology by HE staining in upper urinary tract urothelial carcinoma (UUT-UC) patients. **(A)** Negative urine cytology at a magnification of ×200; **(B)** suspicious urine cytology at a magnification of ×200; and **(C)** positive urine cytology at a magnification of ×200.

TABLE 1 | Demographics and clinicopathologic characteristics of 142 patients treated with RNU for UUT-UC in training cohort.

	Preoperative urine cytology		Total	*P* Value
	Negative group	Positive group		
Gender				0.536
Male	43	36	79	
Female	31	32	63	
Age, years				0.064
Less than 69	42	28	70	
69 or Greater	32	40	72	
Smoking				0.405
Non-smoker	48	49	97	
Current smoker	17	15	32	
Former smoker	9	4	13	
Alcohol use				
Non-drinker	51	43	94	0.484
Current drinker	16	14	30	
Former drinker	7	11	18	
Family history of bladder cancer				0.719
No	71	66	137	
Yes	3	2	5	
Tumor side				0.258
Right	44	34	78	
Left	30	34	64	
Tumor location				0.511
Calix or pelvis	40	41	81	
Ureter	29	25	54	
More than 1	5	2	7	
Tumor focality				0.848
Unifocal	65	59	124	
Mutifocal	9	9	18	
Pathologic stage				**0.001**
T_{is}-T_1	43	20	63	
T_2-T_4	31	48	79	
Histological grade				0.850
Low	25	24	49	
High	49	44	93	
Lymph node status				0.159
N_o	71	60	131	
N_1	3	6	9	
N_x	0	2	2	
Distant metastasis				0.172
M_0	72	68	140	
M_1	2	0	2	
Type of surgery				0.710
Open	49	43	92	
Laparoscopic	25	25	50	

The bold values were applied to highlight P-values which had statistically significance (i.e. P < 0.05).

The outcomes of the univariate and multivariate analyses in the training cohort are shown in **Table 3**. After performing univariate and multivariate Cox regression analyses, preoperative urine cytology (HR 3.283, 95% CI 1.558, 6.920, $P = 0.002$) and histological grade (HR 2.683, 95% CI 1.107, 6.502, $P = 0.029$) were detected as independent predictors correlated with intravesical recurrence in the training cohort. Similarly, **Table 4** demonstrates the results of the univariate and multivariate analyses in the validation cohort. Preoperative positive urine cytology (HR 2.975, 95% CI 1.352, 6.548, $P = 0.007$), higher pathologic stage (HR 5.019, 95% CI 1.180, 21.349, $P = 0.029$), and higher histological grade (HR 2.750, 95% CI 1.087, 6.954, $P = 0.033$) also had the potential to predict poor IV-RFS in the validation cohort.

Nomogram of Prognostic Prediction Based on Preoperative Urine Cytology

As shown in **Figure 3**, we established a novel nomogram using R language. Gender, age, smoking, alcohol use, history of bladder cancer, tumor side, tumor location, tumor focality, histological grade, pathologic stage, lymph node status, distant metastasis, surgery type, and preoperative urine cytology were combined. According to the constructed nomogram model, the clinician can locate the data on the nomogram, sum the score, and obtain a total prognostic point. The 1-, 3-, and 5-year IV-RFS rates of UUT-UC patients can be predicted accordingly.

The calibration and discrimination ability for internal validation was assessed by calibration curves and the C-index. The calibration plots are shown in **Figures 4A–C**. In comparison

TABLE 2 | Demographics and clinicopathologic characteristics of 89 patients treated with RNU for UUT-UC in validation cohort.

	Preoperative urine cytology		Total	*P* Value
	Negative group	Positive group		
Gender				0.873
Male	29	20	49	
Female	23	17	40	
Age, years				0.926
Less than 69	23	16	39	
69 or Greater	29	21	50	
Smoking				**0.047**
Non-smoker	31	31	62	
Current smoker	16	4	20	
Former smoker	5	2	7	
Alcohol use				
Non-drinker	39	28	67	0.833
Current drinker	7	6	13	
Former drinker	6	3	9	
Family history of bladder cancer				0.768
No	50	36	86	
Yes	2	1	3	
Tumor side				0.599
Right	28	22	50	
Left	24	15	39	
Tumor location				0.910
Calix or pelvis	23	16	39	
Ureter	26	18	44	
More than 1	3	3	6	
Tumor focality				0.851
Unifocal	37	27	64	
Mutifocal	15	10	25	
Pathologic stage				0.796
T_{is}-T_1	11	7	18	
T_2-T_4	41	30	71	
Histological grade				0.567
Low	17	10	27	
High	35	27	62	
Lymph node status				0.079
N_o	47	36	83	
N_1	5	0	5	
N_x	0	1	1	
Distant metastasis				0.090
M_0	52	35	87	
M_1	0	2	2	
Type of surgery				0.327
Open	12	12	24	
Laparoscopic	40	25	65	

The bold values were applied to highlight P-values which had statistically significance (i.e. P < 0.05).

with the prediction of 1- and 5-year IV-RFS, we observed that the actual curves were more consistent with the ideal curves in the 3-year nomogram. Compared with AJCC models, the ROC curves of 1-, 3-, and 5-year urine cytology nomogram models revealed better discrimination efficacy, as shown in **Figures 5A–C**. The AUCs of the 1-, 3-, and 5-year AJCC models and the urine cytology nomogram models were equal to 0.489 *vs*. 0.706, 0.583 *vs*. 0.767, and 0.566 *vs*. 0.797, respectively. Good discrimination was also obtained for the bootstrapping method, and the C-index of the nomogram model was equal to 0.673.

For external validation, we evaluated the nomogram by using an independent validation cohort. Good consistency between the predicted and actual curves was demonstrated on calibration plots of the 3-year nomogram (**Figures 6A–C**). The C-index of the external validation was 0.788, indicating that the nomogram model had good discrimination efficacy for predicting IV-RFS in patients with UUT-UC. Compared with 1- and 3-year ROC curves, better predictive performance was shown on the 5-year ROC curves (**Figures 7A–C**). The 1-, 3-, and 5-year AUC values were equal to 0.768, 0.789, and 0.921, respectively.

In summary, we speculate that the prognostic nomogram model has a great potential to predict 1-, 3-, and 5-year IV-RFS.

Meta-Analysis

Method of Systematic Selection of Relevant Publications

The detailed process of selection is shown in **Figure 8**. A total of 723 initial studies were identified from electronic databases and literature searches, and additional records were found from other approaches (n = 3). After removing duplicated publications (n =

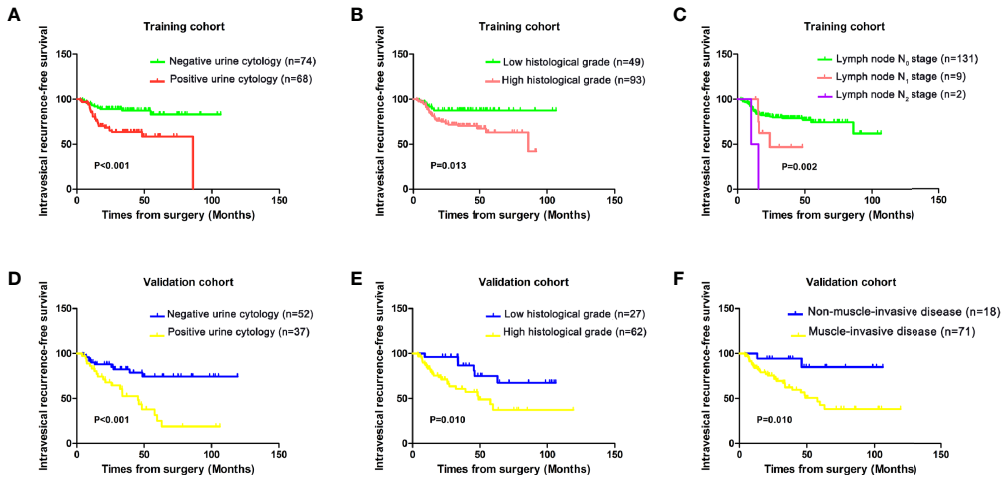

FIGURE 2 | Kaplan–Meier analysis of intravesical recurrence-free survival by **(A)** preoperative urine cytology, **(B)** histological grade, and **(C)** lymph node stage in the training cohort and **(D)** preoperative urine cytology, **(E)** histological grade, and **(F)** muscle invasion in the validation cohort.

274) and excluding articles by title and abstract (n = 238), 36 studies remained. With the careful assessment of the remaining studies, 16 studies were excluded for four reasons: 1) review articles and editorials, 2) unavailable HRs and 95% CIs, 3) animal or molecular biology studies, and 4) not relevant outcomes. Twenty candidate studies remained. Adding these to our cohort study, 21 independent studies were finally included in our meta-analysis. There were two available datasets in two articles about prognosis [Kentaro K, 2019 (33); Long X, 2016 (24)]. We accepted different studies from the same group.

Meta-Analysis of the Correlation Between Preoperative Positive Urine Cytology and Intravesical Recurrence in UUT-UC Patients

The Main Characteristics of Eligible Studies

The major characteristics of 14 studies about intravesical recurrence are summarized in **Table 5**. They were published from 2010 to 2020. One, four, and nine studies assessed patients from Korea (21), China (23, 24, 28), and Japan (30, 33–40), respectively. In addition, one, three, and four studies were RCT (36), case–control (21, 35, 38), and single-arm studies (23, 37, 39, 40), respectively, and the others were retrospective cohort searches (24, 28, 30, 33, 34). The size of the studies varied from 36 to 1,563, with a total number of 6,140 patients. Only one publication did not provide the recruitment period, and demographics were missing from only one publication. Seven studies showed the assessment of positive urine cytology.

Heterogeneity and Sensitivity Analyses

A random-effects model was selected because moderate heterogeneity was detected (I^2 = 49.4%, P = 0.011). A sequential sensitivity analysis was conducted to evaluate heterogeneity in each study, as shown in **Figure 9A**. By removing any of the individual studies, statistical fluctuations of the combined HRs were not

TABLE 3 | Univariate and multivariable Cox regression models predicting intravesical recurrence-free survival in 142 patients treated with RNU for UUT-UC in training cohort.

Characteristic	Univariate		Multivariate	
	HR	*P* Value	HR	*P* Value
Gender	0.616 (0.317–1.194)	0.151		
Age	1.748 (0.890–3.435)	0.105		
Family history of bladder cancer	0.046 (0.001–54.700)	0.393		
Smoking	1.450 (0.935–2.247)	0.097		
Alcohol use	1.338 (0.879–2.037)	0.175		
Preoperative urine cytology	3.577 (1.711-7.477)	**0.001**	3.283 (1.558-6.920)	**0.002**
Tumor laterality	1.292 (0.672–2.486)	0.443		
Tumor location	0.954 (0.556–1.638)	0.865		
Tumor focality	0.996 (0.386–2.570)	0.993		
Pathologic stage	1.140 (0.582–2.234)	0.702		
Histological grade	2.897 (1.204–6.971)	**0.018**	2.683 (1.107-6.502)	**0.029**
Lymph node status	2.672 (1.404–5.086)	**0.003**	1.823 (0.947-3.509)	0.072
Distant metastasis	2.464 (0.335–18.132)	0.376		
Type of surgery	0.848 (0.417–1.727)	0.650		

The bold values were applied to highlight P-values which had statistically significance (i.e. P < 0.05).

TABLE 4 | Univariate and multivariable Cox regression models predicting intravesical recurrence-free survival in 89 patients treated with RNU for UUT-UC in validation cohort.

Characteristic	Univariate		Multivariate	
	HR	*P* Value	HR	*P* Value
Gender	1.327 (0.641–2.744)	0.446		
Age	1.001 (0.490–2.045)	0.997		
Family history of bladder cancer	0.047 (0.001–415.758)	0.510		
Smoking	0.440 (0.203–0.952)	**0.037**	0.519 (0.238-1.130)	0.099
Alcohol use	0.512 (0.253–1.034)	0.062		
Preoperative urine cytology	3.575 (1.678-7.615)	**0.001**	2.975 (1.352-6.548)	**0.007**
Tumor laterality	0.608 (0.291–1.272)	0.186		
Tumor location	0.888 (0.467–1.689)	0.717		
Tumor focality	0.772 (0.314–1.897)	0.572		
Pathologic stage	5.423 (1.291–22.789)	**0.021**	5.019 (1.180-21.349)	**0.029**
Histological grade	3.105 (1.262–7.642)	**0.014**	2.750 (1.087-6.954)	**0.033**
Lymph node status	0.530 (0.083–3.378)	0.502		
Distant metastasis	1.703 (0.229–12.681)	0.603		
Type of surgery	0.570 (0.278–1.167)	0.124		

The bold values were applied to highlight P-values which had statistically significance (i.e. P < 0.05).

observed, indicating that the pooled data combined with the random-effects model have great reliability. The complete I^2 statistical values and *P*-values of the *Q*-test in the leave-one-study-out sensitivity analysis are summarized in **Figure 9B**.

Outcomes Using Random-Effects Models, Publication Bias, and GRADE

Forest plots are depicted in **Figure 10A**, which presents the relative risk estimates from 14 studies for the relationship between preoperative urine cytology and intravesical recurrence. The overall analysis showed that the combined HR was 1.49 (95% CI 1.27, 1.75, $P < 0.001$), suggesting that preoperative positive urine cytology was associated with a 49% increased risk of intravesical recurrence. Visually, the shape of Begg's funnel plots (**Figure 10B**) revealed evidence of obvious asymmetry (*z*-value of Begg's test = 0.005). Statistically, the positive result in Egger's test indicated moderate publication bias ($P = 0.001$). The pooled findings of the GRADE analysis

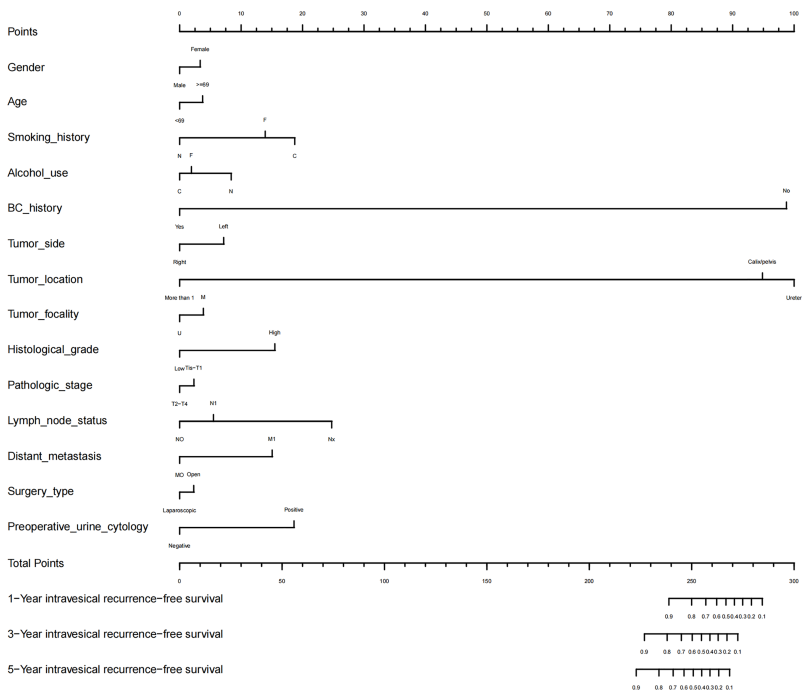

FIGURE 3 | Nomogram for 1-, 3-, and 5-year IV-RFS predictions of UUT-UC and associated predictive performance. The independent prognostic factors (including gender, age, smoking, alcohol use, history of bladder cancer, tumor side, tumor location, tumor focality, histological grade, pathologic stage, lymph node status, distant metastasis, and surgery type and preoperative urine cytology) were combined with the point designations to obtain the total prognostic score. "N" represented non-smoker/drinker, "C" represented current smoker/drinker, "F" represented former smoker/drinker, "U" represented unifocal tumor, "M" represented multifocal tumor, and "BC" represented bladder cancer.

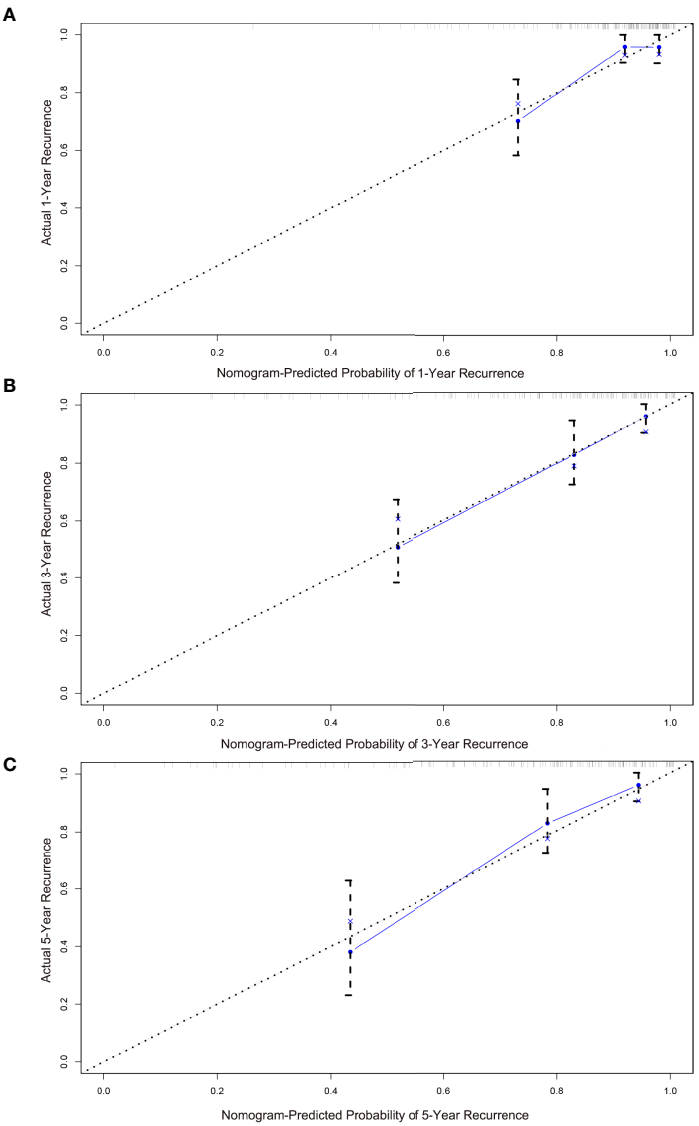

FIGURE 4 | Calibration plots for the nomogram predictions of **(A)** 1-, **(B)** 3-, **(C)** 5-year IV-RFS in the training cohort. The nomogram-predicted probability is plotted on the *x*-axis, and actual recurrence is plotted on the *y*-axis. The gray dotted line represents the ideal curve of the calibration models. The blue broken line demonstrates the actual predictive accuracy, the bootstrap-corrected estimates are visually shown by blue X, and vertical bars represent the 95% CIs.

(**Table 6**) achieved a very low quality of evidence supporting poorer intravesical recurrence-free survival with preoperative positive urine cytology due to indirectness (findings being restricted to limited regions) and imprecision (large width of the confidence interval around the pooled hazard ratios).

Subgroup Analysis

In the subgroup analysis by region, a significant difference was noted between the Japan subgroup (HR 1.32, 95% CI 1.15, 1.52, $P < 0.001$), the China subgroup (HR 1.88, 95% CI 1.26, 2.80, $P = 0.002$), and the Korea subgroup (HR 4.61, 95% CI 1.45, 14.63, $P = 0.01$). Under the analysis of study types, precise evidence was obtained showing that preoperative positive urine cytology was related to an increase in intravesical recurrence, not only in the subgroup of the cohort studies (HR 1.45, 95% CI 1.19, 1.78, $P < 0.001$) but also in the subgroup of the single-arm study (HR 1.63, 95% CI 1.11, 2.41, $P = 0.013$) and RCT study (HR 5.54, 95% CI 1.12, 27.45, $P = 0.036$). In the subgroup analysis by sample size, when the sample size was more than 100, the risk of intravesical recurrence was 1.35 times higher in patients with positive urine cytology than in those with negative cytology (95% CI 1.18, 1.55, $P < 0.001$). When the sample size was 100 or less, the same trend was seen (HR 2.96, 95% CI 1.87, 4.67, $P < 0.001$). In addition, we explored whether the assessment of urine cytology influenced the preoperative urine cytology and intravesical recurrence. The results showed that preoperative positive urine cytology was

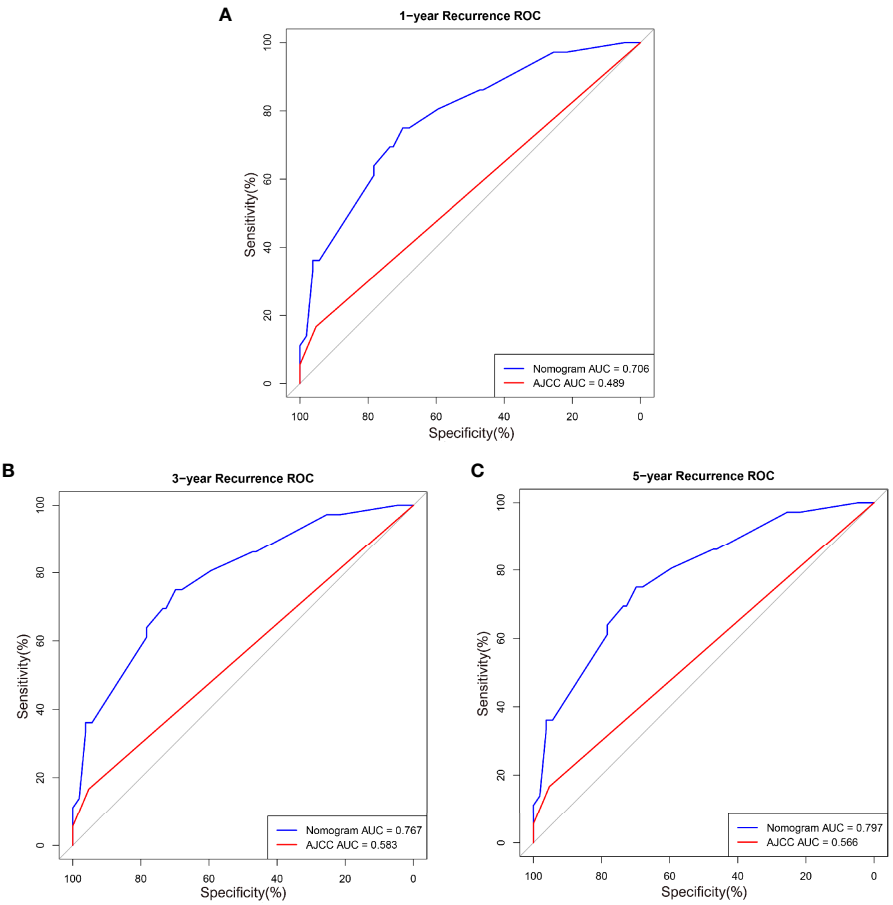

FIGURE 5 | ROC curves comparing the effectiveness of predicting **(A)** 1-, **(B)** 3-, and **(C)** 5-year IV-RFS between the nomogram and the American Joint Commission on Cancer (AJCC) score in the training cohort. Nomogram-predicted intravesical recurrence-free survival rates are indicated by the blue lines, and AJCC-predicted overall survival rates are indicated by the red lines.

associated with an increase in intravesical recurrence, not only in the group with the assessment of urine cytology (HR 2.01, 95% CI 1.41, 2.88, $P < 0.001$) but also in the group without assessment of urine cytology (HR 1.31, 95% CI 1.13, 1.52, $P < 0.001$). **Figures 11A–D** show all the forest plots.

Meta-Analysis of the Correlation Between Preoperative Positive Urine Cytology and Tumor Malignant Biological Behavior for UUT-UC

Article Descriptions

Table 7 outlines the main characteristics of 10 studies about tumor malignant biological behavior. These studies contained 2,463 samples. Considering the selected articles from 2007 to 2021, four studies were retrospective cohort studies (28, 30, 41), and the remaining were case–control studies (29, 42–46). There were four studies from China (28, 42, 45), two studies from America (29, 41), one study from Germany (43), two studies from Japan (30, 46), and one study from Spain (44). In these studies, nine of them involved the risk of high-grade UUT-UC (28–30, 41–45). The risks of muscle-invasive UUT-UC and lymphovascular-invasive UUT-UC emerged in seven studies (30, 41–45) and three studies (28, 30, 46), respectively.

Meta-Analysis of the Correlation Between Preoperative Positive Urine Cytology and the Risk of High-Grade UUT-UC

A total of 2,318 patients were pooled in this analysis. As shown in **Figures 12A, B**, there was substantial heterogeneity overall, indicating that a random-effects model analysis was applied ($I^2 = 84.5\%$, $P < 0.001$). No significant risk of high-grade tumor was observed in the UUT-UC patients with preoperative positive urine cytology (RR 1.23, 95% CI 1.00, 1.51, $P = 0.055$). Due to the high heterogeneity and wide confidence interval, the pooled findings achieved a very low quality of evidence due to their inconsistency and imprecision (**Table 6**).

Meta-Analysis of the Correlation Between Preoperative Positive Urine Cytology and the Risk of Muscle-Invasive UUT-UC

Similarly, the outcomes of relationship and heterogeneity are demonstrated in **Figures 13A, B**. A total of 1,938 patients with

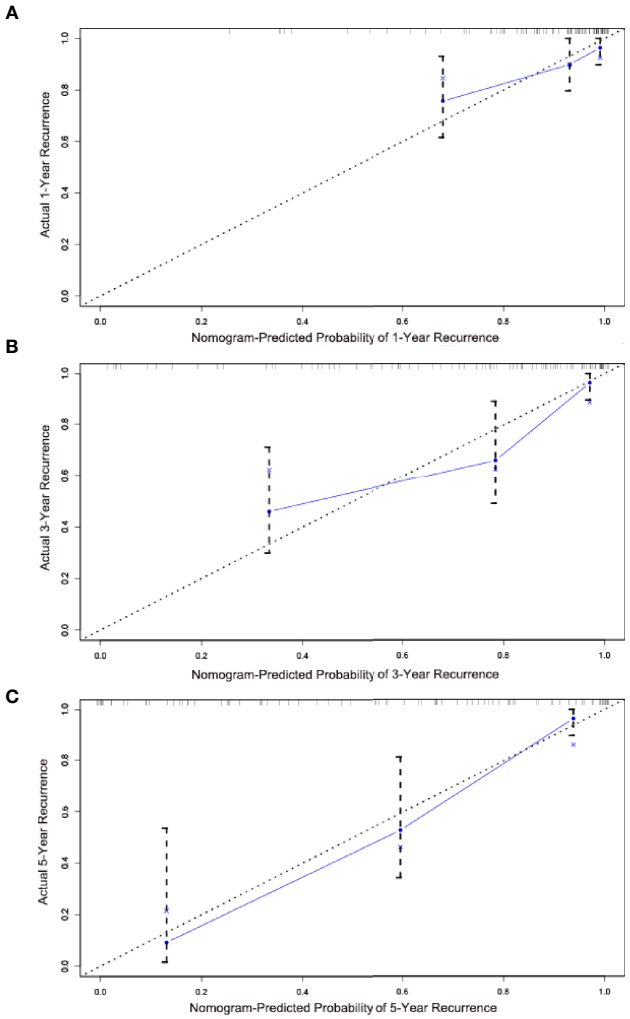

FIGURE 6 | Calibration plots for the nomogram predictions of **(A)** 1-, **(B)** 3- , and **(C)** 5-year IV-RFS in the validation cohort. The nomogram-predicted probability is plotted on the *x*-axis; actual recurrence is plotted on the *y*-axis. The gray dotted line represents the ideal curve of the calibration models. The blue broken line demonstrates the actual predictive accuracy, the bootstrap-corrected estimates are visually shown by blue X, and the vertical bars represent the 95% CIs.

muscle-invasive UUT-UC were enrolled. With severe heterogeneity detected among these studies (I^2 = 54.0%, P = 0.033), the combined analysis using a random-effects model demonstrated that preoperative urine cytology had no obvious association with the risk of muscle-invasive UUT-UC (RR 1.16, 95% CI 0.98, 1.37, P = 0.085). Once again, the quality of evidence regarding muscle-invasive UUT-UC was very low (**Table 6**).

Meta-Analysis of the Correlation Between Preoperative Positive Urine Cytology and the Risk of Lymphovascular-Invasive UUT-UC

We combined the data of 934 patients from two eligible articles and conducted the meta-analysis. The forest plot and the sensitivity test are depicted in **Figures 14A, B**, respectively. A random-effects model analysis was applied because evidence of heterogeneity was observed (I^2 = 74.5%, P = 0.020). The pooled RR was equal to 1.37, but it was not statistically significant (95% CI 0.81, 2.31, P = 0.245). The results showed that the preoperative positive urine cytology was not related to the risk of lymphovascular invasion in UUT-UC. The limitation of the findings to China or Japan and the large ranges of true values in two eligible articles indicated that the evidence of outcomes had a very low quality (**Table 6**).

DISCUSSION

In most studies, the ratio of intravesical recurrence has ranged from 22% to 50% in patients with UUT-UC who underwent RNU (47). Recent studies about the association between preoperative positive urine cytology and intravesical recurrence in patients with UUT-UC in northeast China were relatively rare and controversial. Inspired by the above, we collected data from three clinical centers and conducted a retrospective cohort study. In our Kaplan–Meier survival and univariate and multivariate Cox regression analyses, we found that preoperative urine cytology was a significant factor for bladder cancer recurrence in the training cohort. The use of the validation cohort verified the reliability of the outcomes from the training cohort. Meanwhile, we conducted a nomogram model to further determine the predictive value of preoperative urine cytology. Actually, as various risk factors correlated with intravesical recurrence have been found and analyzed, studies differ significantly in the identification of multifactorial prognosis. For patients with UUT-UC and recurrence of bladder cancer, tumor location in the ureter or tumor multifocality has been validated as prognostic factors in the recurrence of non-muscle-invasive bladder cancer (48–50). The incidence of multiple upper urothelial carcinomas was significantly higher than that of single urothelial carcinomas in patients with urothelial tumor recurrence (51). In addition, tumor size, preoperative hydronephrosis, and ureterorenoscopy are more likely to lead to postoperative recurrence in the bladder (52–54). Some of those conclusions are consistent with ours. The differences among studies explain the diversity of characteristics of urothelial carcinoma, and they have led to uncertainties in clinical follow-up and treatment, which should be studied further in long-term, systematic, large-sample research.

In our meta-analysis, preoperative positive urine cytology was associated with a 49% increased risk of intravesical recurrence. Interestingly, there is an attractive hypothesis that can explain the mechanism of urinary abscission cytology in the prediction of recurrence in the bladder after upper urothelial neoplasms. In a previous study, the dissemination and floating of cancer cells from UUT-UC in the bladder occurred postoperatively (55, 56). During radical nephroureterectomy, the bladder is cut open, and a urethral catheter is placed, resulting in an increased degree of bladder exposure. The injured urothelium is more likely to provide a site for adherence compared with complete

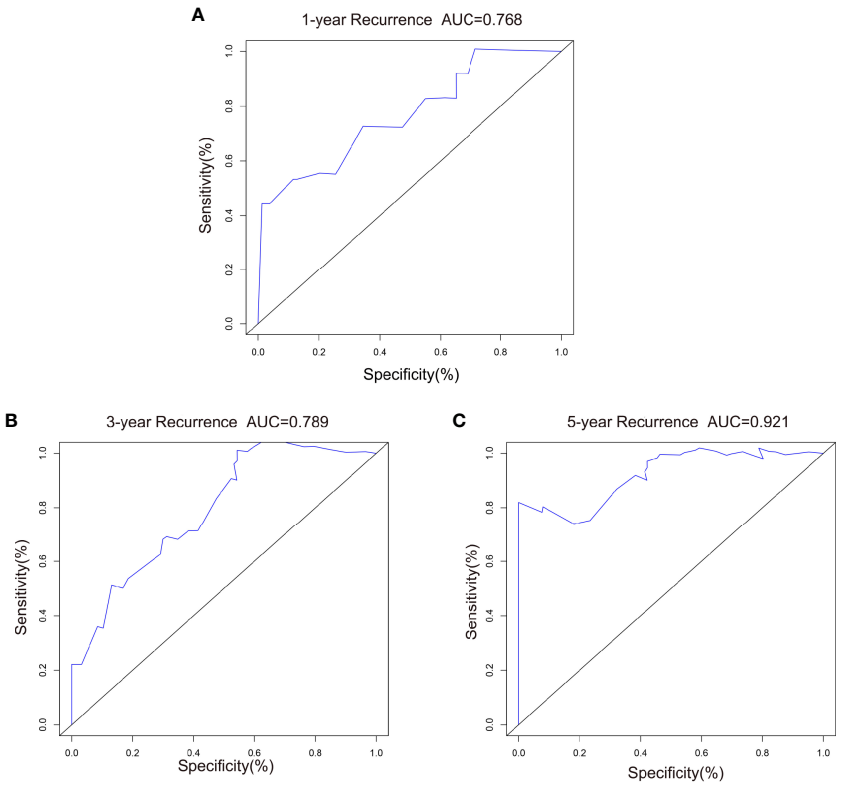

FIGURE 7 | ROC curves comparing the effectiveness of predicting **(A)** 1-, **(B)** 3-, and **(C)** 5-year IV-RFS between the nomogram and the AJCC score in the validation cohort. Nomogram-predicted intravesical recurrence-free survival rates are indicated by the blue lines.

urothelium. Along with continuously monitoring the removal of postoperative remnants, urinary bladder suspensions can be used to determine whether tumor cells have adhered to the damaged urothelium and proliferated (57, 58).

In fact, tumors can occur in one or more sites simultaneously or at different times. Recurrent bladder cancer after upper urinary tract urothelial carcinoma is a kind of multiple-organ carcinoma of the urinary tract epithelium (59, 60). It is universally acknowledged that the multicentricity of lesions plays an essential role in the biological characteristics of urothelial carcinoma (55, 58, 59). Based on our findings and implantation theory, it seems that urothelial multiorgan carcinoma originates from the same cell clone. According to this view, urothelial carcinoma originates from a single cell clone, and urothelial multiorgan carcinoma is caused by tumor cells implanting along the direction of urine flow and migrating intraepithelially to form multiple tumor lesions (55, 61). Many conditions can be explained by the implantation theory, such as unilateral upper urinary tract urothelial carcinoma, tumors formed in the same direction as urine flow, and consistent tumor histologic patterns in multiple organs. Although both UUT-UC and bladder cancer originate from similar transitional epithelium of the urinary tract, next-generation sequencing may provide insights about differences in genetic landscapes between UUT-UC and bladder cancer. In northern China, Yang et al. reported that the distribution of driver genes is significantly different between UUT-UC with PIK3CA, TP53, and FGFR3 mutations and bladder cancer with BRCA1 mutations. Meanwhile, patients with bladder cancer had higher levels of PD-L1 than those with UUT-UC (62). In eastern China, the mutation frequencies of GPR126 intron 6, TERT, and PLEKHS1 promoters in UUT-UC patients were significantly lower than those in patients with bladder cancer (63). The U.S. research team of Petros et al. showed that patients who develop UUT-UC after bladder cancer may predominantly show a more basal-like subtype, while synchronous and primary UUT-UC patients appear to have a luminal-like subtype, which results in a different distribution of fibroblast and immune cell gene expression (64).

From the perspective of tumorigenesis, another theory is the multifocal origin theory. The multicentricity of urothelial carcinoma is caused by chromosome deletion and gene mutation of normal cells through the impact of local carcinogenic factors, thus forming independent cell clones. These clones proliferate to produce multiple tumor lesions at the same or different times. This theory can explain the bilateral upper urinary tract tumors appearing at the same or different times and the long-term neoplasms that occur in the same direction. Some scholars still emphasize that intravesical recurrence and even multiorgan carcinoma in the reverse

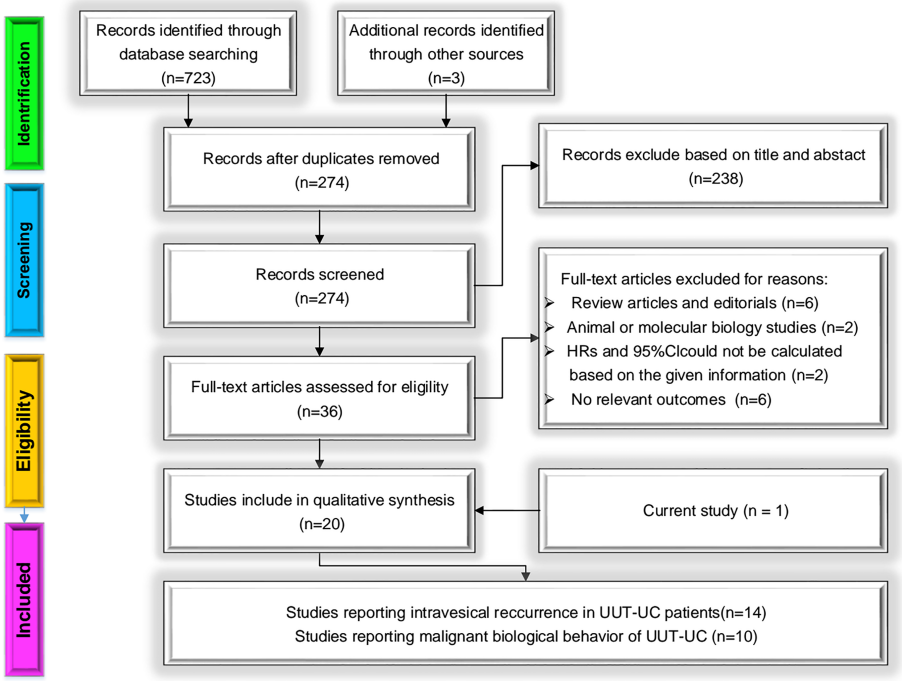

FIGURE 8 | Methodologic flow diagram for the selection of articles.

TABLE 5 | Main characteristics of studies about the intravesical recurrence-free survival included in the meta-analysis.

First author (year)	Country	Study type	Recruitment period	No. of subjects	Demographics (age, sex)	Assessment of positive urine cytology	Reported end-points
Liu W (2020)	China	Cohort study; Retrospective	2012-2019	315	67.0 M:F=192:123	Defined as a positive result and/or a suspicious report	2.210(1.060-4.640)
Kentaro K (2019)	Japan	Cohort study; Retrospective	1995-2009	1563	70.0 M:F=1914:754	NR	1.210(1.020-1.430)
				1245			1.210(1.000-1.480)
Long X (2016)	China	Cohort study; Retrospective	2004-2012	159	62.0 M:F=113:46	NR	2.173(1.084-4.350)
				161	60.8 M:F=120:41		1.143(0.544-2.400)
Ishioka J (2015)	Japan	Case-control study; Retrospective	1995-2010	754	69.0 M:F=526:228	NR	1.259(0.926-1.711)
Narukawa T (2015)	Japan	Case-control study; Retrospective	1995-2012	133	66.0 M:F=101:32	NR	1.080(0.630-1.850)
Fang D (2014)	China	Single arm study	2000-2010	438	NR M:F=187:251	Indication of malignancy and the presence of atypical cells that were highly suggestive of urothelial carcinoma	1.160(0.810-1.660)
Shibuya T (2014)	Japan	Single arm study	2002-2012	54	NR	NR	2.680(1.110-6.450)
Cho DS (2013)	Korea	Case-control study; Retrospective	1994-2009	78	65.0 M:F=58:20	Class 4 or 5 findings according to the Papanicolaou classification	4.606(1.450-14.633)
Ito A (2013)	Japan	RCT	NR	36	69 M:F=43:29	At least one positive finding among multiple examinations	5.540(1.120-27.500)
Tanaka N (2013)	Japan	Cohort study; Retrospective	1994-2010	474	69.0 M:F=346:128	One that reported suspicious or positive results, or both	1.410(1.080-1.850)

(Continued)

TABLE 5 | Continued

First author (year)	Country	Study type	Recruitment period	No. of subjects	Demographics (age, sex)	Assessment of positive urine cytology	Reported end-points
Kobayashi Y (2012)	Japan	Single arm study	2005-2009	288	71.4 M:F=197:91	NR	1.977(1.310-2.983)
Hirano D (2012)	Japan	Cohort study; Retrospective	1995-2010	151	68.0 M:F=121:30	NR	0.948(0.498-1.807)
Takaoka E (2010)	Japan	Single arm study	1989-2007	60	64.7 M:F=40:20	Class 3, 4 or 5 findings according to the Papanicolaou classification	1.560(0.481-5.053)
This study	China	Cohort study; Retrospective	2008-2018	142	67.1 M:F=79:63	One that reported suspicious or positive results, or both	3.283(1.558-6.920)
				89	68.0 M:F=49:40		2.975(1.352-6.548)

RCT, randomized controlled trial; NR, no report; M:F, male-female ratio.

direction from urinary flow are related to tumor cell implantation. In fact, studies have reported that multiple or recurrent urothelial carcinomas likely arise from the same tumor cell implantation rather than from multicentric tumors (55, 59). In recent years, genomic profiles were applied to investigate tumor genomic characterization between primary UUT-UC and subsequent bladder cancer by van Doeveren et al., who found that UUT-UC tissues and paired bladder cancer had clonal relationships by analysis of shared genomic variants (65). Audenet et al. reported that UUT-UC patients with genetic mutations, including FGFR3, CCND1, KDM6A, and TP53, had a higher risk of subsequent bladder recurrence after UUT-UC (1). Above all, whether tumor cell implantation causes recurrent bladder cancer after urothelial carcinoma is worthy of further study for germline and somatic gene alterations (60, 66).

Although we performed a cohort study and meta-analysis of the data in the published literature to overcome the limitation of our single-center sample source to some extent and further confirm the accuracy of our research results further, our study has some potential limitations. First, our limited samples were enrolled from three medical centers, which may restrict the universality of our retrospective findings and cause certain limitations in the statistical analysis of predictors. Meanwhile, because our nomogram model was limited by confined samples, further enlargements on data collection and integration of other prognostic factors will refine reliability and availability for clinical prediction. It is necessary to conduct additional work at more clinical centers with larger included samples to facilitate objective and accurate findings, which will contribute to the impact of preoperative positive urine cytology on the prognostic assessment of UUT-UC patients. Moreover, the meta-analysis results are also problematic. Although we searched for literature from multiple sources and even included unpublished trials and abstracts, which can increase the risk of null results, we could not eliminate potential bias. Publication bias might have been caused by several factors: 1) in these articles, we selected the hazard ratios from the multivariate survival analysis. Univariate data will be adopted when the available results emerge. 2) If the HRs and their variance were not reported, we performed a calculation

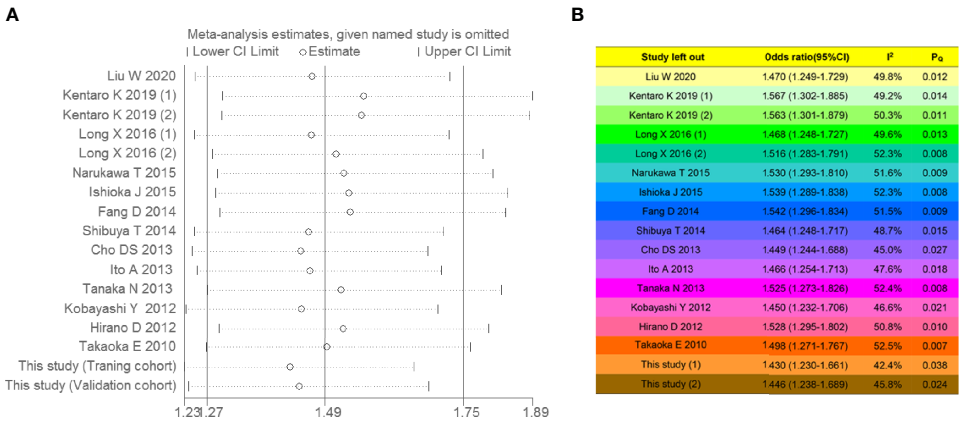

FIGURE 9 | Sensitivity analysis for intravesical recurrence-free survival. **(A)** Forest plot and **(B)** leave-one-study-out sensitivity analysis. If the *P*-values of the *Q*-test (P_Q) were more than 0.05, the individual studies were heterogeneous. Low, moderate, and high heterogeneity corresponded to I^2 ranges of 0%–30%, 30%–50%, and 50%–100%, respectively.

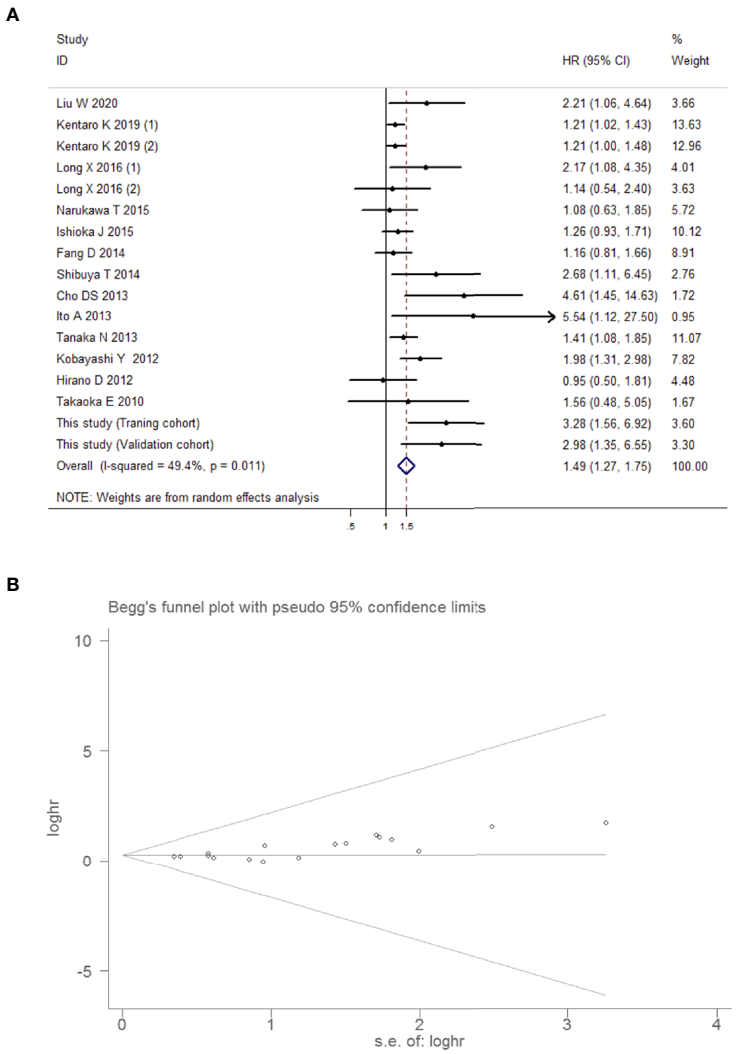

FIGURE 10 | Overall meta-analysis for intravesical recurrence-free survival. **(A)** Forest plot showing intravesical recurrence-free survival in UUT-UC patients with preoperative positive urine cytology in the total analysis. Squares in each trial indicate the hazard ratios, the horizontal line that traverses the square indicates the 95% confidence interval (CI), and diamonds indicate the estimated combined effect. According to the heterogeneity analysis, the Mantel–Haenszel random-effects model was selected. **(B)** Funnel plot for intravesical recurrence-free survival in UUT-UC patients with preoperative positive urine cytology.

TABLE 6 | The overall quality of evidence in pooled findings from eligible studies[a].

Outcomes	No. of studies	Design	Certain quality assessment					Summary of findings	
			Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Relative effect (95% CI)	Quality
Intravesical recurrence-free survival	17	Observational studies	Not serious	Not serious	Serious[c]	Serious[e]	None	HR 1.49 (1.27 to 1.75)	⊕⊖⊖⊖ VERY LOW
Risk of high-grade UUT-UC	10	Observational studies	Not serious	Serious[b]	Not serious	Serious[e]	None	RR 1.23 (1.00 to 1.51)	⊕⊖⊖⊖ VERY LOW
Risk of muscle-invasive UUT-UC	8	Observational studies	Not serious	Serious[b]	Not serious	Serious[e]	None	RR 1.16 (0.98 to 1.37)	⊕⊖⊖⊖ VERY LOW
Risk of lymphovascular-invasive UUT-UC	3	Observational studies	Not serious	Not serious	Serious[d]	Very serious[e]	None	RR 1.37 (0.81 to 2.31)	⊖⊖⊖⊖ VERY LOW

[a]*The quality assessment was based on GRADE approach;* [b]*High levels of heterogeneity in pooled findings;* [c]*Results restricted to China/Japan/Korea;* [d]*Limited findings from China/Japan;* [e]*The range of true values in each pooled studies was too large in varying degrees.*

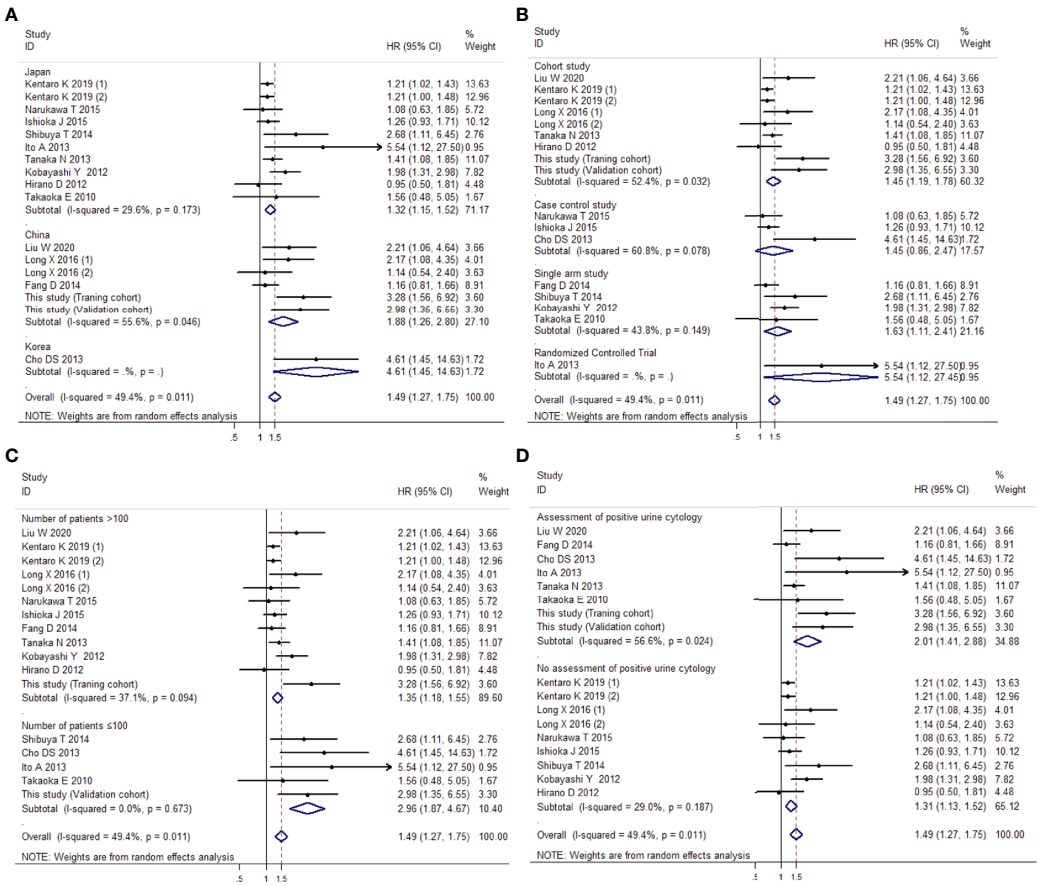

FIGURE 11 | Forest plots showing the risk of intravesical recurrence in UUT-UC patients with preoperative positive urine cytology by subgroup analysis of the **(A)** region, **(B)** study types, **(C)** sample size, and **(D)** assessment of urine cytology. Squares in each trial indicate the hazard ratios, the horizontal line that traverses the square indicates the 95% confidence interval (CI), and diamonds indicate the estimated combined effect. According to the heterogeneity analysis, the Mantel–Haenszel random-effects model was selected.

TABLE 7 | Main characteristics of studies about tumor malignant biological behaviors included in the meta-analysis.

First author (year)	Country	Study type	Recruitment period	No. of subjects	Demographics (age, sex)	Assessment of positive urine cytology	Reported endpoints
Kuroda K (2021)	Japan	Case-control study; Retrospective	NR	145	70.0 M: F=109:36	NR	LVI: 3.439(1.373-8.613)
Liu W (2020)	China	Cohort study; Retrospective	2012-2019	315	67.0 M: F=192:123	Defined as a positive result and/or a suspicious report	HG: 1.190(1.060-1.350) LVI: 0.860 (0.560-1.320)
Maruschke M (2017)	Germany	Case-control study; Retrospective	1996-2011	113	NR	NR	HG: 1.010(0.450-2.250) MI: 0.890 (0.490-1.590)
Wang L (2014)	America	Case-control study; Retrospective	2000-2011	65	69.8 M:F=36:29	One that reported suspicious or positive results, or atypical diagnosis	HG: 1.870(1.240-2.840)
Chen X (2013)	China	Case-control study; Retrospective	2002-2010	693	NR M: F=307:386	Defined as a positive result and/or a suspicious report	HG: 1.365(0.938-1.987) MI: 1.519 (1.043-2.212)
Tanaka N (2013)	Japan	Cohort study; Retrospective	1994-2010	474	69.0 M: F=346:128	One that reported suspicious or positive results, or both	HG: 1.200(1.040-1.380) MI: 1.110 (0.980-1.260) LVI: 1.330(1.050-1.680)

(Continued)

TABLE 7 | Continued

First author (year)	Country	Study type	Recruitment period	No. of subjects	Demographics (age, sex)	Assessment of positive urine cytology	Reported endpoints
Messer J (2011)	America	Cohort study; Retrospective	1997-2008	326	70.0 M: F=202:124	Presence of malignant cells	HG: 0.640(0.530-0.770) MI: 0.820 (0.610-1.110)
Xu C (2010)	China	Case-control study; Retrospective	NR	71	64.8 M:F=54:17	NR	HG: 2.330(1.330-4.070) MI: 1.550 (0.820-2.930)
Marín-Aguilera M (2007)	Spain	Case-control study; Retrospective	2003-2006	30	66.0 M:F=25:5	One that reported suspicious or positive results, or both	HG: 2.310(1.350-3.960) MI: 1.170 (0.600-2.270)
This study	China	Cohort study; Retrospective	2008-2018	142	67.1 M:F=79:63	One that reported suspicious or positive results, or both	HG: 0.980(0.770-1.240) MI: 1.690 (1.210-2.300)
				89	68.0 M:F=49:40		HG: 1.080(0.830-1.420) MI: 1.030 (0.830-1.270)

LVI, lymphovascular invasion; HG, high grade; MI, muscle invasion; NR, no report; M:F, male-female ratio.

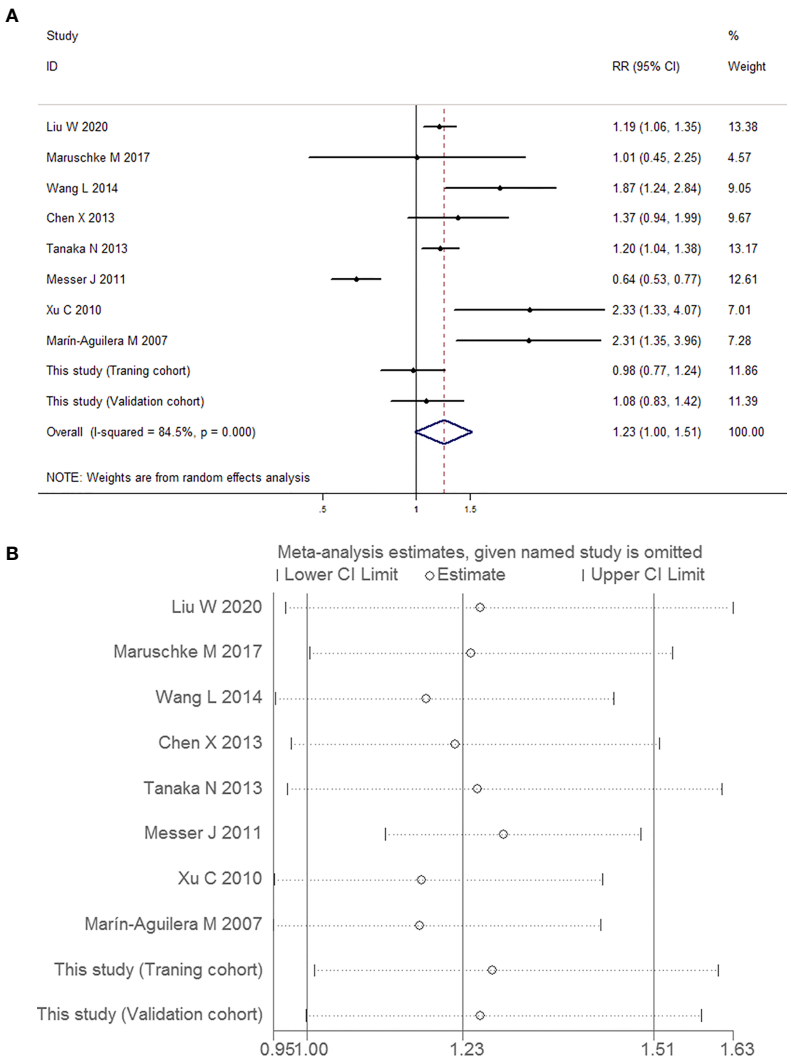

FIGURE 12 | Forest plots for the risk of high-grade UUT-UC in patients with preoperative positive urine cytology. **(A)** Meta-analysis. **(B)** Sensitivity analysis.

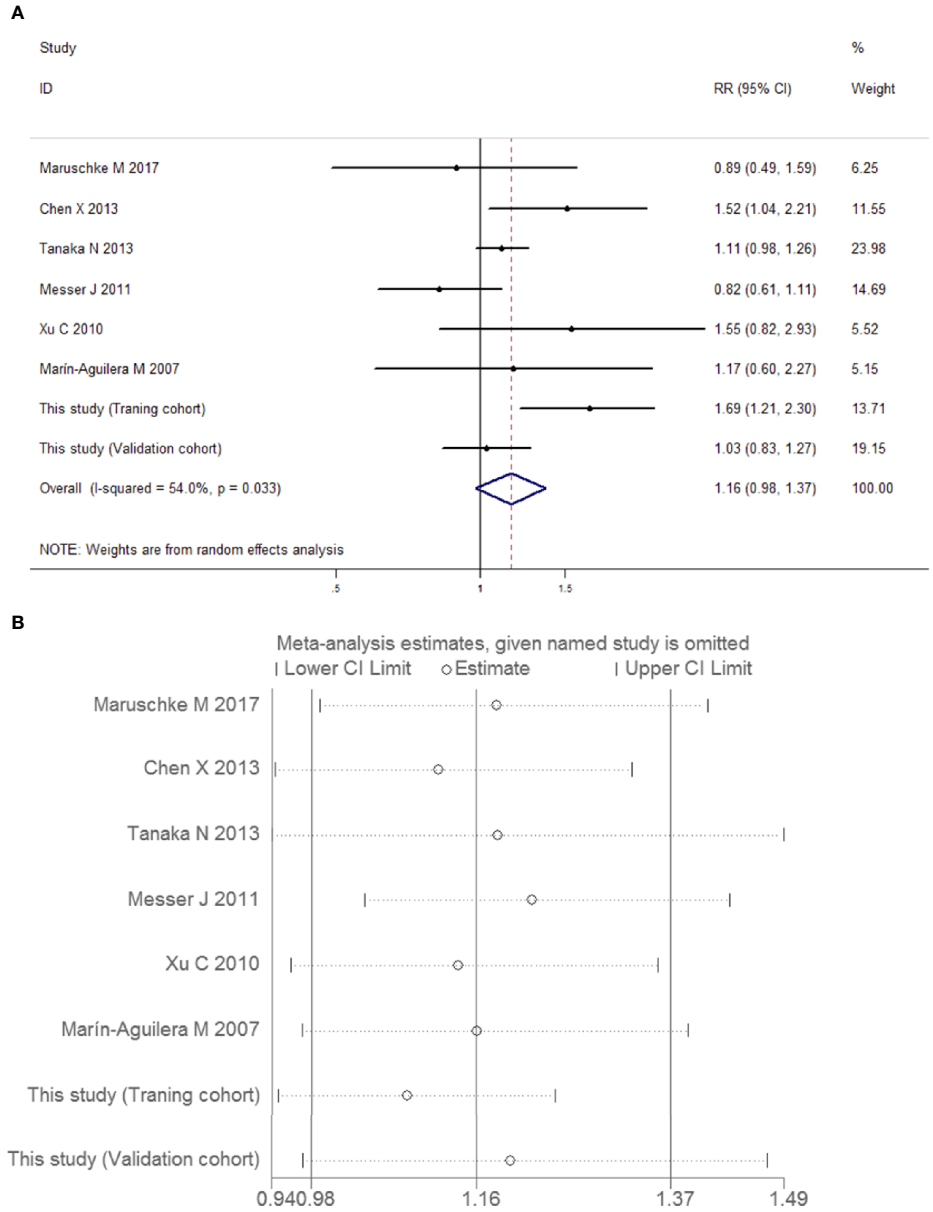

FIGURE 13 | Forest plots for the risk of muscle-invasive UUT-UC in patients with preoperative positive urine cytology. **(A)** Meta-analysis. **(B)** Sensitivity analysis.

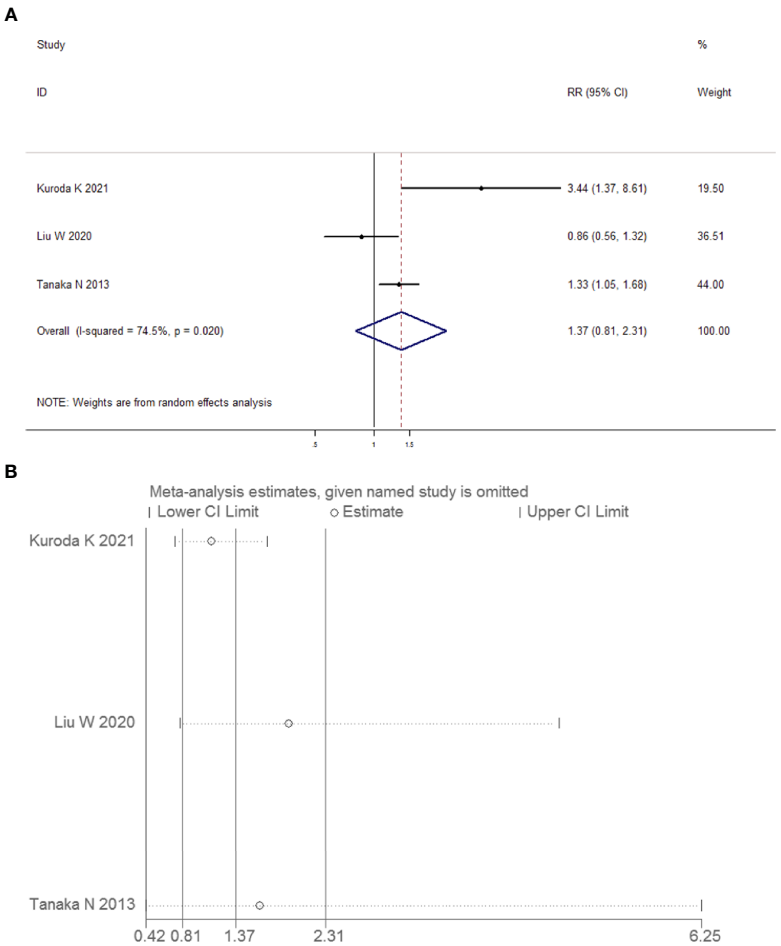

FIGURE 14 | Forest plots for the risk of lymphovascular-invasive UUT-UC in patients with preoperative positive urine cytology. **(A)** Meta-analysis. **(B)** Sensitivity analysis.

from the survival comparison statistics and its variance, which has less reliability than direct data (67–69). In addition, different diagnostic criteria for urinary cytology are used among different countries and regions, and the quality of this study would have been improved by standardized protocols. Furthermore, the number of articles about malignant tumor biological behavior was limited. The included samples could have led to heterogeneity among the groups, which would lead to an inconspicuous association between preoperative urine cytology and malignant tumor biological behavior risk. We expect that more similar studies could be included by other researchers to reach a reasonable conclusion.

CONCLUSION AND OUTLOOK

With the development of molecular biology approaches, the processes of urothelial carcinoma pathogenesis have been widely and deeply explored. We classified bladder cancer by gene sequencing and comprehensive profiling of RNA expression and used DNA sequencing results from biopsy to understand survival distributions and prognostic factors (70–72). This new understanding of heterogeneous diseases, such as UUT-UC and bladder cancer, can improve outcomes and quality of life through customized treatments. For positive tumor cells and moderately or severely abnormal cells, significant differences in genes arise as the disease occurs and progresses. Sequencing of DNA derived from urine had 82.2% sensitivity and 100% specificity, which may have great potential for the diagnosis of UUT-UC (73). In addition, in pathological cancer states, abnormal activation of export machinery, such as exosomes (products of vesicular transport), results in excessive discharge of many vital proteins and misexpression of miRNAs or mRNA, which are molecules with great differences in size (74, 75). The analysis of DNA mutations, including MDM2, TP53, RAS, and FGFR3, is an effective tumor phenotyping tool in defining the molecular subtypes with discrete profiles of gene expression, histology of UUT-UC, and prognostic outcome of patients (73, 76). It is worth focusing on the key molecular processes and analyzing gene and protein expression systematically. We believe that successful exploration of cancer therapeutic targets requires an interdisciplinary understanding of

the processes underlying these cellular activating or inhibiting mechanisms and how secreted biomolecules participate in cell–cell interactions in the tumor microenvironment.

DATA AVAILABILITY STATEMENT

The datasets generated for our case-control study are available on request to the corresponding authors. Additionally, publicly available datasets for meta-analysis in our study could be searched in the PubMed, Medline, Embase, Cochrane Library and Scopus databases.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethical Review Board of the Second Affiliated Hospital of Dalian Medical University, the Ethical Review Board of the Affiliated Dalian Friendship Hospital of Dalian Medical University, and the Ethical Review Board of the Cancer Hospital of China Medical University. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

The author contributions were evaluated by four criteria that were formulated by the guidelines of the International Committee of Medical Journal Editors. BF, YaH, and YW designed the research. BF, YuH, SW, QT, XY, MS, TC, YaH, YW, and ZL acquired the data. BF, SW, and QT analyzed and interpreted the clinical data of the patients. SW and YW performed and interpreted the pathology staining. BF, YuH, and YW reviewed the literature and drafted the manuscript. BF, YuH, QL, YaH, and ZL assisted with image interpretation and formatting and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conditional Intravesical Recurrence-Free Survival Rate After Radical Nephroureterectomy With Bladder Cuff Excision for Upper Tract Urothelial Carcinoma

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Background: To evaluate the conditional intravesical recurrence (IVR)–free (IVRF) survival rate in patients with upper tract urothelial carcinoma (UTUC) who had no history of bladder cancer and no concomitant bladder cancer. Hence, we aimed to analyze a relatively large number of patients with UTUC who underwent radical nephroureterectomy with bladder cuff excision (RNUx).

Methods: We retrospectively analyzed the data of 1,095 patients with UTUC who underwent RNUx. Their baseline characteristics, bladder tumor history, and UTUC features were analyzed to evaluate oncological outcomes. To determine the factors affecting IVR, surgical modality, use of preoperative ureteroscopy, TNM stage, and pathological outcomes were evaluated. Multivariable Cox regression analyses were performed to evaluate the factors affecting IVR. Conditional IVRF survival rate was analyzed using Kaplan–Meier curves.

Results: Among the 1,095 patients, 462 patients developed IVR, and the mean time to the development of IVR was 13.08 ± 0.84 months after RNUx. A total of 30.74% of patients with IVR and 15.32% of those without IVR had a history of bladder cancer ($p < 0.001$). Multivariable analysis showed that a history of bladder cancer, multifocal tumors, use of preoperative ureteroscopy, extravesical bladder cuffing method, lymph node involvement, positive surgical margins, and use of adjuvant chemotherapy were determined to be risk factors for IVR. The conditional IVRF rate was 74.0% at 12 months after RNUx, 87.1% at 24 months after RNUx, 93.6% at 36 months after RNUx, and 97.3% at 60 months after RNUx. The median IVRF survival period was 133.00 months for all patients. In patients with IVRF at 24 months after RNUx, only ureteroscopy was an independent risk factor for IVR [hazard ratio (HR) 1.945, $p = 0.040$]. In patients with IVRF at ≥36 months, there was no significant factor affecting IVR.

Conclusions: Active IVR assessment is required until 36 months after RNUx. In addition, patient education and regular screening tests, such as urine analysis and cytology, are required for patients with IVRF for ≥36 months.

Keywords: urothelium cancer, nephroureterectomy, bladder, recurrence, risk

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INTRODUCTION

Upper tract urothelial carcinoma (UTUC) is a urothelial cancer that occurs in the ureter or pelvocaliceal system. It has an incidence of 1–2 cases per 100,000 patients (1, 2). Recently, the incidence of UTUC has been increasing with the development of diagnostic techniques, such as radiological and endoscopic techniques (3). Although the etiology of UTUC remains unclear, cigarette smoking, herbal medicines, chronic infection, and occupational carcinogenesis are known risk factors for UTUC (3). Its gold standard treatment is radical nephroureterectomy with bladder cuff excision (RNUx), which has excellent oncological outcomes (4). However, an important disadvantage of RNUx is intravesical recurrence (IVR), which occurs in 15%–50% of patients after RNUx (5, 6). Diagnosis of IVR after RNUx is important because additional treatments, such as surgery and chemotherapy, due to bladder cancer are required. Moreover, it may affect the prognosis of UTUC (7).

UTUC and bladder cancer have the same histological subtype and urothelial composition. Nevertheless, the two have embryological, epidemiological, and molecular differences (8). Because of these common features, approximately 13% of patients with UTUC have a history of bladder cancer, and approximately 9% of UTUC cases are diagnosed at the same time as bladder cancer (9). Furthermore, there is a high IVR rate after RNUx (10).

Accurate assessment of risk factors for IVR after RNUx is important in reducing unnecessary examinations and treatments for bladder cancer (11). Several previous studies were conducted with the aim of evaluating and preventing IVR, which occurred after RNUx. In these studies, tumor location, cancer stage, grade, and sex were reported as risk factors for IVR after RNUx (7, 12, 13). However, there are limited guidelines on the follow-up protocol for IVR. Despite the limited evidence, IVR occurs most commonly within 12 months after RNUx (14). Moreover, there are insufficient reports on the probability of recurrence of IVR or follow-up when there is no recurrence within 12 months or no IVR for a specific period. In particular, there is no report of IVR-free (IVRF) survival for patients who did not have a history of bladder cancer or concomitant bladder cancer and UTUC.

Therefore, this study aimed to evaluate the conditional IVRF survival rate of patients with UTUC who had no history of bladder cancer and no concomitant bladder cancer and to determine the incidence and risk factors for IVR by analyzing a relatively large number of patients who underwent RNUx for UTUC.

PATIENTS AND METHODS

Patients and Clinicopathological Parameters

We retrospectively analyzed 1,095 patients who underwent RNUx for UTUC at a single medical center from 1994 to 2018. All patients underwent the standard open or minimal invasive surgery, and specimens were collected for further examination. Preoperative ureteroscopy (URS) was not performed routinely. Lymph node (LN) dissection was not routinely performed on all patients. Nevertheless, it was performed only if LN invasion was suspected based on the radiological evaluation. To evaluate the risk factors for IVR, the 1,095 patients were divided into two groups: patients with IVR (n = 462) and those without IVR (n = 633).

Among the 1,095 patients, 856 patients without a history of bladder cancer and without concomitant bladder cancer were assessed for additional analysis for conditional IVRF survival. To evaluate IVRF survival, these 856 patients were divided into two groups: 536 patients with IVR and 320 without IVR.

Baseline characteristics were evaluated, including age, sex, body mass index (BMI), American Society of Anesthesiologists (ASA) score, smoking history, bladder tumor history, and underlying disease including hypertension (HTN) and diabetes mellitus (DM). In addition, the location, laterality, and multifocality of UTUC were analyzed to evaluate oncological outcomes. Surgical modality, approach methods, use of preoperative URS, TNM stage, and pathological outcomes were subsequently evaluated. Tumors were staged according to the 2010 American Joint Committee on Cancer/International Union Against Cancer TNM classification (15). To evaluate conditional IVRF survival, subanalysis was performed in patients with IVRF for 6, 12, 24, 36, 48, and 60 months.

Statistical Analysis

The groups were compared using the chi-square test for categorical variables and Student's t-test for continuous variables. Multivariable Cox regression analyses were performed to identify risk factors for IVR, and the IVRF survival rate was analyzed using the Kaplan–Meier curves. Statistical analyses were conducted using the SPSS®, version 21.0. For all two-sided tests, p-value <0.05 was considered statistically significant.

Ethics Statement

The study was performed in agreement with the applicable laws and regulations, good clinical practices, and ethical principles described in the Declaration of Helsinki. The Institutional Review Board of the Samsung Medical Center approved the present study (IRB No. 2019-09-115-002). Requirement for informed consent was waived by the Board.

RESULTS

Among the 1,095 patients, 462 patients developed IVR. The mean age of patients with and without IVR was 64.88 ± 10.35 and 65.92 ± 11.28 years, respectively (p = 0.120). The prevalence rates of HTN and DM were 48.05% and 22.29%, respectively, in the IVR group and 41.71% and 16.43%, respectively, in the without IVR group (HTN: p = 0.037, DM: p = 0.014). A total of 30.74% patients in the IVR group and 15.32% in the without IVR group had a history of bladder cancer (p < 0.001). Multifocal tumors were observed in 25.54% patients in the IVR group and 22.75% in the without IVR group (p = 0.003).

Cuffing was performed using the intravesical approach in 51.95% patients in the IVR group and 60.19% in the without IVR

group (p = 0.007). Preoperative URS was performed in 59.53% patients in the IVR group and 44.39% in the without IVR group (p < 0.001). Adjuvant chemotherapy was performed in 81 patients (17.53%) in the IVR group and 159 patients (25.12%) in the without IVR group (p < 0.001) (**Table 1**).

IVR occurred at a mean period of 13.08 (interquartile range, 3.97–14.05) months after RNUx. The median IVRF survival period was 12.30 (95% CI, 7.72–16.88) months in patients with a history of bladder cancer or concomitant bladder cancer and 133.00 (95% CI, 57.89–208.11) months in patients without such a history (p < 0.001) (**Figure 1**). Multivariable analysis revealed a history of bladder cancer [hazard ratio (HR) 2.409, p < 0.001], a multifocal tumor (HR 1.348, p = 0.008), use of preoperative URS (HR 1.733, p < 0.001), extravesical bladder cuffing (HR 1.408, p = 0.009), LN involvement (HR 2.121, p = 0.004), positive surgical margins (HR 1.553, p = 0.026), and adjuvant chemotherapy (HR 0.759, p = 0.033) to be risk factors for IVR (**Table 2**).

Conditional IVRF Survival and Risk Factors for IVR in UTUC Patients Without a History of Bladder Cancer or Concomitant Bladder Cancer

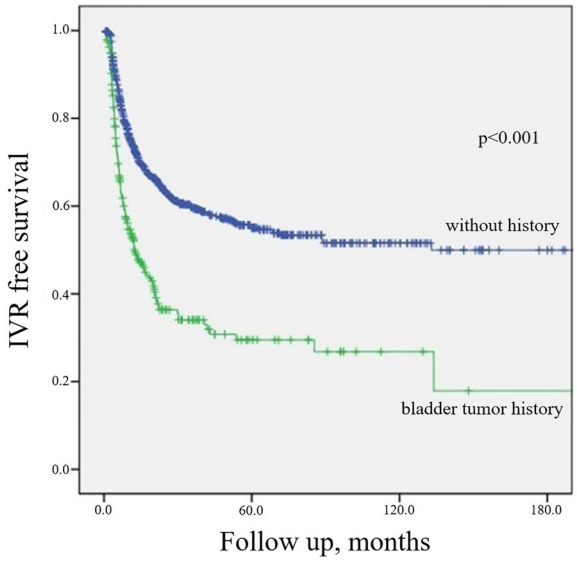
The mean 5-year IVRF survival rate after RNUx was 49.3% ± 0.05% for multifocal tumors and 56.2% ± 0.02% for solitary tumors (p = 0.008). The mean 5-year IVRF survival rate after RNUx was 45.0% ± 0.03% for those who underwent preoperative URS and 65.7% ± 0.03% for those who did not (p < 0.001). In the case of LN involvement, the mean 5-year IVRF survival rate after RNU/Bladder cuff excision (BCE) was 58.5% ± 0.12% and 66.1% ±

TABLE 1 | Baseline characteristics and operative and pathological outcomes.

	IVR group, *n* = 462	Non-IVR group, *n* = 633	p-value
Age, years	64.88 ± 10.35	65.92 ± 11.28	0.120
Sex, male, n (%)	352 (76.19)	454 (71.72)	0.098[a]
BMI, kg/m^2	24.40 ± 3.32	24.21 ± 3.00	0.322
ASA score, ≤2, n (%)	415 (89.83)	572 (90.36)	0.116[a]
HTN	222 (48.05)	264 (41.71)	0.037[a]
DM	103 (22.29)	104 (16.43)	0.014[a]
Smoking, never-smoker, n (%)	215 (46.54)	297 (46.92)	0.771[a]
Ex-smoker	134 (29.00)	176 (27.80)	
Current smoker	111 (24.03)	154 (24.33)	
Gross hematuria	369 (79.87)	475 (75.04)	0.060[a]
History of bladder tumor	142 (30.74)	97 (15.32)	<0.001[a]
Laterality			0.159[a]
Left	239 (51.73)	360 (56.87)	
Right	223 (48.27)	272 (42.97)	
Tumor location			0.057[a]
Renal pelvis	188 (40.69)	302 (47.71)	
Ureter	217 (46.97)	269 (42.50)	
Both renal pelvis and ureter	57 (12.34)	62 (9.79)	
Multifocal tumor, n (%)	118 (25.54)	114 (22.75)	0.003[a]
Surgical modality, n (%)			0.395[a]
MIS	242 (52.38)	348 (54.98)	
Open	220 (47.62)	285 (45.02)	
Extravesical bladder cuffing, n (%)	222 (48.05)	252 (39.81)	0.007[a]
Preoperative ureteroscopy, n (%)	275 (59.53)	281(44.39)	<0.001[a]
pT stage, n (%)			<0.001[a]
pTa	62 (13.42)	73 (11.53)	
pT1	147 (31.82)	175 (27.65)	
pT2	94 (20.35)	90 (14.22)	
pT3-pT4	159 (34.42)	295 (46.60)	
Grade, n (%)			0.002[a]
Grade 3	191 (41.34)	315 (49.76)	
Concomitant CIS, n (%)	52 (11.26)	70 (11.06)	0.906[a]
Lymph node, n (%)			<0.001[a]
pN0	97 (21.00)	179 (28.28)	
pN1	26 (5.63)	75 (11.85)	
pNx	339 (73.38)	379 (59.87)	
Tumor size, cm	3.81 ± 3.15	3.91 ± 2.84	0.578
Lymphovascular invasion, n (%)	78 (16.88)	133 (21.01)	0.087[a]
Surgical margin positive, n (%)	26 (5.63)	22 (3.48)	0.086[a]
Adjuvant chemotherapy, n (%)	81 (17.53)	159 (25.12)	0.003[a]
Follow-up, months	60.14 ± 51.68	44.37 ± 45.54	<0.001

IVR, intravesical recurrence; BMI, body mass index; ASA, American Society of Anesthesiologists; HTN, hypertension; DM, diabetes mellitus; MIS, minimal invasive surgery; CIS, carcinoma in situ.

Student's t-test, [a]chi-square test.



IVR free period	Without history of bladder tumor	Bladder tumor history
No. of patients	856	239
Intravesical recurrence, n (%)	320 (37.38)	142 (59.41)
Median IVR free survival, month (95% CI)	133.00 (57.89 - 208.11)	12.30 (7.72 - 16.88)
Mean IVR free survival, month (SE)	136.84 (5.92)	59.86 (8.72)
5 years IVR free rate, % (SE)	55.0 (0.02)	29.5 (0.04)
10 years IVR free rate, % (SE)	50.0 (0.03)	17.9 (0.08)

FIGURE 1 | Kaplan–Meier curve for intravesical tumor recurrence according to a history of bladder cancer. IVR, intravesical recurrence; CI, confidence interval; SE, standard error.

0.04% for patients with and without involvement, respectively ($p < 0.001$). At 5 years after RNUx, the mean IVRF survival rate of the intravesical bladder cuffing group was 59.9% ± 0.03% and the extravesical bladder cuffing group was 49.3% ± 0.03% ($p = 0.001$) (**Figure 2**).

The IVRF rate was 74.0% at 12 months after RNUx, 87.1% at 24 months after RNUx, 93.6% at 36 months after RNUx, and 97.3% at 60 months after RNUx (**Figure 3**). The mean IVRF survival period was 136.84 months for all patients, 156.24 months for those with 6-month IVRF, 175.38 months for those with 12-month IVRF, 189.14 months for those with 36-month IVRF, and 178.21 months for those with 60-month IVRF (**Table 3**).

Multivariable Cox regression analysis showed that DM (HR 1.589, $p = 0.017$), gross hematuria (HR 1.744, $p = 0.003$), URS (HR 2.207, $p < 0.001$), and extravesical bladder cuff excision (HR 1.614, $p = 0.002$) were found to be risk factors for IVR. In patients with 24-month IVRF survival rate after RNUx, only URS was found to be an independent risk factor for IVR (HR 1.945, $p = 0.040$). In patients with ≥36-month IVRF survival rate, there was no significant risk factor for IVR (**Table 4**).

DISCUSSION

In this study, the risk factors for IVR after RNUx in patients with UTUC are discussed. Moreover, it was confirmed that there were no significant UTUC-related risk factors for IVR among patients who had ≥36-month IVRF survival rate. However, it was confirmed that approximately 5% of patients with an IVRF period of ≥36 months developed IVR.

After RNUx for UTUC, the cause of IVR is not clear yet. However, the possible mechanisms of IVR include the field theory that UTUC is exposed to the urothelium to generate IVR (16) and the intraluminal tumor seeding theory that the cancer cells in the upper tract reach the bladder *via* the urinary stream (17). If URS is performed before RNUx, the intraluminal pressure of the renal pelvis increases and tumor manipulation occurs, resulting in increased intraluminal tumor seeding and eventually increased IVR (18, 19). In the present study, it was found that URS before RNUx was a risk factor for IVR in patients with 0–24-month IVRF survival rate. The efficacy of URS before surgery remains controversial (20, 21). However, reducing the use of preoperative URS based on radiological imaging and laboratory examinations, such as urine analysis and cytology, could improve the IVR rate.

In a recent meta-analysis, the predictors of IVR were classified into three categories: patient-specific factors, tumor-specific factors, and treatment-specific factors (22). In this study, patient-specific factors included male sex, bladder cancer history, and chronic kidney disease; tumor-specific factors included positive urinary cytology, ureteral location, multifocality, invasive pT stage, and necrosis. Furthermore,

TABLE 2 | Results of the univariable and multivariable Cox regression analyses.

	Univariable			Multivariable		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Age (continuous)	1.016	1.007–1.025	0.001	1.000	0.988–1.013	0.959
Sex, male	1.108	0.894–1.373	0.348			
BMI	0.985	0.957–1.014	0.302			
DM	1.424	1.143–1.773	0.002	1.279	0.912–1.794	0.154
HTN	1.248	1.040–1.498	0.017	1.294	0.975–1.717	0.074
Gross hematuria	1.148	0.914–1.441	0.235			
Smoking	0.949	0.851–1.057	0.342			
Bladder cancer history	2.184	1.791–2.665	<0.001	2.409	1.761–3.297	<0.001
ASA score	1.229	1.050–1.438	0.010	1.112	0.861–1.436	0.417
Laterality	0.868	0.723–1.041	0.127			
Location						
Renal pelvis	1	reference	–			
Ureter	1.211	0.996–1.473	0.055			
Both	1.487	1.108–1.997	0.008	1.055	0.610–1.824	0.849
Multifocality	1.503	1.219–1.853	<0.001	1.348	1.082–1.678	0.008
Preoperative URS	1.597	1.325–1.925	<0.001	1.733	1.338–2.244	<0.001
Operation modality						
MIS	0.859	0.454–1.624	0.640			
Bladder cuffing, extravesical	1.320	1.099–1.585	0.003	1.408	1.090–1.818	0.009
pT stage						
pTa	1	reference	–			
pT1	1.164	0.867–1.561	0.312			
pT2	1.311	1.047–1.641	0.018			
pT3+4	1.492	1.156–1.926	0.002	1.245	0.958–1.619	0.102
Grade, low grade	0.902	0.781–1.041	0.158			
Concomitant CIS	0.977	0.743–1.285	0.870			
Lymph node positive						
pN0	1	reference	–			
pN1	1.651	1.238–2.200	0.001	2.121	1.274–3.532	0.004
pNx	0.640	0.384–1.065	0.086			
Tumor size	1.009	0.975–1.044	0.602			
Margin positive	2.009	1.350–2.988	<0.001	1.553	0.819–2.947	0.026
Lymphovascular invasion	0.927	0.727–1.183	0.545			
Adjuvant chemotherapy	0.697	0.548–0.886	0.003	0.759	0.589–0.979	0.033

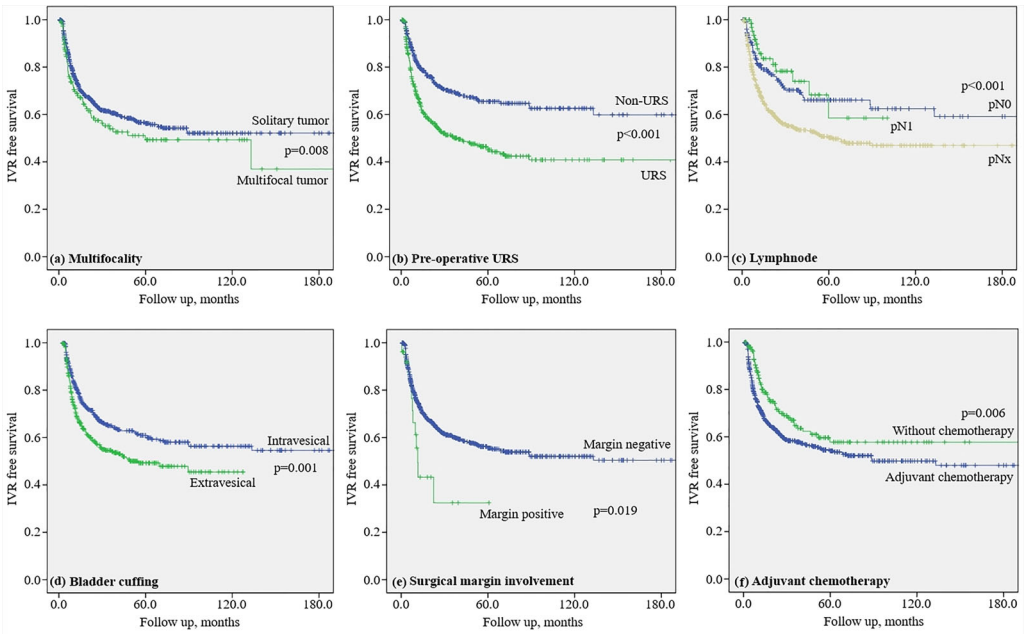
HR, hazard ratio; CI, confidence interval; BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; CVA, cerebrovascular accident; ASA, American Society of Anesthesiologists; URS, ureteroscopy; CIS, carcinoma in situ.

treatment-specific factors included laparoscopic surgery, extravesical bladder cuff excision, and positive surgical margins. In the present study, bladder cancer history, preoperative URS, multifocality, LN invasion, bladder cuffing, and surgical margin involvement were identified as risk factors for IVR after RNUx. The present study was also conducted in patients with no history of bladder cancer. In these patients, DM, gross hematuria, URS, and extravesical bladder cuff excision were evaluated as predictive factors of IVR after RNUx possibly owing to the difference between the groups. However, it is clear that avoiding preoperative URS and extravesical bladder cuff excision can reduce IVR.

Previous studies reported that DM is an independent risk factor for IVR (23, 24). Moreover, a previous study reported that DM corresponded to a poor prognosis in patients with UTUC. Chronic exposure to hyperinsulinemia or hyperglycemia is a possible factor that induced tumor cell proliferation (25, 26). Hashimoto et al. (27) reported that gross hematuria was a significant risk factor for IVR. They suggested that hemorrhagic tumor cells could be seeded easily in the mucosal epithelium. Nevertheless, the methods of management for bladder cuff remain controversial. Some studies reported that the method of bladder cuff excision was not associated with the IVR rate (28, 29). However, when intramural ureter was not appropriately resected, the reported recurrence rate was 33%–75% (30).

Recently, Katims et al. (31) reported the risk factors for IVR after minimally invasive RNUx. They reported that IVR occurred in 22.7% of patients who underwent RNUx. In addition, URS, transurethral resection of the bladder cuff and positive surgical margin were suggested to be risk factors for IVR. In the present study, 37.38% of patients without prior or concurrent bladder cancer developed IVR. The different cancer stage of the enrolled patients might have affected higher IVR rate.

Due to concerns about IVR, bladder examination, including cystoscope and urinary cytology, is recommended for 5 years after RNUx for UTUC (4). However, due to the lack of studies, optimal follow-up strategies for IVR after RNUx cannot be concluded (32, 33). Recently, Shigeta et al. (34) reported on conditional IVRF survival after RNUx of the 364 patients with Ta-T3 UTUC. According to this study, IVR was identified in 48.4% patients; the 5-year conditional IVRF survival rate



	Median IVR free survival, mo (95% CI)	5-year IVR free survival rate, % (SE)	10-year IVR free survival rate, % (SE)
Multifocality			
Solitary tumor	not reached	56.2 (0.02)	52.1 (0.03)
Multifocal tumor	59.70 (4.64 - 114.77)	49.3 (0.05)	37.0 (0.11)
Pre-operative URS			
URS	39.20 (20.40 - 58.00)	45.0 (0.03)	40.7 (0.03)
non-URS	not reached	65.7 (0.03)	62.7 (0.03)
Lymphnode			
pN0	not reached	66.1 (0.04)	62.4 (0.05)
pN1	not reached	58.5 (0.12)	N/A
pNx	133.00 (N/A)	50.3 (0.02)	47.0 (0.03)
Bladder cuffing			
Intravesical	200.00 (N/A)	59.9 (0.03)	56.3 (0.03)
Extravesical	88.40 (N/A)	49.3 (0.03)	N/A
Surgical margin invoivement			
Margin negative	not reached	55.6 (0.02)	52.1 (0.02)
Margin positive	11.40 (9.20 - 13.60)	32.4 (0.13)	N/A
Adjuvant chemotherapy			
Adjuvant chemotherapy	200 (4.23 - 395.78)	54.1 (0.02)	49.9 (0.03)
Without adjuvant chemotherapy	not reached	57.4 (0.05)	57.4 (0.05)

FIGURE 2 | Kaplan–Meier curve for intravesical recurrence in patients without a history of bladder tumor.

increased from 41.5% to 60.5%, 73.4%, 79.5%, and 96.7% in patients with 1-, 2-, 3-, and 4-year IVRF survival rate, respectively. In the present study, each conditional IVRF survival was evaluated for patients with 0.5-, 1-, 2-, 3-, 4-, and 5-year IVRF survival rate. Our results are consistent with those of a previous study. Moreover, gross hematuria, URS, retroperitoneal approach, and a low grade were identified as risk factors for IVR in patients with 1-year IVRF survival rate, and only URS was evaluated as a risk factor for IVR in patients with 2-year IVRF survival rate. In addition, for patients with >3-year IVRF survival rate, these risk factors were deemed to have no significant effect on IVR. However, IVR occurred in 13.1% of patients with 3-year IVRF survival rate, and only 5.6% of patients with 5-year IVRF survival rate were diagnosed with IVR. Although these could not be clearly identified as IVR associated with UTUC, it was found to be a higher incidence than that in the general population. Therefore, patients with UTUC require IVR follow-up even following 5 years after RNUx.

Conditional survival analysis is a method of assessing additional survival at a specific time point after initial diagnosis or treatment (35). It is widely performed in cancer research because it can transmit additional important and diverse information during follow-up (36, 37). In this study, conditional IVRF survival according to the IVRF period was found in a large number of UTUC patients without a history of bladder cancer. According to the conditional IVRF survival rate in the present study, in the case of patients whose IVRF period is <36 months, the IVR rate after the IVRF period is >10%; hence, active assessment is required until 3 years after RNUx. In addition, since IVR occurs in 3%–5% of patients with an IVRF period of ≥36 months, patient education and screening tests, such as regular urine analysis and cytology, are required.

This study has several limitations. First, it was a retrospective study; thus, fixed criteria for diagnosis, treatment, and patient follow-up could not be used. Second, the indications for the use of URS, lymphadenectomy, and adjuvant chemotherapy were

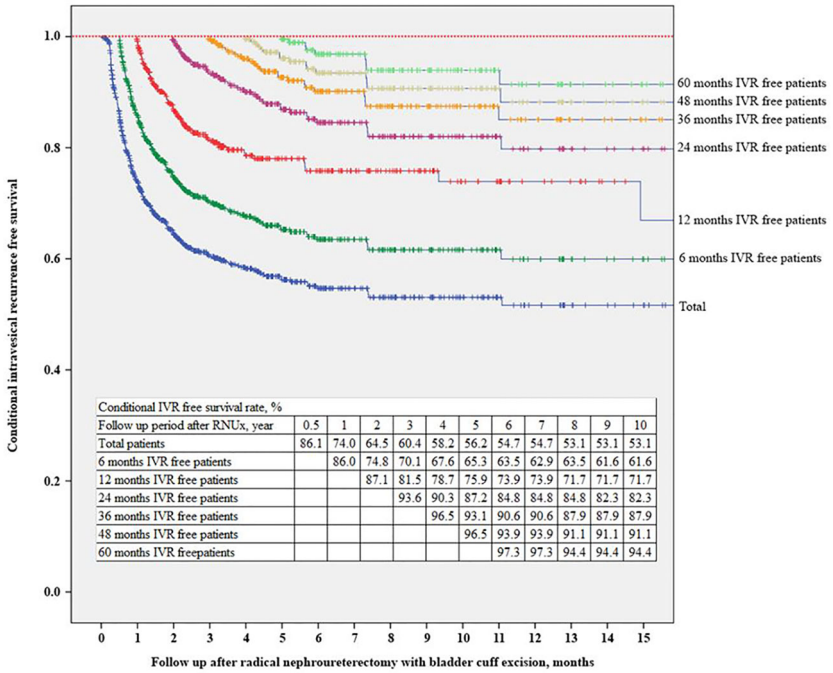


FIGURE 3 | Conditional intravesical recurrence-free survival. IVR, intravesical recurrence; RNUx, radical nephroureterectomy with bladder cuff excision.

TABLE 3 | Conditional intravesical recurrence-free survival after radical nephroureterectomy with bladder cuff excision.

Conditional IVR-free period	Total	0.5 year	1 year	2 years	3 years	4 years	5 years
No. of patients	856	696	544	381	285	228	174
Intravesical recurrence, n (%)	320 (37.38)	205 (29.45)	111 (20.40)	47 (12.34)	25 (8.77)	16 (7.02)	9 (5.17)
Median IVR-free survival, months (95% CI)	133.00 (57.89–208.11)	not reached	not reached	not reached	not reached	not reached	not reached
Mean IVR-free survival, months (SE)	136.84 (5.92)	156.24 (6.34)	175.38 (6.83)	188.67 (7.07)	189.14 (7.03)	183.84 (6.92)	178.21 (6.73)
5 years IVR-free survival rate, % (SE)*	56.2 (0.02)	63.5 (0.02)	73.9 (0.02)	84.8 (0.02)	87.9 (0.03)	91.1 (0.02)	94.4 (0.02)
10 years IVR-free survival rate, % (SE)*	53.1 (0.02)	61.6 (0.02)	71.7 (0.03)	80.1 (0.03)	85.5 (0.03)	88.7 (0.03)	91.9 (0.03)

CI, confidence interval; SE, standard error.
**After conditional IVR-free period.*

TABLE 4 | Risk factors for IVR in patients with UTUC who had no history of bladder cancer.

	Total			0.5 year IVRF			1 year IVRF			2 years IVRF		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
Diabetes mellitus	1.589	1.088–2.323	0.017	1.281	0.814–2.017	0.284	1.105	0.609–2.002	0.743	1.173	0.484–2.841	0.724
Gross hematuria	1.744	1.209–2.515	0.003	2.258	1.425–3.580	0.001	2.299	1.252–4.219	0.007	2.083	0.881–4.926	0.095
Ureteroscopy	2.207	1.633–2.985	<0.001	2.14	1.502–3.049	<0.001	2.082	1.324–3.274	0.001	1.945	1.030–3.672	0.040
Bladder cuffing, extravesical	1.614	1.193–2.183	0.002	1.557	1.091–2.220	0.015	1.336	0.847–2.105	0.213	1.208	0.624–2.341	0.575
Grade	0.77	0.589–1.007	0.057	0.643	0.466–0.887	0.007	0.595	0.394–0.900	0.014	0.683	0.384–1.215	0.194
Adjuvant chemotherapy	0.746	0.510–1.092	0.131	1.253	0.819–1.917	0.298	1.407	0.808–2.448	0.227	1.671	0.773–3.612	0.192
	3 years IVRF			4 years IVRF			5 years IVRF					
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value			
Diabetes mellitus	1.237	0.337–4.535	0.749	2.213	0.563–8.704	0.255	1.137	0.126–10.294	0.909			
Gross hematuria	1.088	0.408–2.903	0.866	0.638	0.201–2.018	0.444	0.945	0.168–5.309	0.948			
Ureteroscopy	1.688	0.724–3.935	0.225	1.548	0.532–4.505	0.422	1.632	0.388–6.861	0.504			
Bladder cuffing, extravesical	0.858	0.349–2.109	0.738	0.427	0.112–1.634	0.214	0.79	0.139–4.509	0.791			
Grade	0.717	0.332–1.549	0.398	0.816	0.311–2.144	0.680	0.848	0.228–3.160	0.806			
Adjuvant chemotherapy	1.493	0.526–4.242	0.452	0.991	0.248–3.967	0.991	0.618	0.067–5.708	0.671			

Multivariable Cox regression analysis.
IVRF, intravesical recurrence-free; HR, hazard ratio; CI, confidence interval.

not clear. Although this study could not suggest an optimal follow-up strategy for IVR after RNUx for UTUC, it was considered that follow-up for IVR could be optimized through systematic analysis if additional data would be collected in the future. Moreover, notably, to the best of our knowledge, it is the first large-scale study on the conditional survival of patients with UTUC who had no history of bladder cancer.

CONCLUSION

In this study, a history of bladder cancer, multifocal tumors, preoperative URS, LN invasion, extravesical bladder cuffing, and surgical margin involvement were identified as risk factors for IVR. However, in patients with no history of bladder cancer, DM, gross hematuria, preoperative URS, and extravesical bladder cuffing were risk factors. In patients whose IVRF period is <3 years, the IVR rate is ≥10%; hence, active IVR assessment is required until 3 years after RNUx. In addition, since 3%–5% of patients with an IVRF survival period of ≥3 years develop IVR, patient education and regular screening tests, such as urine analysis and cytology, are required for patients with ≥3-year IVRF survival rate.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Samsung medical center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

HS contributed to the conceptualization. WS, MK, HJ, BJ, SS, SJ, and HL contributed to the methodology. JC contributed to the formal analysis. JC and HS contributed to the data curation. JC contributed to writing–original draft preparation. HS contributed to writing–review and editing and supervision. All authors contributed to the article and approved the submitted version.

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Knowledge of and Compliance With Guidelines in the Management of Non-Muscle-Invasive Bladder Cancer: A Survey of Chinese Urologists

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Background: Non-muscle-invasive bladder cancer (NMIBC) still poses a heavy load for resulting in many new cases which contribute significantly to medical costs. Although many NMIBC guidelines have been developed, their implementation remains deficient.

Objective: This study was conducted in order to analyze the knowledge of and compliance with the guidelines for NMIBC of Chinese urologists and to identify associated factors.

Methods: We conducted an online survey between August 2019 and January 2021. Respondents who were more than 65 years old or did not give informed consent were excluded. Linear/logistic regressions were performed to identify factors associated with the knowledge of and compliance with the guidelines of urologists, respectively. McNemar's tests were used to explore the divergence between knowledge and compliance.

Results: A total of 814 responses were received, and 98.77% of urologists acknowledged the positive effects of high-quality guidelines. The average knowledge score was 6.10 ± 1.28 (out of a full score of 9), and it was positively associated with educational level and the number of guidelines consulted. Only 1.61% and 39.36% of the respondents realized that the guidelines did not recommend further chemotherapy or BCG infusion for low-risk patients. There were 38.87% and 51.84% respondents "often" or more frequently utilizing BCG therapy for intermediate- and high-risk NMIBC patients, respectively. Divergence between knowledge and compliance in performing a second TURBT after incomplete initial resection reached statistical significance ($p < 0.001$).

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Conclusions: Although the vast majority of urologists acknowledged the positive effects of guidelines, knowledge of and compliance with some recommendations of NMIBC guidelines are still inadequate. Factors associated with guidelines, individual professionals, patients, organizations, and the environment jointly contributed to the non-compliance.

Keywords: guideline, guideline adherence, urinary bladder neoplasms, surveys and questionnaires, professional practice

INTRODUCTION

Ranking as the 10th most common cancer worldwide, bladder cancer caused an estimated 549,000 new cases and 200,000 deaths worldwide in 2018 (1). For therapeutic purposes, papillary tumors confined to the mucosa (Ta) or submucosa (T1) and carcinoma *in situ* (CIS, Tis) are classified as non-muscle-invasive bladder cancer (NMIBC) (2), which accounts for approximately 75% of bladder cancer cases (3). NMIBC still poses a heavy load due to its high 5-year recurrence (up to 78%) and progression (up to 45%) risk (4), hence incurring high medical costs.

For the universal coverage of high-quality medical services, institutions and academies worldwide have invested substantial resources in developing treatment guidelines. The European Association of Urology (EAU), National Comprehensive Cancer Network (NCCN), and American Urological Association (AUA) have published guidelines relating to NMIBC, and all of them updated their guidelines last year. However, although the positive effects of guidelines have been demonstrated (5, 6), many studies have reported that guideline-prescribed care was not universally introduced into clinical management of NMIBC (5, 7, 8), especially the use of intravesical adjuvant therapy (8–10).

The knowledge, attitude, and behavior of urologists have a great impact on the process from guideline to practice (11). However, currently, there are insufficient studies on variations in quality of bladder cancer care, and limited attention has been paid to barriers that urologists meet with in clinical practice. In this study, we collected responses of urologists with the following aims: i) to identify the knowledge and compliance rate with NMIBC guidelines; ii) to reveal the variation between knowledge and compliance, guideline, and clinical practice; and iii) to find reasons and explanations for non-compliance. To remove the impact of preference of the patient, the compliance with the guidelines of a urologist was defined as the extent to which his clinical suggestion coincided with guideline recommendations (12). It was noted that guidelines did not have any legislative influence or override the responsibility to make decisions.

METHODS

Study Design

Applying convenience and snowball sampling, we conducted an online survey on the knowledge of and compliance with NMIBC guidelines of Chinese urologists from October 2019 to January 2021. The questionnaire link was sent *via* a QR code posted on several national urological specialist forums in China. Meanwhile, we requested respondents to help us disseminate the questionnaire. Information for informed consent included the statement that individual responses would remain anonymous. Respondents who were more than 65 years old or did not give informed consent were excluded. No incentive was offered for participation.

Development of the Questionnaire

An exhaustive online literature review of NMIBC guidelines worldwide was conducted. We had appraised the quality of NMIBC guidelines within the past 5 years and compared the similarities and differences of therapeutic recommendations between guidelines previously (13). Based on this, six core interventions pertaining to the management of NMIBC were formulated, which addressed the initial transurethral resection of bladder tumor (TURBT), second resection, immediate postoperative intravesical instillation of chemotherapy, additional adjuvant intravesical chemotherapy instillations, intravesical bacillus Calmette–Guerin (BCG) immunotherapy, and radical cystectomy. After several rounds of group meetings, the draft was sent to three clinical urological experts and two methodologists for consultation to verify the face validity. Based on the valuable opinions of experts, we deleted the item of "device-assisted chemotherapy" and added the interpretation for "BCG failure." Through these efforts, we enhanced the acceptability and clarity of the questionnaire and also improved its expression to promote understanding.

Finally, the questionnaire consisted of the following six sections: i) demographic characteristics of urologists; ii) attitude toward guidelines in general, where the information of preferred guideline developers were gathered; iii) clinical utilization of chemotherapy and BCG; iv) knowledge of guidelines, in which the knowledge score of recommendations in NMIBC guidelines was assessed by nine items, while each item scored 1 point. Regarding the indications for surgery, given that all of them were extracted from the guidelines, 1 score was divided equally among each option (for example, if there were five indications for a second TURBT, then 0.2 point was given for each indication selected); v) compliance with guidelines, which focused on the strategy of chemotherapy and BCG for patients of each risk classification (low risk, intermediate risk, and high risk); and vi) barriers in clinical practice, which involved the barriers that respondents met in the clinical practice of a second TURBT, BCG, and radical cystectomy (**Appendix 1**). Urologists were asked to use yes/no/unsure or check choices of medical

interventions to indicate whether they thought a medical intervention was recommended by guidelines or whether they would comply with it. Questions about barriers to guideline implementation were multiple choice. When asking about frequency, Likert-scale questions were adopted on a 5-point scale as never, seldom, sometimes, often, and always. Text forms were offered where extra information was needed.

Statistical Analysis

Descriptive statistics were used to summarize the data, where categorical variables were expressed as counts and percentages, and continuous variables were expressed as mean and standard deviation. McNemar's tests were used to compare the knowledge and compliance in each item. To identify characteristic factors associated with knowledge, we performed univariable and multivariable linear regressions. While univariable and multivariable logistic regressions were conducted for factors associated with compliance, Firth's logistic regressions were applied for unbalanced event with potential complete or quasi-complete separation issue. Statistical analyses were performed using SAS software, version 9.4 TS1M6 (SAS Institute Inc., Cary, NC), and a two-sided p-value of ≤0.05 was considered statistically significant.

RESULTS

Characteristics of the Respondents

A total of 814 responses were received, 2 of which were excluded for overage and informed disconsent, respectively. The vast majority of respondents were working in tertiary hospitals (78.45%), had been in practice for more than 15 years (63.18%), and had a master's degree or above (56.28%) (**Table 1**). Although only 10.71% respondents reported having had prior experience in guideline development, most respondents (98.77%) agreed that high-quality guidelines were efficacious in improving healthcare quality, standardizing the clinical procedures and quality assurance. Respondents preferred guidelines developed by the Chinese Medical Association (CMA) (72.78%), European Association of Urology (EAU) (60.96%), and American Urological Association (AUA) (48.77%). Respondents usually referred to an average of 2.78 (± 1.46) NMIBC guidelines.

TABLE 1 | Characteristics of the respondents.

	n	%
Gender		
Male	796	98.03%
Female	16	1.97%
Age		
<40	285	35.10%
40–59	515	63.42%
≥60	12	1.48%
Years of clinical practice		
<15	299	36.82%
15–29	404	49.75%
≥30	109	13.42%
Level of healthcare institute		
3	637	78.45%
2	174	21.43%
1	3	0.37%
Education background		
Doctor	195	24.01%
Master	262	32.27%
Bachelor	345	42.49%
College	10	1.23%
Professional title		
Senior title	233	28.69%
Vice-senior title	299	36.82%
Middle title	119	14.66%
Primary title	61	7.51%
Preferred guideline developer		
Chinese Medical Association (CMA)	591	72.78%
European Association of Urology (EAU)	495	60.96%
American Urological Association (AUA)	396	48.77%
National Comprehensive Cancer Network (NCCN)	298	36.70%
Canadian Urological Association (CUA)	267	32.88%
Chinese Medical Doctor Association (CMDA)	236	29.06%
National Institute for Health and Care Excellence (NICE)	86	10.59%
Chinese Research Hospital Association (CRHA)	51	6.28%
Have participated in guideline development	87	10.71%
Recognized the positive function of guidelines	802	98.77%

Clinical Utilization of Chemotherapy and BCG

There were 79.25% of the respondents who reported that low-risk NMIBC patients agreed to have chemotherapy for immediate course together with maintenance course. Respondents who reported that they "often" or more frequently utilized BCG therapy for intermediate- and high-risk NMIBC patients were only 38.87% and 51.84%, respectively.

For patients from each risk classification, at least 49.32% respondents scheduled induction course of intravesical chemotherapy instillations of 4 to 8 weeks, and 47.62% or more respondents scheduled a maintenance course of 6 to 12 months (**Appendix 2**). As for BCG, many respondents (from 42.80% to 48.14%) scheduled induction course of BCG instillations of 6 to 8 weeks, while a maintenance course of 4 weeks or more was chosen by at least 61.98% respondents. The median length of maintenance installations was 1 year. Moreover, a majority of respondents (from 76.39% to 90.21%) approved of a standard dose of BCG.

Knowledge of Guideline Recommendations

The average knowledge score of respondents was 6.10 ± 1.28 (out of a full score of 9). "The range of initial TURBT" (97.40%) and "whether to perform an immediate postoperative instillation" (96.77%) got the highest correct rate (**Table 2**). However, for low-risk patients, only 3.97% respondents realized that further chemotherapy was not recommended, and 43.08% respondents recognized that BCG immunotherapy was also not recommended.

Compliance With Guideline Recommendations

There were 98.39% of the respondents who tended to utilize further chemotherapy instillations for patients with low-risk

TABLE 2 | Knowledge of and compliance with guideline recommendations.

No.	Recommendations	*N* (%)		*p*-value
		Knowledge	Compliance	
1	The presence of detrusor muscle in the specimen is necessary for the NMIBC patients having had initial TURBT	786 (97.40)	/	/
2	Perform a single postoperative instillation of intravesical chemotherapy after TURBT	780 (96.77)	/	/
	Further chemotherapy instillations			
3	Not recommended for low-risk NMIBC patients	32 (3.97)	13 (1.61)	<0.001
4	Provide induction and maintenance chemotherapy instillations for intermediate-risk NMIBC patients	735 (91.99)	743 (92.99)	0.243
5	Provide induction and maintenance chemotherapy instillations for high-risk NMIBC patients	/	704 (89.00)	/
	BCG immunotherapy			
6	Not recommended for low-risk NMIBC patients	336 (43.08)	307 (39.36)	0.181
7	Provide induction and maintenance chemotherapy instillations for intermediate-risk NMIBC patients	513 (66.97)	518 (67.62)	0.685
8	Provide induction and maintenance chemotherapy instillations for high-risk NMIBC patients	608 (79.89)	614 (80.68)	0.451
9	Indications for a second TURBT			
	After incomplete initial TURBT	640 (79.60)	542 (67.41)	<0.001
	There is no muscular layer in the first resected specimen (except for TaLG/G1 tumor and carcinoma *in situ*)	598 (74.38)	595 (74.00)	0.863
	In T1 tumors	347 (43.16)	357 (44.40)	0.474
	In G3/high-grade tumors (except for CIS)	456 (56.72)	441 (54.85)	0.228
	Pathology analysis results of initial TURBT failed to determine stage or risk grading	485 (60.32)	443 (55.10)	0.001
10	Indications for radical cystectomy			
	High-grade T1 with histological variation (micropapillary, sarcoma, small cell type)	466 (58.54)	398 (50.00)	<0.001
	High-grade T1 with lymphatic vessel infiltration, multiple and/or large high-grade T1, high-grade T1 with bladder/prostate CIS	654 (82.16)	634 (79.65)	0.111
	Pathology analysis results of a second TURBT is still high-grade T1	491 (61.68)	545 (68.47)	<0.001
	High-grade NMIBC with early recurrence within 3 months	532 (66.83)	542 (68.09)	0.440
	NMIBC involving the bladder diverticulum	331 (41.58)	384 (48.24)	<0.001
	High-risk NMIBC patients with BCG failure	590 (74.12)	526 (66.08)	<0.001

NMIBC (**Table 2**). Notably, the difference between the knowledge and compliance was statistically significant in items of further chemotherapy for low-risk patients ($p_{low} < 0.001$). For intermediate-risk patients, although BCG was widely recommended in the guidelines, the compliance rate (67.62%) was much lower than that for chemotherapy (92.99%). More respondents (89.00%) suggested further chemotherapy for high-risk patients even though it was not supported by many guidelines.

Divergence between knowledge rate and compliance rate also existed in some items of surgery indications. In a second TURBT, the compliance rate was relatively lower than knowledge rate for the indication of "after incomplete initial TURBT" (79.60% vs. 67.41%, $p < 0.001$), so did "pathology analysis results of initial TURBT failed to determine stage or risk grading" (60.32% vs. 55.10%, $p < 0.0011$).

Characteristic Factors Associated With Knowledge and Compliance

We used multiple linear regressions to assess characteristic factors associated with knowledge score of the respondents (**Table 3**). Knowledge scores were positively and independently associated with the number of guidelines that respondents usually referred to, and the adjusted coefficient was 0.17 (95% CI, 0.10 to 0.23), $p < 0.001$. Compared with bachelors, masters and PhDs were more likely to gain higher knowledge scores ($B = 0.67$, $p < 0.001$; $B = 0.42$, $p < 0.001$). Besides, respondents from the Middle may gain lower scores than those from the East and the Northeast (we combined the data of respondents from these two regions for regression analysis) ($B = -0.28$, $p = 0.022$).

Univariable and multivariable logistic regressions were performed to identify factors associated with the compliance of the respondent with guideline recommendation (**Tables 4**, **5**). Knowledge of the recommendation was demonstrated to affect the compliance positively ($p < 0.001$). In some items, respondents who consulted more guidelines were more likely to comply with the guidelines, such as further chemotherapy and BCG therapy for patients with intermediate-risk NMIBC (OR = 1.32, $p = 0.047$; OR = 1.22, $p = 0.015$). However, the result of BCG therapy for low-risk patients was reversed (OR = 0.87, $p = 0.034$). Regarding further chemotherapy for intermediate-risk patients (OR = 0.24, $p = 0.033$) and BCG for high-risk patients (OR = 0.36, $p = 0.016$), respondents from the Middle were more likely to gain less score than those from the East and the Northeast.

Barriers in Clinical Practice

There were 70.63% of the respondents who reported that "patients rejected the operation because of risk or side effects" prevented the implementation of a second TURBT in clinical practice, followed by "patients rejected the operation for economic reasons" (50.43%) and "urologist didn't suggest it because of risk or complications" (37.55%) (**Appendix 3**). As data about the frequency of BCG utilization and radical cystectomy in clinical practice had been collected, we excluded respondents who "usually" or "always" implemented them in barrier analysis. For barriers that hindered the implementation of BCG, "drug was not accessible" was the dominant barrier (74.89%). Besides, there were 62.22% respondents who thought "patients rejected the operation for economic reasons" "often", "usually", or "always" hindered the implementation. With regard

TABLE 3 | Multivariable linear regressions of knowledge of guidelines.

Variables	Level	Multivariable	
		Coefficient (95% CI)	*p*-value
Hospital level	Tertiary	0.20 (–0.06, 0.46)	0.128
	Secondary and below	Reference	
Years of practice	11~20	0.12 (–0.16, 0.39)	0.401
	21~30	–0.05 (–0.40, 0.30)	0.786
	≥31	–0.14 (–0.60, 0.32)	0.551
	≤10	Reference	
Education background	PhD	0.67 (0.39, 0.95)	<0.001
	Master	0.67 (0.39, 0.95)	<0.001
	Bachelor or college	Reference	
Professional title	Senior	0.30 (–0.05, 0.66)	0.094
	Vice-senior	0.12 (–0.15, 0.38)	0.391
	Middle and below	Reference	
Having participated in guideline development	Yes	–0.00 (–0.30, 0.29)	0.973
	No	Reference	
Region	The Middle	–0.28 (–0.51, –0.04)	0.022
	The West	–0.10 (–0.33, 0.14)	0.431
	The East and the Northeast	Reference	
Number of guidelines consulted		0.23 (0.07, 0.39)	0.005

CI, confidence interval.

TABLE 4 | Multivariable logistic regression of compliance with recommendation about chemotherapy.

Independent variables	Level	Dependent variables					
		Low-risk patients		Intermediate-risk patients		High-risk patients	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Hospital level	Tertiary	1.93 (0.08, 45.97)	0.684	0.38 (0.13, 1.11)	0.077	1.80 (0.63, 5.15)	0.272
	Secondary and below	Reference					
Years of practice	11~20	0.24 (0.03, 2.21)	0.207	1.77 (0.54, 5.86)	0.348	0.46 (0.16, 1.33)	0.15
	21~30	0.22 (0.02, 3.20)	0.271	0.87 (0.18, 4.23)	0.86	1.24 (0.29, 5.38)	0.773
	≥31	0.20 (0.01, 4.52)	0.31	0.51 (0.07, 3.58)	0.496	1.07 (0.18, 6.51)	0.942
	≤10	Reference					
Education background	PhD	4.42 (0.37, 52.56)	0.239	5.76 (1.30, 25.49)	0.021	2.48 (0.67, 9.16)	0.174
	Master	6.11 (0.65, 57.62)	0.114	1.23 (0.47, 3.21)	0.674	0.94 (0.36, 2.44)	0.901
	Bachelor or college	Reference					
Professional title	Senior	3.39 (0.25, 46.34)	0.361	3.25 (0.61, 17.31)	0.167	2.33 (0.53, 10.23)	0.263
	Vice-senior	0.71 (0.09, 5.50)	0.744	1.28 (0.41, 4.04)	0.671	2.91 (0.98, 8.62)	0.054
	Middle and below	Reference					
Having participated in guideline development	Yes	0.61 (0.11, 3.32)	0.566	0.15 (0.05, 0.47)	0.001	0.62 (0.16, 2.49)	0.503
	No	Reference					
Region	The Middle	1.12 (0.22, 5.60)	0.894	0.24 (0.07, 0.89)	0.033	1.14 (0.38, 3.47)	0.816
	The West	1.96 (0.48, 7.96)	0.347	0.39 (0.10, 1.46)	0.164	0.66 (0.22, 2.01)	0.464
	The East and the Northeast	Reference					
Number of guidelines consulted		1.12 (0.75, 1.68)	0.573	1.32 (1.00, 1.74)	0.047	0.79 (0.60, 1.05)	0.099
Knowledge of this recommendation		57.71 (16.77, 198.60)	<0.001	101.59 (44.85, 230.12)	<0.001	254.97 (104.95, 619.46)	<0.001

to radical cystectomy, 57.73% respondents indicated that "patients rejected it because of decrease in life quality" often or more frequently hindered the implementation.

Besides, studies of similar themes or about determinants of practice were also reviewed to analyze the reason for non-compliance. After reviewing studies about compliance with the guidelines, determinants of practice, and others, we developed a flowchart to incorporate the sequence of compliance and corresponding influencing factors (**Figure 1**) (11, 14, 15).

DISCUSSION

Our findings demonstrated the discrepancy between guideline and practice, inadequate knowledge of guidelines, and deficient compliance with guidelines, all of which were also reported by previous studies (8–10). In the analysis of findings, we found that factors associated with guidelines, individual professionals, patients, organizations, and the environment all contributed to the transition from guideline to practice (11, 14).

TABLE 5 | Multivariable logistic regression of compliance with recommendation about BCG immunotherapy.

Independent variables	Level	Dependent variables					
		Low-risk patients		Intermediate-risk patients		High-risk patients	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Hospital level	Tertiary	0.88 (0.53, 1.47)	0.622	1.54 (0.81, 2.95)	0.19	2.85 (1.33, 6.12)	0.007
	Secondary and below	Reference					
Years of practice	11~20	1.51 (0.87, 2.60)	0.14	1.13 (0.56, 2.27)	0.734	0.77 (0.33, 1.82)	0.559
	21~30	1.08 (0.54, 2.16)	0.82	1.41 (0.58, 3.44)	0.449	0.49 (0.16, 1.50)	0.212
	≥31	1.06 (0.42, 2.67)	0.907	2.01 (0.62, 6.53)	0.243	0.50 (0.12, 2.05)	0.333
	≤10	Reference					
Education background	PhD	1.00 (0.57, 1.74)	0.991	0.92 (0.45, 1.88)	0.827	0.54 (0.21, 1.40)	0.204
	Master	1.30 (0.81, 2.08)	0.271	0.91 (0.50, 1.65)	0.76	0.53 (0.25, 1.10)	0.09
	Bachelor or college	Reference					
Professional title	Senior	0.96 (0.48, 1.92)	0.903	0.56 (0.23, 1.38)	0.206	1.03 (0.34, 3.08)	0.963
	Vice-senior	1.15 (0.69, 1.93)	0.593	0.64 (0.33, 1.26)	0.198	0.91 (0.41, 2.03)	0.813
	Middle and below	Reference					
Having participated in guideline development	Yes	0.67 (0.38, 1.20)	0.183	0.68 (0.33, 1.41)	0.3	1.57 (0.54, 4.50)	0.405
	No	Reference					
Region	The Middle	1.32 (0.82, 2.10)	0.251	0.66 (0.36, 1.20)	0.173	0.36 (0.16, 0.83)	0.016
	The West	1.35 (0.84, 2.17)	0.209	0.72 (0.38, 1.35)	0.301	0.70 (0.29, 1.65)	0.409
	The East and the Northeast	Reference					
Number of guidelines consulted		0.87 (0.77, 0.99)	0.034	1.22 (1.04, 1.43)	0.015	1.20 (0.99, 1.45)	0.068
Knowledge of this recommendation		10.47 (7.22, 15.17)	<0.001	38.01 (24.44, 59.12)	<0.001	77.17 (42.21, 141.08)	<0.001

Guideline Self-Related Factors

Guidelines need to reflect current research (16), and one out of five recommendations could be out of date after 3 years (17). CMA amended and published their guideline last October, while its previous version was published in 2014. Given that it took time for the guideline to disseminate, the CMA guideline that was familiar to respondents when filling in questionnaires might be the 2014 version. From the aspect of timeliness, it could have less credibility compared with the EAU and NCCN guidelines, which were updated every year. After all, these results show an evident preference of respondents for native guideline. A survey conducted in China explored the barriers and enablers for the implementation of guidelines, which reported that 27.3% of their respondents encountered language barriers associated with English guidelines (18). Language might be an important reason for the gap of utilization between guidelines in Chinese and non-native language. Efforts should be devoted to increase readability of guideline for non-native speakers, and to provide translated executive summaries of guidelines could be a solution. Meanwhile, it requires guideline users to build trust in non-native guidelines and improve linguistic proficiency.

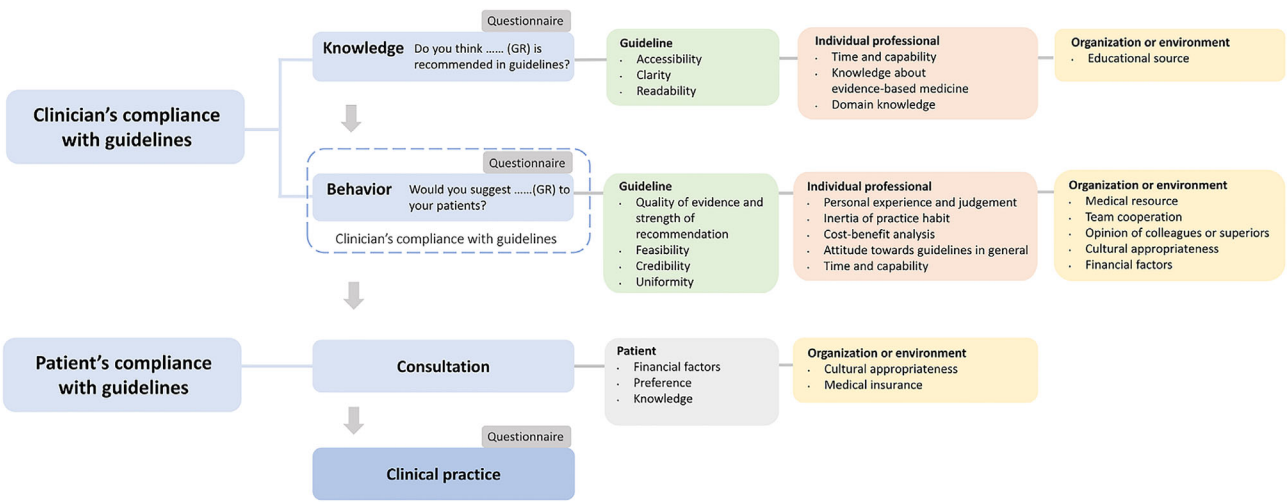

FIGURE 1 | Sequence of clinician's compliance and associated factors.

In addition, the feasibility of the guidelines is crucial for urologists to determine whether to recommend the guidelines to patients in actual clinical situations. As mentioned above, although some respondents knew that "an incomplete resection" was an indication for a second TURBT in the guidelines, they would not comply with it. Two respondents indicated that there was no objective criteria for TURBT to judge whether it was "a complete resection" or not. Hence, urologists were confused with this indication when applying guidelines. To enhance feasibility, guideline developers could give a definition to "complete resection" (for example, there was no residue with the naked eyes during operation or with cystoscopy), or detail the situations for the recommendation, such as "clear visual field in operation" and "the histopathology of the tumour base was negative".

Individual Professional-Related Factors

Knowledge of guideline is an essential prerequisite for compliance (11). For patients with low-risk NMIBC, it was stated in the guidelines that a single postoperative intravesical instillation was considered to be standard and complete treatment (19), while an additional chemotherapy course did not confer benefit but increase the risk of side effects (20). Our results indicated disturbing overuse of further intravesical instillations for low-risk patients, which reveals insufficient knowledge. Moreover, we found that respondents who referred to more guidelines were more likely to comply with guideline recommendations about the utilization of BCG for low-risk patients. Reminding urologists of the need to devote more time in acquiring guideline recommendations is essential. On the other hand, educational source about guidelines needs to be developed to benefit more urologists.

We got feedback from the respondents that the capability of communication with patients is of vital importance in clinical practice, especially when persuading patients to agree to have a second TURBT, or eschew further chemotherapy installations for low-risk NMIBC. Maintaining a harmonious relationship between patients and clinicians based on mutual trust and communication was essential (12).

Furthermore, the capability and training of urologists determined whether they could implement the recommendation in practice. In the study of Witjes et al., only 17.3% of intermediate-risk patients received BCG therapy in their group, which conformed to our results (10). Under the premise of the high toxicity and treatment cessation rate of BCG, one of the various barriers to its utilization was the lack of experience and confidence in the prevention and management of BCG-associated adverse events. As the management of BCG toxicity has been expounded in the EAU guideline (19) and practical recommendations for the prevention and management of adverse events had been acknowledged, these efforts might increase knowledge and finally contribute to clinical practice.

Organization- or Environment-Related Factors

In our study, the lack of drug accessibility was the primary barrier to the utilization of BCG. Not only some respondents reported no local supply of BCG, but also the requirements for the transportation and storage of BCG are strict, which presents a challenge to the ability of a hospital to source the medicine. Actually, BCG shortage was a global problem, especially comparing with increasing demands (21). Desouky indicated that the ongoing clinical trials using BCG against COVID-19 could aggravate the shortage and influence our urology practice (22). Under the circumstances, both the AUA and NCCN provided strategies for urologists to help mitigate the conflicts, such as using chemotherapy as a substitute in intermediate-risk NMIBC patients when BCG was second-line therapy (20, 23).

Patient-Related Factors

A survey conducted in Europe also reported wide overuse of additional adjuvant intravesical chemotherapy instillations for low-risk NMIBC patients (8). For patients without supporting medical knowledge, it was firmly believed that chemotherapy was an essential measure for the management of all tumors. If the tumor recurs or progresses, the urologist would be severely blamed by patients and their relatives for his "negative" attitude. Hence, medical education for patients was necessary to improve their knowledge of disease management, especially their knowledge of and confidence in the guidelines. At this point, some guidelines have included patients in their targeted users, or a specially designed patient version has been published.

Meanwhile, the financial resources of patients also influenced the implementation of recommendations. The price of BCG was much higher than chemotherapy, but it was included into the scope of reimbursement only in a few provinces of China. Our study showed that a second TURBT and radical cystectomy were also limited by the financial resource of patients. For instance, patients need to pay the cost of operation, postoperative urine collection bag, skin protection materials, and others. However, BCG therapy could achieve cost savings by decreasing the risks of local recurrence and its attendant treatments (24). A cost-effectiveness study found that the implementation of BCG intravesical therapy decreased costs by $3,900 per 5-year recurrence-free interval compared with the total costs attributable to recurrences (25).

Strengths and Limitations

This is the first nationwide survey on the compliance of urologists with NMIBC guidelines in China, and it reveals the reasons for non-compliance in detail. The questionnaire development and distribution were carried out under the guidance of methodological experts. Through detailed analysis, it provided reliable information for guideline developers to improve the guideline implementation. However, this study still has some limitations that have to be considered. Recall bias might exist as the results were based on the subjective feedback of urologists, especially in the self-reported barriers to guideline implementation. Respondents might overstate the impact of patients and reduce their own responsibilities in non-compliance. Meanwhile, the results might be affected by the sample size of the questionnaire as well as the unknown response rate.

CONCLUSIONS

This survey revealed that although the vast majority of urologists acknowledged the positive effects of guidelines, the knowledge of and compliance with some recommendations in NMIBC guidelines were still not high enough. It highlights the overuse of further intravesical instillations for low-risk NMIBC patients and the underuse of BCG for intermediate-risk and high-risk patients. Guideline factors (lack of readability and feasibility), individual professional factors (insufficient knowledge, lack of capability and training), organization or environment factors (lack of educational and medical resources), and patient factors (preference and financial sources) jointly resulted in the non-compliance.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Wuhan University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Study concept and design: Y-HJ, D-QW, and X-TZ. Acquisition of data: Y-XS, Y-YW, S-YY, LY, and B-HL. Analysis and interpretation of data: QH, D-QW, and XH. Drafting of the manuscript: D-QW, QH, and Y-HJ. Critical revision of the manuscript for important intellectual content: XH, Y-HJ, and T-ZL. Obtaining funding: Y-HJ and X-TZ. Administrative, technical, or material support: LY and X-TZ. Supervision: T-ZL and X-TZ. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Comprehensive Analysis of N6-Methylandenosine-Related Long Non-Coding RNAs Signature in Prognosis and Tumor Microenvironment of Bladder Cancer

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To investigate the role of N6-methyladenosine (m6A)- related long non-coding RNAs (lncRNAs) in bladder cancer (BC). 50 m6A-related lncRNAs were screened out and were correlated with prognosis from BC samples in The Cancer Genome Atlas (TCGA). The lncRNAs were subdivided into cluster 1 and cluster 2 with consensus cluster analysis, and it was found that lncRNAs in cluster 2 were associated with poor prognosis and increased PD-L1 expression. Gene set enrichment analysis (GSEA) revealed tumor-related pathways in cluster 2. Through least absolute shrinkage and selection operator (LASSO) Cox regression analysis, univariate and multivariate Cox regression, and ROC analyses, 14 prognostic lncRNAs were selected and used to construct the m6A-related lncRNA prognostic signature (m6A-LPS), furthermore, that m6A-LPS was as a valuable independent prognostic factor. Interestingly, the m6A-LPS risk score was positively correlated with the immune score, PD-L1 expression, and the infiltration of immune cell subtypes in BC. SNHG16, a member of the high-risk group based on m6A-LPS, was highly expressed in BC tissues and cell lines and interfered with siRNA resulted in suppressed proliferation, migration, and invasion *in vitro*. Our study illustrates the role of m6A-related lncRNAs in BC. The m6A-LPS may be an important regulatory target of the tumor microenvironment (TME) in BC.

Keywords: bladder cancer, N6-methyladenosine, prognostic signature, immune infiltration, long non-coding RNA

INTRODUCTION

Bladder cancer (BC) is the 10th most common cancer globally (1) and is characterized by high morbidity and mortality rates (2). Most BCs are urothelial carcinomas, of which approximately 75% are non-muscle-invasive and 25% are muscle-invasive or metastatic cancers (3). Non-muscle-invasive bladder cancer (NMIBC) has a high recurrence rate after resection and can develop into muscle-invasive bladder cancer (MIBC). MIBC has a poor prognosis and often recurs after the first resection (4). Although some advanced urinary assays could detect the early-stage bladder cancer leading to

early treatment, the approach to predict the prognosis of bladder cancer and help clinical decision making is lacking and unsatisfied (5, 6). Despite the application of targeted therapy, immunotherapy, and antibody-drug conjugates for the treatment of advanced BC, the objective response rate (ORR) is still low (7). The immune checkpoint inhibitors (ICIs) treatments are currently applied during or flowing platinum-based chemotherapy, and also as the first-line therapy for the platinum ineligible metastatic bladder cancer (mBC). About 15-30% mBC is the response to ICIs treatment (8), indicating that the majority of patients do not benefit from ICIs. It is still a challenge to identify patients who will benefit from ICIs treatment and to improve the ORR. In addition, it is of great significance to predict and differentiate tumors of different subtypes based on tumor heterogeneity to individualize treatment approaches and improve the therapeutic effect while reducing the application of unnecessary treatment. However, the existing pathology-based diagnosis strategy does not reflect the intrinsic characteristics of the tumor. For example, even in the same pathological stage and grade of BC, the biological behavior of the tumor may be completely different (9). Although the molecular typing system can better reflect the intrinsic characteristics of BC than traditional pathological typing, it is not viable for clinical application because of its high cost and complexity. Therefore, the search for new economically viable and effective prediction methods is of great significance for improving the prognosis of BC and realizing individualized treatment.

N6-methyladenosine (m6A) is one of the most important modifications of RNA and mediates more than 60% of RNA methylation events (10). In general, m6A methylation plays an important role in the regulation of gene expression by modulating RNA splicing, translation efficiency, and mRNA stability (11). m6A modification is dynamic and reversible and consists of methyltransferase complexes (m6A "writers"), demethylases (m6A "erasers"), and m6A-binding proteins (m6A "readers") (12, 13). In recent years, scientists have reported that m6A modification can affect tumor initiation and progression in various cancers, including BC (14). For example, METTL3 accelerates the maturation of pri-miR-221/222 in an m6A-dependent manner and promotes the proliferation of BC cells (14).

Long non-coding RNAs (lncRNAs) are non-protein-coding RNA molecules more than 200 nucleotides in length (15). In patient tumors, the abnormal expression of lncRNAs is a common biological phenomenon that is closely related to patient prognosis. For instance, the lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was initially discovered in lung cancer, and the overexpression of MALAT1 is associated with a poor prognosis among patients with lung cancer (16). LncRNA LBCS inhibited the SOX2, stem cell factor, to suppress the self-renewal and enhance chemosensitivity in bladder cancer. In addition, lncRNA DANCR, BLACAT2, and LNMAT2 promote the lymphatic metastasis of bladder cancer (17–20). However, little research has been conducted on whether the expression of lncRNAs through m6A modifications affects the occurrence and development of BC. Therefore, understanding the role of lncRNAs modulated by m6A in BC helps identify biomarkers that can be used as meaningful therapeutic targets. However, the relationship between m6A-related lncRNAs and the response of BC to ICIs is still not fully understood, and the interaction between these lncRNAs and the tumor microenvironment (TME) needs to be further explored.

This study aimed to evaluate the correlation between m6A-related lncRNAs and the prognosis of BC. We identified 50 m6A-related lncRNAs related to the prognosis of BC and constructed a prognostic model for BC using bioinformatics methods in The Cancer Genome Atlas (TCGA) dataset. Furthermore, we investigated the possible roles of the selected m6A-related lncRNAs in the progression of BC by gene set enrichment analysis (GSEA) and single-sample gene set enrichment analysis (ssGSEA). In addition, we studied the relationships among these lncRNAs, programmed death-ligand 1 (PD-L1) expression, and the TME to determine whether the signature could be used to predict the response of BC patients to ICIs and immunotherapy.

MATERIALS AND METHODS

Raw Data and m6A Gene Acquisition

We acquired BC transcriptome data and clinical data from TCGA (https://portal.gdc.cancer.gov/). The data for 434 BC samples, including 414 tumor samples and 19 normal samples, were downloaded. Nineteen normal samples were the paracancerous tissues of 19 of the 414 BC patients in the TCGA dataset. After excluding 6 duplicate samples and 2 samples without complete survival time and status, 406 of 414 tumor samples had complete clinical information and were included for further study. The clinical characteristics of these 406 patients with BC are shown in **Table 1**. According to previously published literature, we identified 23 m6A RNA methylation regulators based on TCGA BC dataset gene expression information. These 23 m6A regulators comprised 8 writers (METTL3, METTL14, METTL16, KIA1499, WTAP, RBM15, RBM15B, and ZC3H13), 2 erasers (ALKBH5 and FTO), and 13 readers (YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2, HNRNPA2B1, HNRNPC, IGFBP1, IGFBP2, IGFBP3, FMR1, LRPPRC, and RBMX) (21).

Bioinformatic Analysis

Pearson correlation ($|\text{Pearson R}| > 0.4$ and $P < 0.01$) was implemented to identify lncRNAs correlated with m6A genes to identify m6A-related lncRNAs. Based on clinical survival data from TCGA datasets, we further identified m6A-related lncRNAs related to prognosis ($p<0.01$).

Patients with BC were divided into different subgroups by the ConsensusClusterPlus package (22) according to the expression of m6A-related lncRNAs related to prognosis. GSEA was performed, and hallmark gene sets were downloaded from the Molecular Signatures Database (MSigDB) (23); next, the enrichment results were selected based on the normalized enrichment score (NES) and a false discovery rate (FDR) value

TABLE 1 | The clinical characteristics of BC patients in the TCGA datasets.

Characteristic	Type	n	Proportion (%)
Age	≤65	160	39.41%
	>65	246	60.59%
Gender	Female	107	26.35%
	Male	299	73.65%
Grade	High grade	383	94.33%
	Low grade	20	4.93%
	Unknown	3	0.74%
TNM stage (stage)	Stage I	2	0.49%
	Stage II	129	31.77%
	Stage III	140	34.49%
	Stage IV	133	32.76%
	Unknown	2	0.49%
T stage	T0	1	0.25%
	T1	3	0.74%
	T2	118	29.06%
	T3	193	47.54%
	T4	58	14.29%
	Unknown	33	8.13%
M stage	M0	195	48.03%
	M1	11	2.71%
	Unknown	200	49.26%
N stage	N0	236	58.13%
	N1	46	11.33%
	N2	75	18.47%
	N3	7	1.72%
	Unknown	42	10.34%

<0.05 to explain the survival differences between the different BC subtypes.

Next, the least absolute shrinkage and selection operator (LASSO) Cox regression was performed with the R package "glmnet" to construct the m6A-related lncRNA prognostic signature (m6A-LPS) (24). The risk score was calculated with the following equation: Risk score = $\sum_{i=1}^{n} Coefi \times xi$ ($Coefi$ represents the coefficients, and xi represents the fragments per kilobase of transcript per million mapped reads (FPKM) value of 50 m6A-related lncRNAs related to prognosis). According to this equation, we calculated the risk score for each BC patient. Then, taking the median risk score as the cutoff point, the patients were divided into a high-risk group and a low-risk group.

To estimate the prognostic capability of the risk score for 1-, 3-, and 5-year overall survival (OS), receiver operating characteristic (ROC) curves were carried out to assess the area under the curve (AUC) values (25). To determine the independent prognostic factors in BC, we analyzed the prognostic relationships between the risk score and gender, age, WHO grade, and stage based on univariate and multivariate Cox regression analyses. We then constructed a nomogram with integrated prognostic features to predict the 1-, 3-, and 5-year survival rates of patients with BC.

The immune score and stromal score of each sample were calculated in TCGA datasets using Estimation of Stromal and Immune cells in Malignant Tumor tissues using Expression data (ESTIMATE) algorithm via the "estimate" R package (26). For each sample, we quantified 22 types of infiltrating immune cells with cell type identification by estimating relative subsets of RNA transcripts (CIBERSORT) (27) and then selected samples with a CIBERSORT P-value <0.05 for subsequent analysis to compare differences in immune infiltration levels between the subgroups grouped by cluster subtype and risk score. In addition, we used ssGSEA to investigate the differences in immune cell infiltration and immune function between the high-risk and low-risk groups (28).

Samples and Quantitative Real-Time Polymerase Chain Reaction

Thirteen nonneoplastic and neoplastic samples from patients who underwent surgical treatments were obtained from the Renmin Hospital of Wuhan University in 2019. To evaluate SNHG16 expression, we extracted total RNA from clinical BC samples using RNA TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was carried out using the iScript cDNA Synthesis Kit from Bio-Rad. qRT-PCR was conducted on a LightCycler® 480 Real-Time PCR System (Roche, Germany). The relative lncRNA expression levels were calculated using the 2^-ΔΔCt method with GAPDH as an endogenous control. The primer sequences used were as follows: SNHG16 forward 5'- CC TCGTGCCAGTAACTCTGAAATC-3' and reverse 5'- CT CAGTCACCAGAAACGAAACAC-3'; GAPDH forward 5'- G GAAGCTTGTCATCAATGGAAATC-3' and reverse 5'- TG ATGACCCTTTTGGCTCCC-3'.

Cell Culture

The human BC cell lines 5637 and T24 and the immortalized human bladder epithelial cell line SV-HUC-1 were purchased from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences, Shanghai Institute of Biochemistry and Cell Biology. 5637 and T24 cells were cultured in RPMI 1640, and SV-HUC-1 cells were cultured in F12K medium with 10%

(Gibco, Grand Island, NY, USA) and penicillin-streptomycin (100 U/mL) at 37°C with 5% CO_2.

siRNA Transfection of Cell Line

5637 cells were transfected with small-interfering RNA (siRNA), and a negative control siRNA (Sangon Biotech, Shanghai, China) using 5637 was transfected with siRNA (Sangon Biotech, Shanghai, China) using Lipofectamine 2000 transfection reagent (Invitrogen). The siRNA sequences were 5'-UGGAAGAGCCUAAGAGGAATT-3' (sense) and 5'-UUCCUCUUAGGGCUCUUCCATT-3' (antisense). A negative control (NC) was transfected simultaneously with the siRNA. The NC sequences were 5'-UUCUCCGAACGUGUCACGUTT-3' (sense) and 5'-ACGUGCCACGUUCGGAGAATT -3' (antisense). The knockdown of SNHG16 expression following siRNA transfection was verified by qRT-PCR.

CCK-8 Cell Viability, Scratch Wound Healing, and Transwell Invasion Assays

A total of 5637 cells were seeded into 96-well and 6-well plates. When the cells reached 30–50% confluence, they were transfected with either SNHG16 siRNA or the NC. At 0, 1, 2, 3, and 4 days after transfection, CCK-8 solution was added at a ratio of 1:9 to the 96-well plates, and optical density (OD) values at a wavelength of 450 nm were measured by a microplate reader. Transwell invasion assays were performed to detect the invasive ability of the cells. The upper layer of the transwell chamber was coated with Matrigel (BD Biosciences, USA); the cells were seeded into the upper chamber with medium without FBS, and the lower chamber was filled with complete culture medium. The cells were cultured in a humidified 5% CO2 incubator at 37°C for 24 hours. The invaded cells were fixed in 4% paraformaldehyde for 5 minutes and then stained with 0.3% crystal violet (Solarbio, China). The invading cells or migrating cells were counted under a light microscope.

Statistical Analysis

Data were analyzed using R software version 4.0.2. Correlations between m6A genes and lncRNAs were analyzed by Pearson's test, and a *P*-value <0.01 was considered significant. The log-rank test was used to compare Kaplan-Meier survival curves between various subgroups, including cluster subtypes and low- and high-risk subgroups. Group comparisons for continuous data were accomplished by Student's t-test and one-way ANOVA. Categorical variables were compared using chi-square tests. Univariate and multivariate Cox regression analyses were used to validate the independent prognostic factors for BC. A P <0.05 was considered significant.

RESULTS

Acquisition of m6A-Related LncRNAs in BC

We identified 14,086 lncRNAs in the TCGA datasets by analyzing the annotated files downloaded from the GENCODE website. Then, 23 m6A gene expression matrices were extracted from the TCGA datasets. LncRNAs associated with one or more m6A genes (|Pearson R| > 0.4 and P < 0.01) were defined as m6A-related lncRNAs. Through Pearson's correlation analysis, 414 lncRNAs were found to be significantly related to the m6A genes in the TCGA datasets. The specific process of this study is shown in **Figure 1A**. We obtained the coexpression network of m6A genes and m6A-related genes, as shown in **Figure 1B**. Next, according to the clinical survival information from the TCGA datasets, we screened 50 m6A-related lncRNAs with a significant

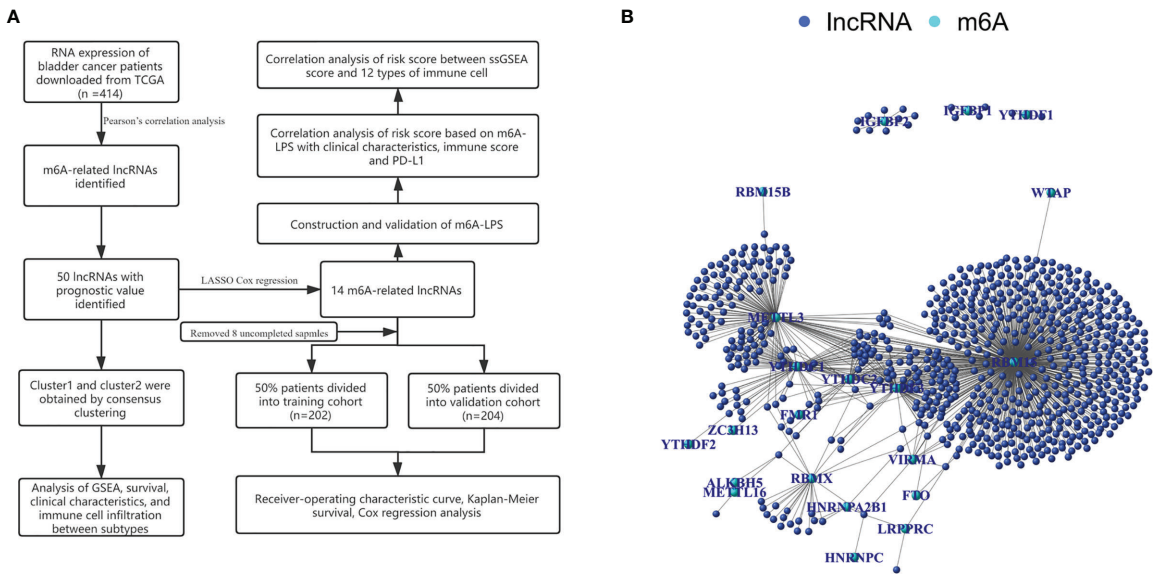

FIGURE 1 | **(A)** Research flow chart. **(B)** The coexpression network of m6A-related genes and m6A-related lncRNAs.

prognostic value from the 411 m6A-related lncRNAs ($P < 0.01$). The risk ratio forest plot revealed that there were 40 low-risk and 10 high-risk m6A-related lncRNAs (**Figure 2A** and **Supplementary Table S1**). **Figures 2B, C** show the differences in the expression of m6A-related lncRNAs between 406 tumor tissues and 19 tumor-adjacent normal pairs from the TCGA dataset. Except for RAP2C-AS1, AC025280.1, ATP1B3-AS1, AC087286.2, AC012568.1, AC007686.3 and BDNF-AS, the other m6A-related lncRNAs were highly expressed in BC tissues ($P < 0.05$). These results suggested that these 50 m6A-related lncRNAs possess essential biological roles in BC development.

Consensus Clustering Showed That m6A-Related LncRNAs Were Closely Associated With the Clinical Characteristics and Survival Rates of Patients With BC

To verify the prognostic value of m6A-related lncRNAs, we conducted consensus clustering analysis on 406 BC samples using 50 m6A-related lncRNAs. Based on the similarity of the expression of m6A-related lncRNAs, we determined that K = 2 had the best clustering stability (K = 2 to 9). Then, we divided 406 BC samples into two subgroups, cluster 1 and cluster 2, for further analysis (**Figure 3A**). **Figure 3B** shows that most of the 50 m6A-related lncRNAs were highly expressed in cluster 2. The clinicopathological features between the two subtypes were then compared (**Figure 3B**). Cluster 2 mainly contained BC patients aged over 65 years ($P < 0.05$) and was preferentially associated with stage III–IV ($P < 0.01$). As shown in **Figure 3C**, the OS of cluster 2 was shorter than that of cluster 1 ($P = 0.008$), which indicated that the prognosis of the cluster 2 subgroup was poor compared with that of the cluster 1 subgroup. The similarity of the expression levels of 50 m6A-related lncRNAs in cluster 1 and cluster 2 samples showed that the expression level of these lncRNAs was closely related to the heterogeneity of the 406 BC patients in the TCGA dataset. Our results also confirmed the prognostic value of 50 m6A-related lncRNAs.

GSEA of Cluster Subtypes and the Association Between PD-L1 and m6A-Related LncRNAs

GSEA was performed to explore the potential regulatory mechanisms that led to the differences in survival rates between the cluster 1 and cluster 2 subgroups. The FDR of the enrichment analysis of cluster 1 was not <0.05. The enrichment

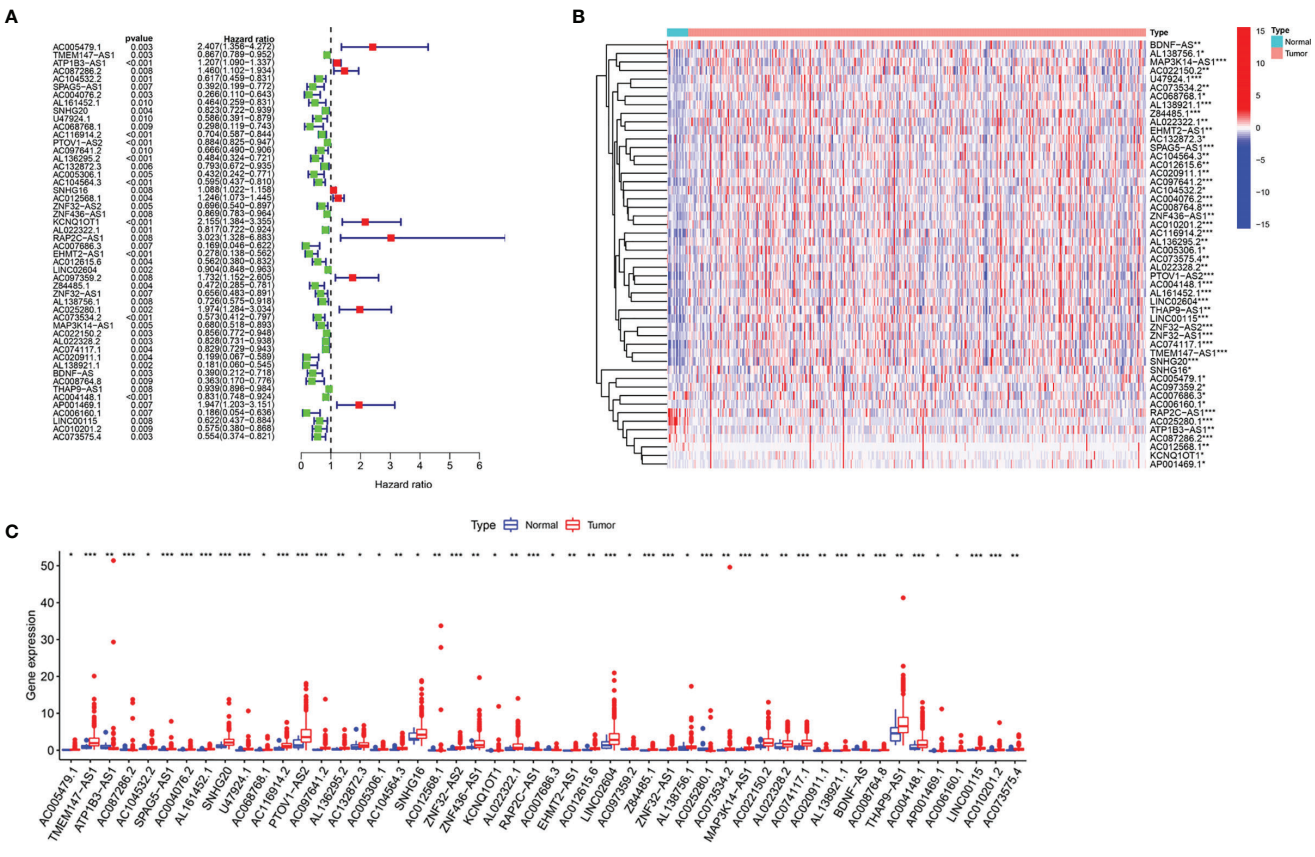

FIGURE 2 | The differences in the expression of m6A-related lncRNAs in the TCGA datasets. **(A)** Forest plot of the hazard ratio for m6A-related lncRNAs. **(B, C)** Heatmap **(B)** and expression levels **(C)** of 50 m6A-related lncRNAs in tumor and adjacent normal tissues. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

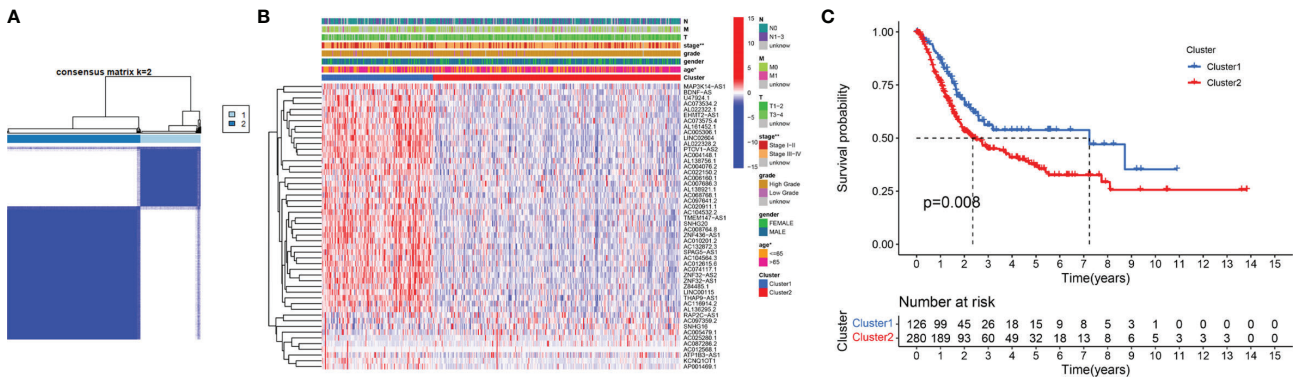

FIGURE 3 | Differential clinicopathological features and survival rates of BC patients in cluster 1 and cluster 2. **(A)** Consensus clustering matrix for k = 2. **(B)** The heatmap of cluster 1 and cluster 2 along with clinicopathological features that included gender, age, grade, T stage, N stage, M stage, and stage. **(C)** Kaplan-Meier curves of OS. *$P < 0.05$ and **$P < 0.01$.

analysis results of the cluster 2 subgroup revealed that allograft rejection, apical junctions, apoptosis, coagulation, complement, epithelial-mesenchymal transformation, delayed estrogen response, hypoxia, IL2-STAT5 signaling, IL6-JAK-STAT3 signaling, the inflammatory response, the interferon γ response, KRAS signaling, mTORC1 signaling, the P53 pathway and TNFA signaling through NFkB (FDR <0.05; **Figure 4A**) were enriched. Based on the correlation between the IL6/JAK/STAT3 signaling pathway and PD-L1 (29, 30), we analyzed the differential expression of PD-L1 between cluster 1 and cluster 2. Compared with that in cluster 1, PD-L1 expression in cluster 2 was significantly higher (p <0.001; **Figure 4B**). To explore the potential relationship between PD-L1 and m6A-related lncRNAs, we further studied the expression correlation between PD-L1 and m6A-related lncRNAs. The results showed that PD-L1 expression was correlated with that of most of the m6A-related lncRNAs, while the m6A-related lncRNAs showed a significant positive correlation with each other (**Figure 4C**). To analyze the potential relationship between m6A-related lncRNAs and the TME of BC, the level of immunocyte infiltration in cluster 1 and cluster 2 was evaluated, and we found that the infiltration level of naïve CD4 T cells, regulatory T cells (Tregs), memory B cells and plasma cells was relatively high in cluster 1, and that of neutrophils and activated memory CD4 T cells was relatively high in cluster 2 (**Figure 4D**). Hence, the IL6/JAK/STAT3 signaling pathway might be implicated in the distinct TME of cluster 1/2.

Construction and Validation of the m6A-LPS

To accurately predict the clinical prognosis of m6A-related lncRNAs in BC patients, the 50 lncRNAs related to m6A genes were analyzed by LASSO Cox regression. We identified the m6A-LPS, which accounted for the expression levels of the 14 m6A-related lncRNAs and their respective coefficients (**Figures 5A–C**). Then, based on the expression of the 14 lncRNAs and their risk coefficients, we calculated the risk score for each patient in the TCGA training cohort and the validation cohort. We divided patients in the TCGA training and validation cohorts into high-risk and low-risk groups based on the median risk score. **Figures 5D, E** show the risk scores, OS rates, vital statuses, and expression profiles of m6A-LPS components in the TCGA training and validation cohorts. The heatmap indicated that AC005479.1, ATP1B3-AS1, SNHG16, AC025280.1, and AP001469.1 were highly expressed in the high-risk group. In the TCGA training and validation cohorts, there was a significant difference in OS rates between the low-risk and high-risk groups ($p < 0.0001$, **Figures 5F, G**).

We analyzed the ROC curves for 1-year, 3-year, and 5-year survival by comparing AUC values to evaluate the prognostic accuracy of the 14 risk lncRNAs. We found that the 1-, 3- and 5-year AUC values of the 14-lncRNA signature were 0.757, 0.746, and 0.740, respectively, in the training cohort (**Figure 5H**) and 0.667, 0.639, and 0.653, respectively, in the validation cohort (**Figure 5I**). The AUC values indicated that the 14-lncRNA signature has a good ability to differentiate prognosis in BC patients.

m6A-LPS Was an Independent Prognostic Factor in Patients With BC

To confirm whether the risk score based on m6A-LPS can be used as an independent prognostic factor for patients with BC, we performed univariate and multivariate Cox regression analyses on the TCGA training and validation cohorts. Univariate analysis showed that in the TCGA training cohort, age (P =0.10), stage (P <0.001), and risk score (P <0.001) correlated with the OS rate. The multivariate Cox regression analysis of the training cohort showed that age (P = 0.005), staging ($P < 0.001$), and risk score ($P < 0.001$) were still closely related to the OS rate (**Figures 6A, B**). Similarly, we conducted the same analysis for the TCGA validation cohort, and the results showed that age, stage, and risk score were independent prognostic factors (**Figures 6C, D**). We found that the risk score based on the m6A-LPS could be used as an independent prognostic factor for the TCGA training and validation cohorts, indicating that the m6A-LPS may have potential value in the process of assessing clinical prognosis.

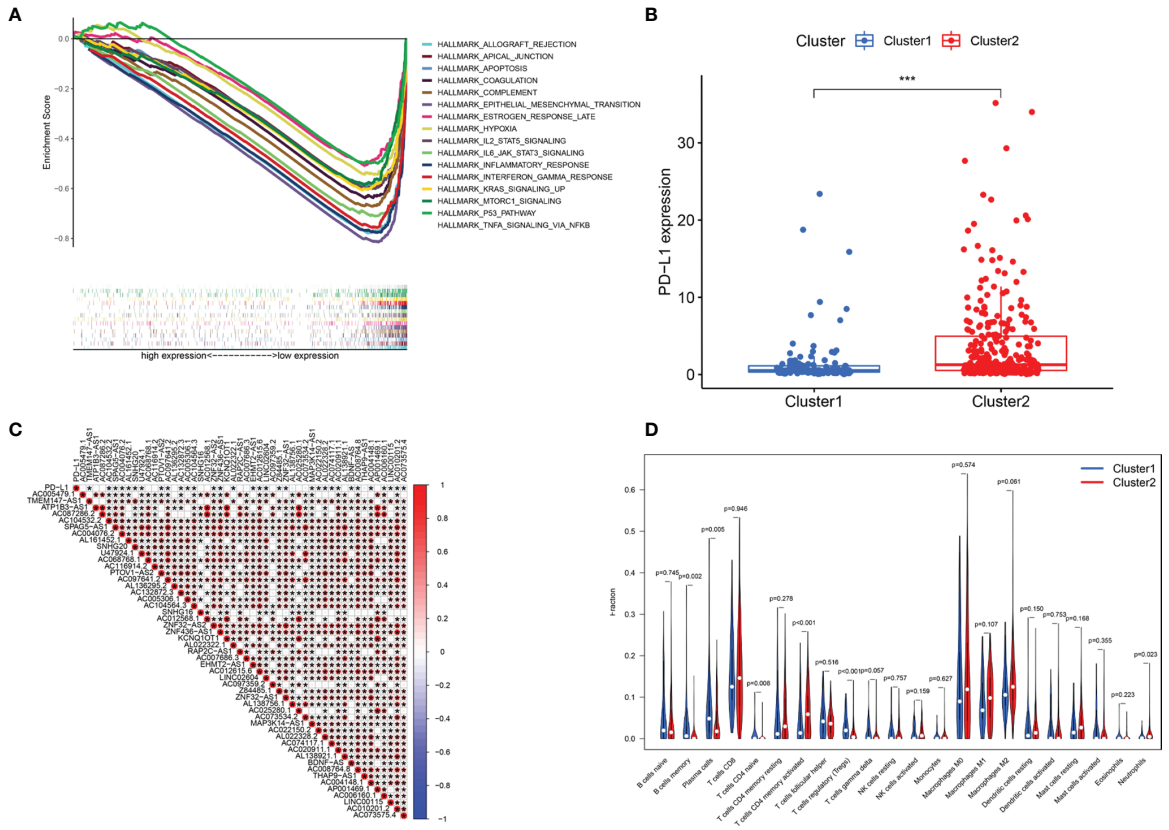

FIGURE 4 | Association of PD-L1 with m6A-related lncRNAs and the landscape of immune cell infiltration in BC cluster 1 and cluster 2. **(A)** GSEA indicating that tumor hallmarks were enriched in cluster 2. **(B)** PD-L1 expression in BC cluster 1 and cluster 2. **(C)** The correlation of PD-L1 with m6A-related lncRNAs. **(D)** The infiltration levels of 22 immune cell types in BC cluster 1 and cluster 2. *$P < 0.05$; ***$P < 0.001$.

In addition, we assembled a nomogram based on risk score, age, sex, grade, and stage to predict the prognosis of BC (**Figure 6E**). In our nomogram, the calibration curve has a good ability to predict the prognosis of 1-year, 3-year, and 5-year OS rates (**Figure 6F**). The AUC of the nomogram in the ROC curve is 0.731, 0.721, and 0.745 at 1-, 3-, and 5-year, respectively, which is more accurate than risk score, age, sex, grade, and stage (**Figures 6G–I**). In general, the risk score signature we established can provide the most useful and accurate guidance for predicting the survival of these prognostic indicators.

Analysis of the Correlation Between the Risk Score and Clinical Characteristics of BC Patients

We further estimated the relationship between the risk score and clinical characteristics in TCGA datasets. The heatmap in **Figure 7A** shows the differences in the expression levels of 14 m6A-related lncRNAs in the TCGA datasets in the low-risk and high-risk groups. There were significant differences in T stage ($P < 0.01$), stage ($P < 0.001$), cluster subtype ($P < 0.001$), grade ($P < 0.01$) and immune score ($P < 0.001$) between the low-risk group and the high-risk group. The risk scores of the T3-4, late-stage, cluster 2, high grade, and high immune score groups were significantly higher than those of the T1-2, early-stage, cluster 1, low grade, and low immune score groups (**Figures 7B–F**). In addition, we found that PD-L1 expression in patients with high-risk scores was significantly higher than that in patients with low-risk scores ($P < 0.001$; **Figure 7G**). These findings revealed that the risk score based on m6A-LPS was significantly associated with subtype, grade, T stage, stage, immune score, and PD-L1 expression in BC patients, also suggesting a potential correlation between the risk score and the TME of BC.

Immune Cell Enrichment Analysis

To explore the potential correlation between the risk score and the TME of BC, we further studied the correlation between the risk score and immune infiltrating cells and immune functions by ssGSEA in TCGA datasets. We found significant differences in 16 types of immune cells between the high- and low-risk groups (**Figure 8A**): activated dendritic cells (aDCs); macrophages; neutrophils; NK cells; immature dendritic cells (iDCs); dendritic cells (DCs); mast cells; B cells; CD8+ T cells; plasmacytoid dendritic cells (pDCs); T helper cells; T helper 2

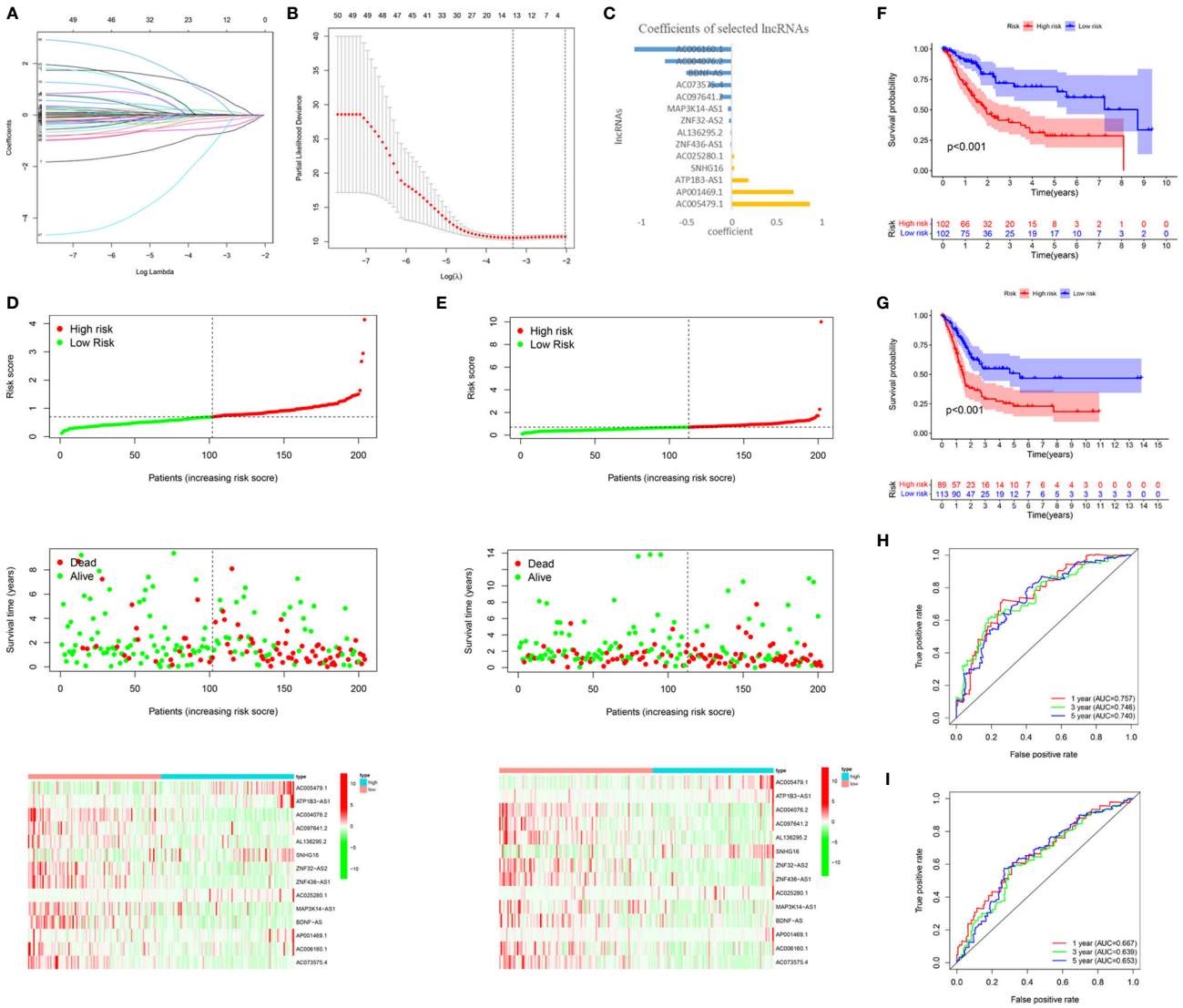

FIGURE 5 | Construction and validation of the m6A-LPS in the TCGA cohort. **(A–C)** LASSO regression analysis was performed, and the minimum criteria **(A, B)** and coefficients **(C)** were calculated. **(D, E)** Distribution of risk scores, OS rates, and OS statuses and the heatmap of the 14 prognostic m6A-related lncRNAs in the TCGA training cohort **(D)** and the TCGA validation cohort **(E)**. **(F, G)** Kaplan-Meier curves of OS rates for bladder cancer patients based on the risk scores in the TCGA training cohort **(F)** and the TCGA validation cohort **(G)**. **(H, I)** Time-dependent ROC curves for the TCGA training cohort **(H)** and the TCGA validation cohort **(I)**.

(Th2) cells; tumor-infiltrating lymphocytes (TILs); Tregs; T helper 1 (Th1) cells; and follicular T helper (Tfh) cells. In addition, the scores of immune functions, such as cytolytic activity, HLA, cytokine, and cytokine receptor (CCR), T cell coinhibition, T cell costimulation, APC inhibition, APC stimulation, the type I IFN response, and checkpoints, were significantly higher in the high-risk group, implying that these immune functions are more active in patients in the high-risk group (**Figure 8B**). These results indicated that the risk score based on m6A-LPS was closely related to immune infiltrating cells and immune functions in BC, which further suggested that m6A-LPS had a potential influence on the TME of BC.

Effect of m6A-LPS on Immune Cell Infiltration

To investigate whether m6A-LPS can regulate the TME of BC, we analyzed the correlation between the risk score and the infiltration of 12 types of immune cells in the TCGA datasets. We found that the infiltration levels of memory B cells, activated dendritic cells, plasma cells, follicular helper T cells, and gamma delta Tregs were negatively correlated with the risk score ($P < 0.05$, **Figures 9A–G**). Moreover, the infiltration levels of eosinophils, resting memory CD4 T cells, M0 macrophages, M1 macrophages, and activated mast cells were positively correlated with the risk score ($P < 0.05$, **Figures 9H–L**).

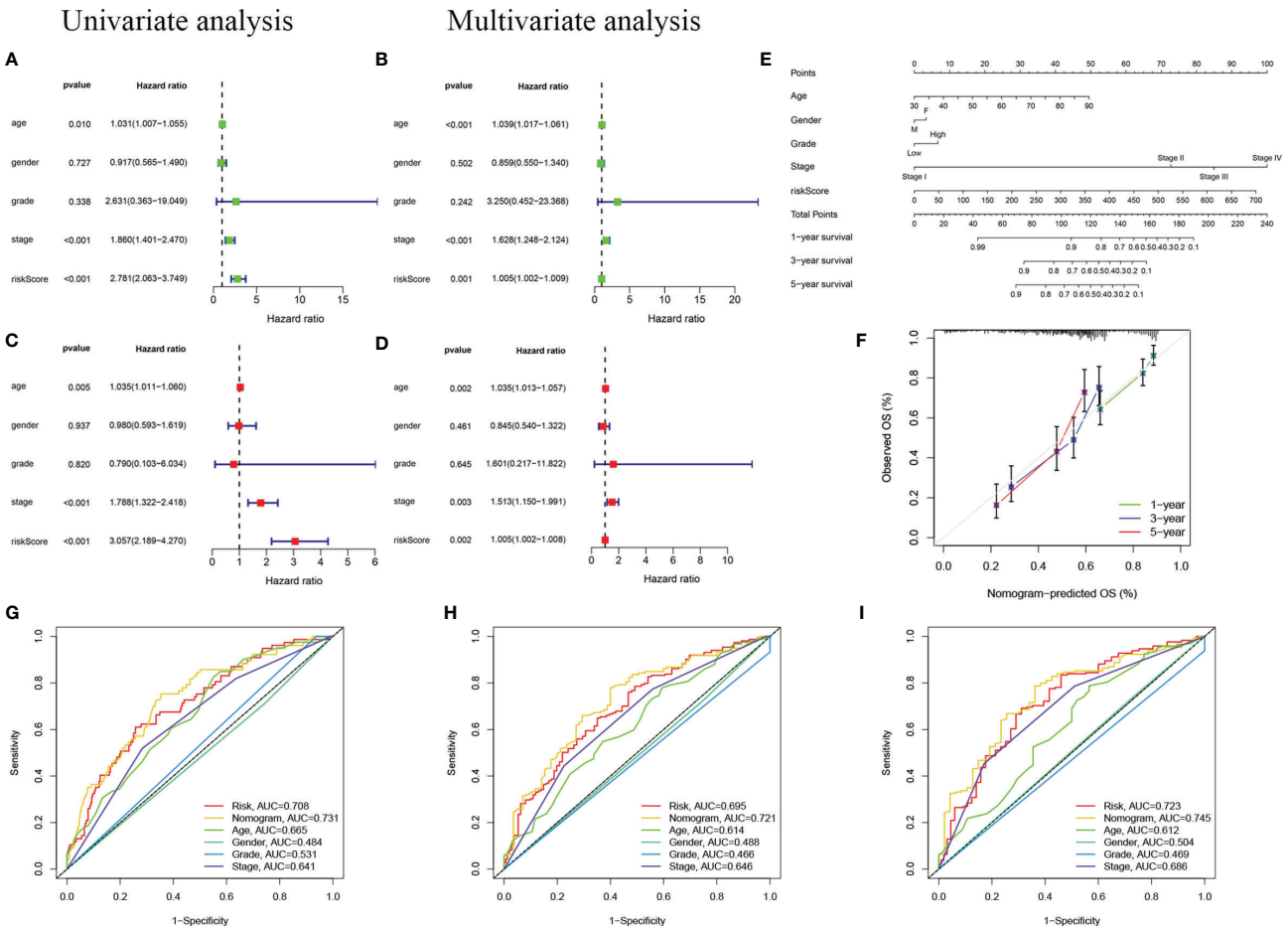

FIGURE 6 | Independent prognostic analysis. **(A, B)** Univariate **(A)** and multivariate **(B)** Cox regression analyses in the TCGA training cohort. **(C, D)** Univariate **(C)** and multivariate **(D)** Cox regression analyses in the TCGA validation cohort. **(E)** Nomogram based on age, gender, grade, stage, and risk score. **(F)** Calibration plot of a nomogram to predict 1-,3- and 5-year OS in the TCGA cohort. The x-axis represents the nomogram-estimated probabilities and the y-axis represents the observed probabilities. **(G–I)** Discriminatory accuracy for predicting OS assessed by receiver operator characteristics (ROC) analysis calculating the time-independent area under the curves (AUCs).1- **(G)**, 3- **(H)**, and 5-year **(I)** in the TCGA cohort.

Our results indicate that m6A-LPS has pivotal regulatory effects on the TME in BC patients.

SNHG16 Was Highly Expressed in BC Samples and Affected BC Cell Proliferation, Migration, and Invasion

For verification, we detected SNHG16 expression in 13 samples of BC patient tissues, including 8 BC tissues and 5 paracancerous tissues, by RT-qPCR assay. Our results showed that SNHG16 was upregulated in BC tissues compared with normal tissues (P <0.05, **Figure 10A**). The result was consistent with those in **Figure 2B**. In addition, we detected SNHG16 expression in the immortalized human bladder epithelial cell line SV-HUC-1 and the BC cell lines 5637 and T24 by qPCR. SNHG16 expression was higher in 5637 and T24 cells than in SV-HUC-1 cells, and its expression level in 5637 cells was higher than that in T24 cells (P <0.05, **Figure 10B**). As shown in **Figure 7A**, SNHG16 was highly expressed in the high-risk group, which suggested that SNHG16 promotes the development of BC. Therefore, the role of SNHG16 in BC was further explored. We then selected 5637 cells with higher SNHG16 expression levels for further study. After knocking down SNHG16 by siRNA (**Figure 10C**), our study showed that the proliferation, migration, and invasion ability of BC cell lines with low SNHG16 expression were decreased compared with that in cells with high SNHG16 expression (P <0.05, **Figures 10D–F**); however, the proliferation ability of 5637 cells with SNHG16 knockdown was still higher than that of SV-HUC-1 cells (P <0.05, **Figure 10D**). Our results showed that SNGH16, which is relatively highly expressed in the high-risk group, is highly expressed in BC tissues and can promote BC cell proliferation, migration, and invasion.

DISCUSSION

Because of its poor prognosis and high recurrence rate, BC is considered a serious threat to health (31). It is critical to identify

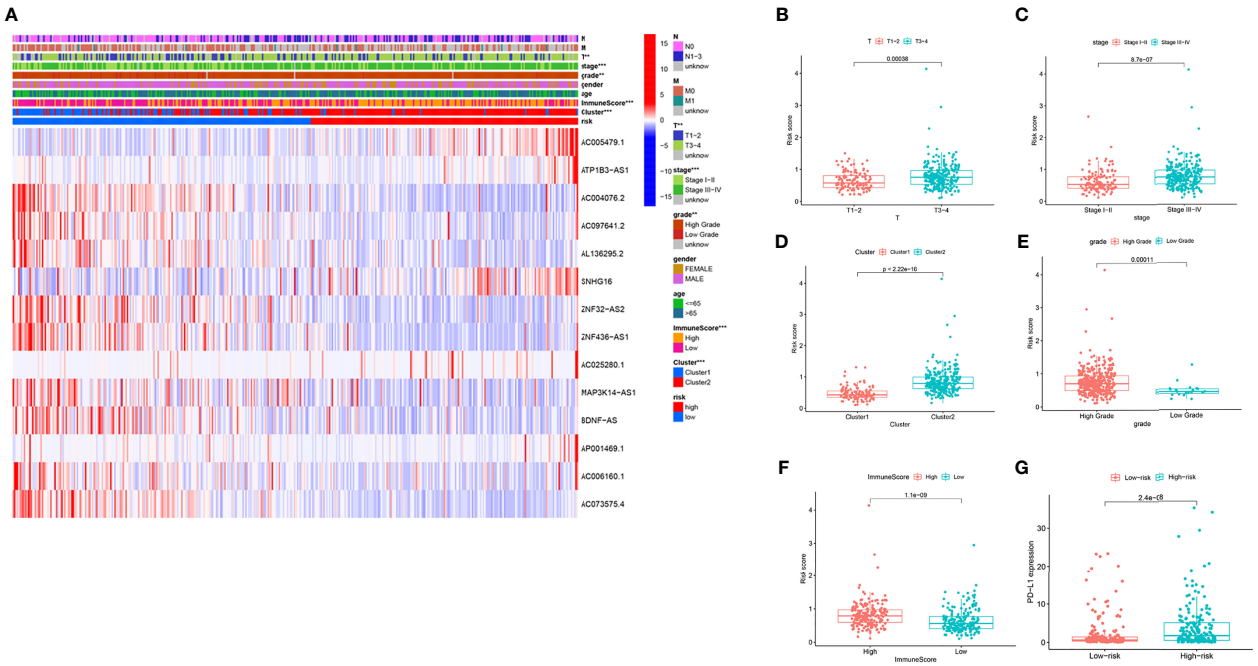

FIGURE 7 | The prognostic risk score was correlated with clinicopathological features and immune score in the TCGA datasets. **(A)** Heatmap and clinicopathologic features for the high- and low-risk groups. **(B–F)** Distribution of risk scores stratified by T stage **(B)**, stage **(C)**, cluster **(D)**, grade **(E)** and, immune score **(F)**. **(G)** PD-L1 expression by risk score group in the TCGA datasets. $^{**}P < 0.01$; $^{***}P < 0.001$.

specific and sensitive biomarkers to improve the prognosis of patients with BC. Recently, the expression levels and mechanisms of mRNAs and miRNAs have been extensively studied in BC, and many of these factors have been identified as prognostic markers in BC patients (32–36). With further research on BC, the role of lncRNAs has been gradually uncovered and is helpful for comprehensively understanding the characteristics of the occurrence and development of BC (37–39). Many studies have confirmed that m6A methylation may play regulatory roles in the tumorigenesis, development, and progression of BC (40, 41); however, it is not clear how m6A modification plays a role in BC progression in a lncRNA-dependent manner.

We first identified 50 m6A-related lncRNAs with prognostic value in TCGA datasets and then performed consensus

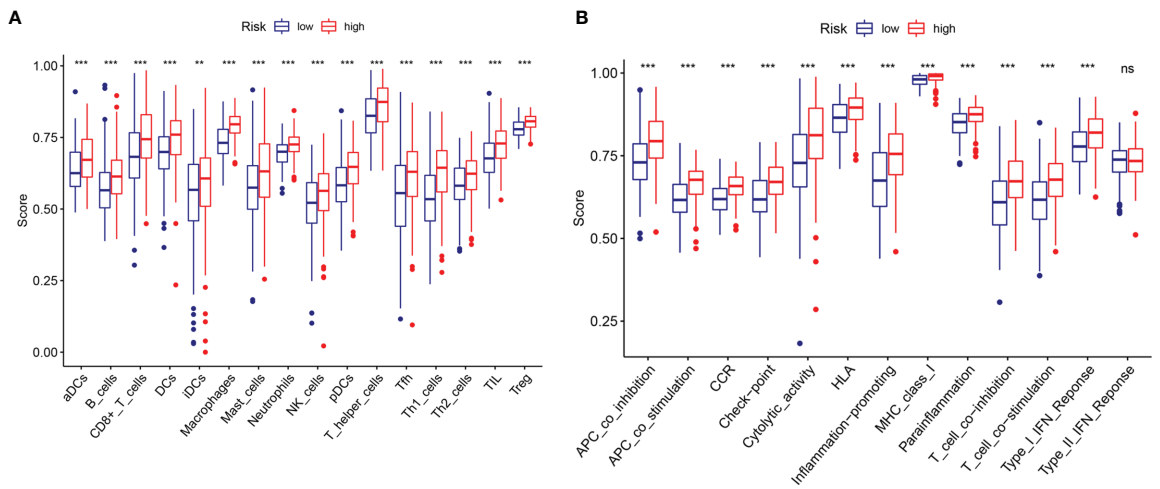

FIGURE 8 | Comparison of the ssGSEA scores between the high- and low-risk groups. The scores for 16 immune cells **(A)** and 13 immune-related functions **(B)** are displayed in boxplots. ns, not significant. $^{**}P < 0.01$; $^{***}P < 0.001$.

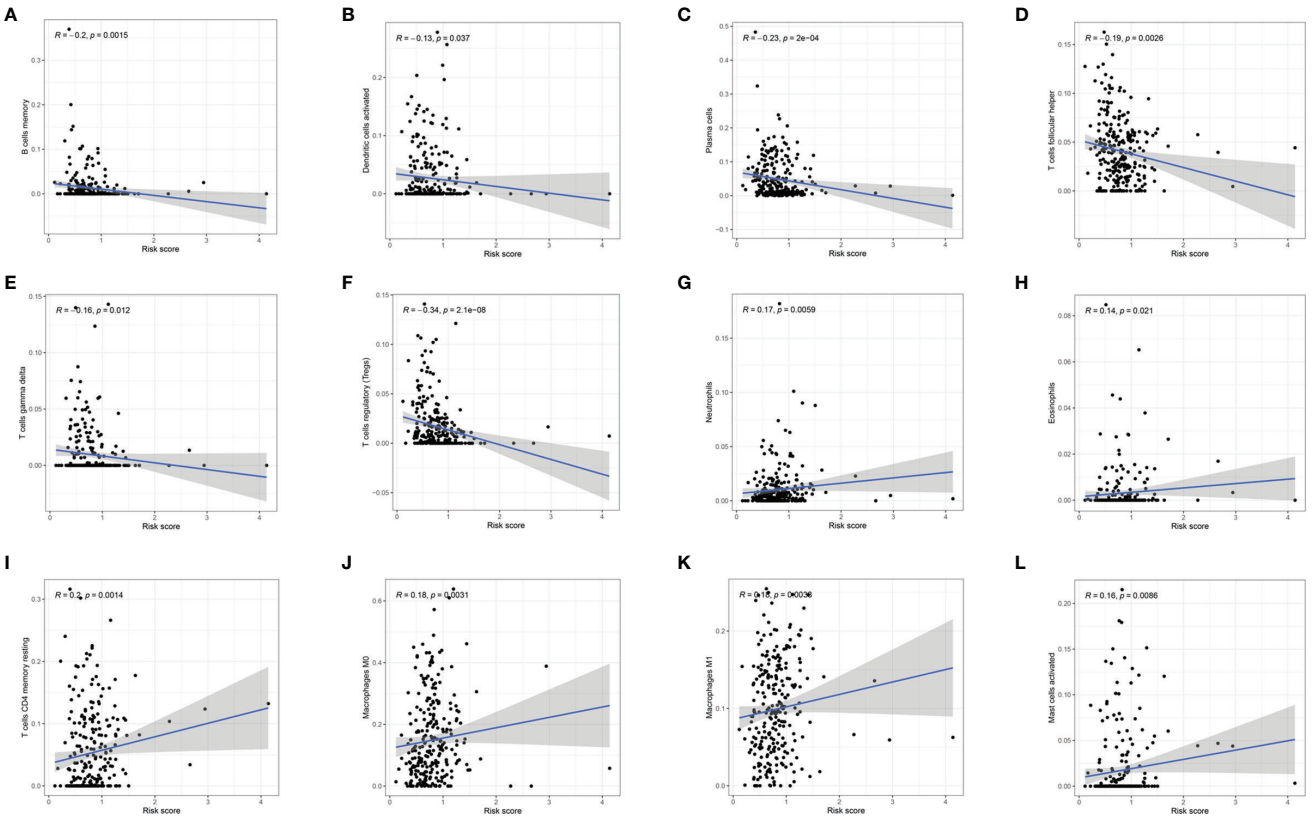

FIGURE 9 | Relationships between the risk score and the infiltration levels of 12 immune cell types. **(A–F)** The infiltration levels of memory B cells **(A)**, aDCs **(B)**, plasma cells **(C)**, Tfh cells **(D)**, gamma delta T cells **(E)**, and Tregs **(F)** were negatively correlated with risk score. **(G–L)** The infiltration levels of neutrophils **(G)**, eosinophils **(H)**, resting memory CD4 T cells **(I)**, M0 macrophages **(J)**, M1 macrophages **(K)**, and activated mast cells **(L)** were positively correlated with the risk score.

clustering on these m6A-related lncRNAs to identify two BC subtypes, namely, cluster 1 and cluster 2. Compared with the cluster 1 subgroup, the prognosis of the cluster 2 subgroup was worse ($p = 0.008$). Next, we performed GSEA on cluster 1 and cluster 2 to identify the possible major functional pathways. GSEA results showed that hallmarks of malignant tumors (including allograft rejection, apical junction, apoptosis, coagulation, complement, epithelial-mesenchymal transition, late estrogen response, hypoxia, IL2/STAT5 signaling, IL6/JAK/STAT3 signaling, inflammatory response, interferon gamma response, KRAS signaling, mTORC1 signaling, P53 pathway and TNFA signaling *via* NFKB) were significantly enriched in cluster 2, in which the IL6/JAK/STAT3 signaling pathway was shown to induce the expression of PD-1 and/or PD-L1 (29, 30). There are more and more studies on the regulation of PD-L1 in BC. For example, recent studies have shown that WDR5 activates PD-L1 expression through H3K4me3 modification, and OICR-9429 targets WDR5 to inhibit immune escape by blocking PD-L1 (42). PD-L1 has been shown to weaken the antitumor immune response and plays an important role in a variety of tumors (43). Then, we analyzed PD-L1 expression in cluster 1 and cluster 2 and found that PD-L1 was significantly more highly expressed in cluster 2 ($p < 0.001$), which indicates a correlation between PD-L1 and the IL6/JAK/STAT3 signaling pathway. The analysis revealed an unexpected correlation between PD-L1 expression and m6A-related lncRNAs, suggesting that these specific m6A-related lncRNAs are involved in the development of BC and the expression of PD-L1 in BC through the IL6/JAK/STAT3 signaling pathway. In our study, different subtypes were related to the prognosis and some clinicopathological characteristics of BC and were tightly associated with the expression of PD-L1 and the degree of immune cell infiltration.

Through subtype cluster analysis, we found that the 50 m6A-related lncRNAs we selected were indeed closely related to the prognosis of patients with BC, and this result may provide useful information for predicting the prognosis of BC patients in the clinic. However, more convenient and effective predictors are required; thus, we constructed m6A-LPS. Fourteen prognostic risk factors were obtained by LASSO Cox regression analysis. After correlation calculation, the risk score of each patient was obtained. Among these risk factors, lncRNA SNHG16 showed significantly upregulated expression in hepatocellular carcinoma, lung cancer, colorectal cancer, glioma, and other tumor tissues

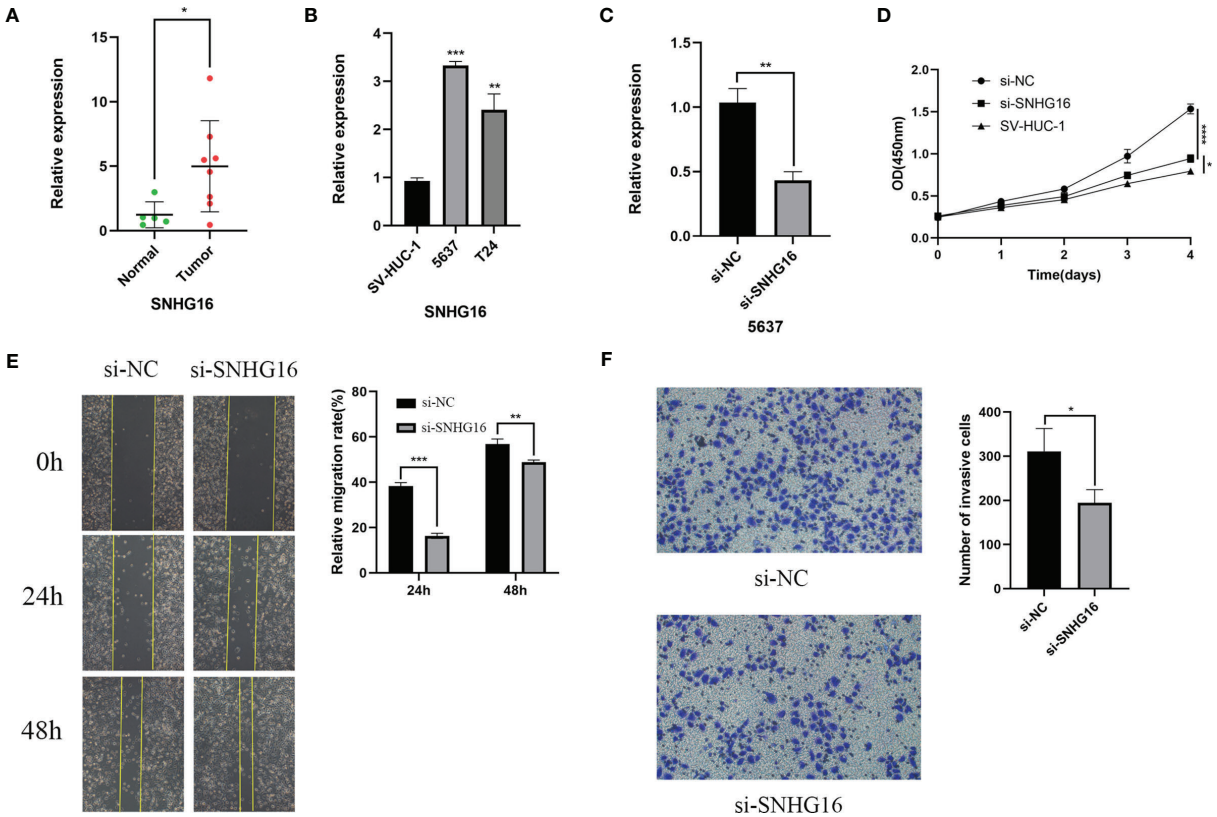

FIGURE 10 | SNHG16 was highly expressed in BC tissues and cell lines and interfered with siRNA resulted in suppressed proliferation, migration, and invasion *in vitro*. **(A)**The SNHG16 expression in BC tissues and paracancerous tissues. **(B)** SNHG16 expression level was assessed in the immortalized human bladder epithelial cell line SV-HUC-1 and the BC cell lines 5637 and T24 by qPCR. **(C)** The efficiency of siRNA knockdown of SNHG16 was assessed in the BC cell line 5637 by qPCR. **(D)** Cell proliferation was determined in SV-HUC-1 cells and 5637 cells transfected with si-SNHG16 or si-NC by CCK-8 assay on days 0, 1, 2, 3, and 4. **(E)** Scratch wound assay: The scratch was imaged after transfection, and cell mobility was measured by determining the extent of wound closure at 24 and 48 h (× 100). **(F)** The invasion of 5637 cells transfected with si-SNHG16 or si-NC was evaluated by transwell assay with Matrigel (× 200). **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001.

and cell lines, and the overexpression of SNHG16 often indicates a poor prognosis (44–47). Subsequently, we proved that SNHG16 expression in BC tissues was higher than that in adjacent tissues, and high SNHG16 expression could promote BC cell proliferation and migration, which is consistent with the results of a previous study (48). Our results showed that SNHG16 was highly expressed in the high-risk group, which was consistent with the above analysis.

Then, the patients were subdivided into high- and low-risk groups according to the median value in the training and validation TCGA cohorts. We found that patients in the high-risk group had a poorer prognosis in both cohorts. Based on univariate and multivariate Cox regression analyses, we confirmed that the risk score was an independent prognostic factor for patients with BC. Within the nomogram integrating independent prognostic factors, the risk score made a significant contribution and performed better than other factors in survival prediction. The risk score was also related to the T stage, different cluster subtypes, grade, immune score, and PD-L1 expression level. We also observed that cluster 2 had a higher risk score and that PD-L1 expression was higher in the high-risk group, which was consistent with the findings of previous studies. Moreover, the immune score of the high-risk group was higher than that of the low-risk group. We further explored the relationship between the risk score and immune cells and immune functions by ssGSEA. The results showed that the expression levels of 16 types of immune cells in the high-risk group were significantly higher than those in the low-risk group. Rosenberg et al. (49) found that the expression of PD-L1 in BC was more closely associated with immune infiltrating cells than with tumor cells. In addition, some studies have shown that responses to ICIs are often associated with high tumor mutation loads, resulting in the production of a large number of mutated neoantigens that support extensive immune cell infiltration and render tumors sensitive to ICIs (50, 51). The results of the above analysis may explain the correlation between the high expression of PD-L1 and the high immune cell infiltration in the high-risk group in BC and indicate that ICIs are more effective for patients in the high-risk group.

Immune cell infiltration in the TME may affect the survival, metastasis, and therapeutic resistance of tumor patients (52–54).

Previous studies have shown that neutrophils in tumor immune cell infiltration are associated with a poor prognosis (50, 55), while BC patients with high levels of Treg infiltration have a better prognosis (56). In this study, the risk score was negatively correlated with activated dendritic cells, plasma cells, memory B cells, Tfh cells, gamma delta T cells, and Tregs and positively correlated with neutrophils, eosinophils, resting memory CD4 T cells, M0 macrophages, M1 macrophages, and activated mast cells. Taken together, these results suggest that the 14 risk m6A-related lncRNAs in BC were associated with the infiltration of these immune cell subtypes. Our results were consistent with previous results.

However, our results need to be further verified before they can be used to assist the clinical treatment of patients with BC, such as determining whether it is appropriate to apply PD-L1 blockade therapy and to finally achieve the purpose of predicting and improving the prognosis of patients with BC. Moreover, the different immune characteristics of the subtypes established by the m6A-LPS classification system indicate that patients from different subgroups may have different responses to immunotherapy. More studies are needed to confirm the accuracy of the prediction system and whether personalized treatment for these subtypes can improve patient prognosis.

CONCLUSIONS

In summary, this study methodically assessed the prognostic value of m6A-related lncRNAs in BC from TCGA datasets and studied the correlation between these lncRNAs and PD-L1 expression and the TME. m6A-related lncRNAs might be involved in the regulation of PD-L1 expression and the TME of BC in synergy with the IL6/JAK/STAT3 signaling pathway. The risk score based on the m6A-LPS was shown to be an independent prognostic indicator and could effectively predict the prognosis of patients with BC. Therefore, identifying m6A-related lncRNAs related to the signaling pathway affecting the TME and further studying their regulatory mechanism may provide a promising target for improving the responsiveness of BC to immunotherapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Ethics Committee of Renmin Hospital of Wuhan University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KC designed this study. KC, SZ, WY, and YX performed the data analysis, plotted the figures, and wrote the manuscript. JX revised the content. JG and FC were responsible for confirming the authenticity of the data. All the authors read and approved the final manuscript.

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Prospective Clinical Trial of the Oncologic Outcomes and Safety of Extraperitoneal Laparoscopic Extended Retroperitoneal Lymph Node Dissection at Time of Nephroureterectomy for Upper Tract Urothelial Carcinoma

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Purpose: To determine the safety and feasibility of extraperitoneal laparoscopic extended lymph node dissection (LND) at the time of extraperitoneal laparoscopic radical nephroureterectomy (RNU).

Materials and Methods: Between May 2018 and March 2019, 39 patients with upper tract urothelial carcinoma (UTUC) received extraperitoneal laparoscopic RNU and concomitant extraperitoneal laparoscopic extended LND. All patients were followed for at least 90 days. Perioperative and pathological data including nodal status and perioperative complications were collected.

Results: Among all 39 patients, 12 patients had pT1, 6 had pT2, 20 had pT3 disease, and 1 had T4 disease. The median (range) lymph node count was 10 (5–22), with 8 patients having pathologically proven lymph node metastasis. The median (range) operating time was 225 (165–430) min, and the median estimated blood loss was 200 (60–800) ml. The median postoperative hemoglobin loss was 1.6 (0–4.2) g/dl. The median (range) postoperative hospital stays were 6 (3–26) days. Overall, 7 patients experienced minor (Clavien Grade I–II) postoperative complications with five patients having Clavien Grade I complications and two patients having Clavien Grade II complications. No major complication (Clavien grade III–IV) occurred. With a median follow-up of 38 months, a total of 8 patients (20.5%) developed local or distant recurrence and no regional LNs where extended LND were performed had recurrence.

Conclusions: The present prospective study demonstrated that extraperitoneal laparoscopic extended LND during extraperitoneal laparoscopic RNU for UTUC is a

feasible and safe procedure which provides minimal invasion, rapid recovery, and potentially lower risk of regional LN recurrence. Larger prospective clinical trials with survival endpoints are needed to further determine its potential therapeutic benefits.

Trial Registration: ClinicalTrials.gov identifier NCT 03544437 www.clinicaltrials.gov.

Keywords: lymph node dissection, oncologic outcomes, upper urinary tract, laparoscopy, urothelial carcinoma

INTRODUCTION

Upper tract urothelial carcinoma (UTUC) is an uncommon but aggressive disease that accounts for approximately 5% to 10% of all urothelial neoplasms (1, 2). Overall, 60% of UTUCs are invasive at presentation, of which 15%–30% have involvement of regional lymph nodes at the time of surgery (2). Lymph node metastasis is a powerful prognostic predictor for survival outcomes in UTUC (3). It has been demonstrated that regular imaging is limited in accurately assessing nodal involvement in UTUC (4). However, standardized dissection templates of lymphadenectomy in UTUC have been inadequately defined and often left at the surgeon's discretion in practice, which hinders the most accurate staging and brings great variation among studies exploring its benefits. Moreover, since vast lymphatic drainage routes and great variation of lymphatic spread exist in UTUC of different primary sites, extended lymphadenectomy may also be needed for eradication of all potential metastasis (5, 6). Thus, thorough and extensive lymphadenectomy represents the most accurate staging method.

Previous mapping studies have demonstrated a lymphatic metastatic pattern of UTUC, suggesting more extended dissection (5, 6). More recently, Martin et al. showed frequent lymphatic metastases to the paracaval and para-aortic regions from middle and distal ureteral tumors and downward migration to the common or internal iliac regions from those of the mid-ureter (7). Also, it is noteworthy that a secondary involvement of interaortocaval nodes can be omitted guided by frozen section analysis during surgery in the absence of lymphadenopathy. These results established refined regional lymph node dissection (LND) boundaries and suggested the need of more extensive and thorough LND.

While extended pelvic lymph node dissection of urothelial carcinoma in the bladder has been a fundamental component of surgical intervention, providing accurate staging and possible survival benefits (8), extended lymph node dissection in UTUC, despite a histologically similar phenotype, remains far less studied. To date, few prospective studies have explored the concomitant extended LND in UTUCs regarding its safety and potential clinical benefit. One prospective study has offered a preliminary baseline of modified retroperitoneal lymph node dissection during nephroureterectomy (9). The procedure was performed by heterogeneous techniques including an open and minimally invasive way in a transperitoneal route. Since emerging evidence has advocated modified laparoscopic retroperitoneal templates of regional LND over strengths such as decreased risks of ileus and peritoneal tumor seeding (10, 11), it is extrapolated that extended LND of UTUC may also be achieved with merits of reliable safety and comparable oncologic efficacy based on this technique. Therefore, we were prompted to determine the feasibility, safety, and potential impacts on disease outcomes of extraperitoneal laparoscopic extended retroperitoneal LND during nephroureterectomy for UTUC. In this study, a prospectively recruited cohort of patients underwent preoperatively specified extended retroperitoneal LND during laparoscopic radical nephroureterectomy (RNU).

PATIENTS AND METHODS

The present single-arm study was designed to prospectively recruit patients diagnosed with UTUC for laparoscopic extraperitoneal RNU with bladder cuff excision with concomitant laparoscopic extended retroperitoneal LND conducted by two surgeons of the urology department at Renji Hospital. The clinical trial was approved by the Institutional Ethics Committee of Shanghai Renji Hospital, School of Medicine, Shanghai Jiao Tong University. All patients were informed of the study in details and had their written informed consent. A total of 39 patients were included between May 2018 and March 2019.

Eligibility criteria were as follows:

1. 15–80-year-old patients clinically diagnosed with upper tract urothelial carcinoma;
2. patients who had no distant metastasis;
3. patients who had an Eastern Cooperative Oncology Group performance (ECGO) status of 0 to 2;
4. patients who were expected to receive radical nephroureterectomy.

Exclusion criteria included previous abdominal surgeries, contraindications to laparoscopic surgery (e.g., severe chronic obstructive pulmonary disease), and cT4 disease before surgery.

Outcome Measures

The primary outcome was the perioperative complication rate. Perioperative complications were evaluated up to 90 days after surgery and were graded by Clavien–Dindo classification (12). Secondary outcomes include operating time, estimate blood loss, and length of stay.

Surgical Approach

All patients underwent extraperitoneal laparoscopic RNU with bladder cuff excision with concomitant laparoscopic extended retroperitoneal LND conducted by two surgeons of the urology department at Renji Hospital.

The extraperitoneal laparoscopic RNU and extended retroperitoneal LND were adapted from a previous reported

technique (10). Briefly, the patient was positioned in a modified supine position with the affected side rotated up to 15° and the surgeon stood at the tumor side. As shown in **Supplementary Figure 1**, port A was located 2 cm superior from the anteriosuperior iliac spine for lens. Port B was placed at the umbilicus level alongside the lateral margin of the rectus abdominis, port C was placed at the umbilicus level alongside the anterior axillary line. Additional port D could be placed alongside the lateral margin of the rectus abdominis at the surgeon's discretion.

The anatomical boundaries of the lymph node dissection were defined by the ipsilateral side of UTUC. In patients with right-sided UTUC, the template of dissection consisted of (i) right perihilar lymph nodes, (ii) paracaval lymph nodes, (iii) interaortocaval lymph nodes, and (iv) right pelvic lymph nodes (common, external, and obturator lymph nodes).

In patients with left-sided UTUC, the template of dissection included (i) left perihilar lymph nodes, (ii) para-aortic lymph nodes, and (iii) left pelvic lymph nodes (common, external, and obturator lymph nodes).

For right-sided UTUC patients, lymph nodes including right perihilar lymph nodes, paracaval lymph nodes, interaortocaval lymph nodes, and common iliac lymph nodes were dissected in laparoscopy. The right external and obturator lymph nodes were dissected by open technique *via* a 10–12-cm midline lower abdominal incision. For left-sided UTUC patients, lymph nodes including left perihilar lymph nodes, para-aortic lymph nodes, and common iliac lymph nodes were dissected in laparoscopy. Left external and obturator lymph nodes were dissected by open technique *via* a 10–12-cm midline lower abdominal incision.

Lymph node specimens were sampled "en bloc" with surrounding adipose tissue and were sent for pathological examination as individual packets with the surrounding adipose tissue.

The video clip demonstrating surgical steps and intraoperative views after completion of lymph node dissection is provided in **Supplementary Material**. Patients included in the video were informed of the study and video distribution in details and had their written informed consent.

RESULTS

Baseline demographic characteristics are summarized in **Table 1**. The median (range) age of patients at diagnosis was 67 (42–80) years. According to the tumor location, 23 patients had disease located in the renal pelvis, 3 in the proximal ureter, 6 in the middle ureter, and 7 in the distal ureter.

On pathological examination, 12 patients had pT1 disease, 6 had pT2, 20 had pT3, and 1 had pT4 disease. Low-grade tumors were found in 5 patients with pT1 and high-grade tumors in other 34 patients. Furthermore, 8 patients were found harboring lymph node metastases, which is shown in **Table 2**.

Surgical Outcomes

As shown in **Table 3**, median lymph node harvest was 10 (5-22). The median (range) operating time was 225 (165–430) min, and the median (range) intra-operative blood loss was 200 (60–800) ml. The median hemoglobin loss one day post-surgery was 1.6 (0–4.2) g/dl. The median postoperative hospital stay was 6 (3–26) days. The median (range) follow-up from time of surgery was 90 days.

TABLE 1 | Baseline characteristics of the cohort.

Clinical variables	Total
Case no. (%)	39
Age, years	67 (42–80)
BMI, kg/m^2	22.9 (16.0–34.6)
Side, no.	
Left	21
Right	18
Gender, no.	
Male	25
Female	14
ASA score	
1	3
2	36
Pathological stage, no. (%)	
pTa	
pT1	12 (30.8%)
pT2	6 (15.4%)
pT3	20 (51.3%)
pT4	1 (2.6%)
Pathological grade, no. (%)	
Low grade	5 (12.8%)
High grade	34 (87.2%)
Lymph node status	
pN0	31 (79.5%)
pN1	8 (20.5%)
Location of tumor, no. (%)	
Renal pelvis	23 (59.0%)
Upper ureter	3 (7.7%)
Middle ureter	6 (15.4%)
Distal ureter	7 (17.9%)
Preoperative hydronephrosis, no. (%)	17 (43.6%)

Complications

No injuries to major vessels occurred intraoperatively.

All other postoperative complications that occurred were classified according to the Clavien grading system, as shown in **Table 3**.

A total of seven patients had postoperative complications. Two patients who had chylous lymphatic leak were managed medically with prolonged drainage time. One patient developed thrombus in the left lower limb 1 day postoperation, and one patient had cerebral infarction 3 days after surgery. Two patients experienced prolonged postoperative fever. One patient had severe postoperative vomiting. No severe complications occurred.

Metastatic Patterns of LN

A total of 8 patients with 18 metastatic LNs were identified in the present study. Of all patients with pathologically confirmed lymph node metastasis (LNM), two were clinical N0 stage without enlarged LNs over 1 cm in preoperative contrasted computed tomography while the other six had clinically metastatic LNs. The distribution of metastatic lymph nodes based on location of primary tumors is detailed in

TABLE 2 | Baseline characteristics of patients with lymph node metastasis.

Clinical features	Total
Case no.	8
Age (IQR), years	64 (63–69)
Tumor side, no.	
Left	4
Right	4
Gender, no.	
Male	4
Female	4
Pathological stage, no. (%)	
pT1	1
pT2	1
pT3	5
pT4	1
Pathological grade, no. (%)	
Low grade	0
High grade	8
Location of tumor, no. (%)	
Renal pelvis	6
Upper ureter	0
Middle ureter	2
Distal ureter	0

TABLE 3 | Surgical outcomes of the cohort.

Clinical variable	Median (range)
Total lymph node count	10 (5–22)
Surgical time (min)	225 (165–430)
Blood loss (mL)	200 (60–800)
Hemoglobin loss (g/dL)	1.6 (0–4.2)
Postoperative hospital stays (days)	6 (3-26)
Clavien grading, no. (%)	
I	5
II	2
IIIa and higher	0

Supplementary Table 1 and anatomically illustrated in **Supplementary Figure 2**.

Oncological Outcomes

With a median follow-up of 38 months, a total of 8 patients (8/39, 20.5%) developed local or distant recurrence. The median time to first recurrence was 8 months (range 5–24). All recurrence sites and frequencies include intravesical recurrence (4/10, 40.0%), lung (1/10, 10.0%), osseous sites (2/10, 20%), distant lymph nodes (1/10, 10.0%), psoas major area (1/10, 10.0%), and inguinal lymph node (1/10, 10.0%). The characteristics of recurrent patients are shown in **Table 4**.

TABLE 4 | Baseline characteristics of recurrent patients.

Clinical features	Total
Case no.	8
Age (IQR), years	65.5 (60.5–73.5)
Tumor side, no.	
Left	4
Right	4
Gender, no.	
Male	3
Female	5
Pathological stage, no. (%)	
pT1	2
pT2	1
pT3	5
Pathological grade, no. (%)	
Low grade	2
High grade	6
Lymph node status	
pN0	7
pN1	1
Location of tumor, no. (%)	
Renal pelvis	3
Upper ureter	1
Middle ureter	2
Distal ureter	2
Preoperative hydronephrosis, no. (%)	6
Margin	
Positive	0
Negative	8
Multifocality	
Yes	0
No	8
Adjuvant therapy	
Yes	4
No	4

DISCUSSION

The present study represents the first prospective trial to explore the safety, feasibility, and impact on the disease outcome of extraperitoneal laparoscopic extended retroperitoneal lymph node dissection at the time of retroperitoneoscopic nephroureterectomy for UTUC. We showed that this procedure in the retroperitoneal route by the laparoscopic approach could be performed with low complication rates and desirable surgical outcomes despite an extended lymph node dissection. The present study established the viability and safety of this procedure, which allows for the most accurate lymph node staging without increased perioperative morbidities.

In high-risk UTUC, radical nephroureterectomy (RNU) remains the standard of care with segmental ureterectomy as an alternative conservative approach (13). While lymphadenectomy for UTUC has been debated with regard to its therapeutic effects, increasing evidence in literature has advocated the potential benefits of staging and treatment that retroperitoneal LND brings to UTUCs, especially in patients with advanced UTUCs (14, 15). A recent meta-analysis exploring outcomes of LND on UTUC has also shown improved survival, particularly for locally advanced tumors (16). In fact, the desirable oncological efficacy of lymphadenectomy has been suggested depending on the complete and adequate dissection of LN. One study has highlighted an adequate dissection of LN defined as eight or more to achieve a probability of 75% in finding one or more positive nodes (17). In pN0 UTUC, the number of removed LN has also been proved to be a predictive factor for cancer-specific mortality (17). In concordance, it was shown that patients with clinical non-metastatic urothelial carcinoma in the renal pelvis undergoing complete lymphadenectomy could improve cancer-specific survival and recurrence-free survival compared to those with incomplete or

no lymphadenectomy (18). However, lymphatic patterns of UTUC are poorly defined because of its great variation and complication, which in turn led to major discrepancy in clinical practice. Previous mapping studies have identified additional regional LNs for UTUC and suggested more extensive LND. In the present study, concomitant extended retroperitoneal laparoscopic RPLND was performed in a laparoscopic RNU with median harvested lymph nodes of 10, which indicated an adequate removal of LNs by the standard of literature. Although laparoscopic RNU has been criticized for inadequate lymphadenectomy (19, 20), our results demonstrated feasible extended LN removal. Moreover, results of dissected LN in the present study were comparable to the previous prospective study exercising extended retroperitoneal LND by open or minimally invasive methods in UTUC with a median lymph node count of 7 (9). Based on existing evidence, it is reasonable to hypothesize that the extended LND procedure in the present study is viable and effective (21). Also, it is notable that one of pathologically confirmed lymphatic metastatic patients with pT1 disease in the right renal pelvis had pathological lymphatic involvement confirmed in extended LND. With adequacy of LND, this procedure can improve local control by eradicating potential nodal micro-metastases not identified in routine pathological examination and offering possible therapeutic benefits.

As previously reported, strengths of laparoscopic radical nephroureterectomy have been well established, including shortened convalescence and cosmetic preference with similar oncological efficacy compared to the open RNU (22, 23). However, concomitant extended laparoscopic retroperitoneal LND has actually been underused in LRUN in daily practice due to technical difficulties and concerns over postoperative morbidities. It has been reported in a multi-institutional study that patients undergoing laparoscopic RNU were less likely to receive a concomitant LND with only 7.7% patients receiving an adequate LND of more than 8 lymph nodes when compared to 18.2% of an adequate LND in patients with open RNU (24). While laparoscopic RNU and LND are typically preferred in a transperitoneal approach by offering a wide surgical field, the transperitoneal route involves interference with abdominal organs and increases risks of postoperative ileus. These risks can be mitigated by retroperitoneal methods. Extrapolating from laparoscopic retroperitoneal LND in testicular cancer, common complications include vascular injury with a rate of 2.2% to 20%, chylous lymphatic leak, and lymphocele with reported rates up to 6.6% and 13.2%, respectively (25). In the present study, all complications in seven patients were minor (Clavien Grades I–II). Commonly reported complications such as vascular injury did not occur in our cohort. However, two patients had chylous lymphatic leak and were managed medically with prolonged drainage time. This was likely to be attributed to extended LND. In comparison to the previous study performing extended retroperitoneal LND and RNU in open, laparoscopic, and robot-assisted approaches, rates of chylous lymphatic leak were comparable (9). Notably, no patients developed postoperative ileus which could be partly attributed to our retroperitoneal approach. As for perioperative outcomes, our study reported a shorter median surgical time, fewer blood loss, and fewer complications as well as similar hospitalization length and lymph node numbers in comparison to previously mentioned study (9). Indeed, extraperitoneal laparoscopic RNU can deliver a direct control of the renal pedicle and also minimize tumor seeding in peritoneal cavity. Moreover, during concomitant laparoscopic LND, the extraperitoneal cavity can also be clearly exposed in the modified supine position. These results suggested safer and equally effective performances in our study using the laparoscopic retroperitoneal technique. The survival benefits need further validation in randomized prospective studies.

With prospectively designed extended LND, the current study also added new evidence to metastatic patterns of UTUC. Previous mapping studies identified rare metastasis to pelvic lymph nodes in primary tumors of the renal pelvis or upper ureter (5, 7). However, our study showed iliac LNM in one metastatic UTUC in the right renal pelvis, supporting the rationale of extended LND including pelvic LNs. Also, one patient with primary tumor in the right renal pelvis had only interaortocaval LNM without other sites affected, which is uncommon in other mapping studies (5, 7). Such metastatic patterns shown in the present study need to be recognized since the knowledge of secondary involvement of interaortocaval LN could lead to omission of dissection of interaortocaval LN guided by frozen section during surgery. Although limited by the small sample size of LNM patients in the current study, our results are in accordance with previous studies and expand perspectives to patterns of LNM.

Further, the present study also explored disease outcomes of UTUC patients with nephroureterectomy and extraperitoneal laparoscopic extended retroperitoneal lymph node dissection. With a median follow-up of 38 months, 8 patients (20.5%) developed local or distant recurrence. Several previous studies have described the recurrence pattern of UTUC patients with radical nephroureterectomy (21, 26, 27). One study including 389 UTUC patients with radical nephroureterectomy demonstrated that 73 patients (18.7%) developed local recurrence within a median follow-up of 41 months. Moreover, the para-aortic lymph node region was the most common recurrence area for all the patients (24). The study also showed that left-sided UTUC had over 70% recurrent lymph nodes in the left para-aortic region (LPA) while right-sided UTUC patients have recurrent para-aortic lymph nodes mostly distributed in the aortocaval regions (41.5%). Another multi-institutional study on relapse analysis also demonstrated that 76 of 293 patients developed disease relapses with regional lymph node recurrence as the most common site (21). In our study, the recurrent rate was 20.5% although some patients have adjuvant treatment due to adverse pathological features. It has been demonstrated that adjuvant chemotherapy could yield possible survival benefits for locally advanced UTUC with adverse pathological features including pathological lymph node metastasis and high tumor stage (28). Also, multiple retrospective studies have also shown that neoadjuvant chemotherapy in UTUC could deliver tumor downstaging and lower the risk of disease recurrence (29). However, conclusive evidence of perioperative therapy is further needed. Notably in

our study, no regional LN where extended LND was performed was among recurrent sites. This could be attributed to the extended retroperitoneal lymph node dissection in the present study, which might block the potential tumor metastasis route. The impact of such procedure on distant metastasis pattern and survival benefits remains unknown, which requires randomized trials with a large sample size.

The present study has some limitations. The sample size is small due to a monocentric recruitment. As a non-randomized study, the lack of control group may result in the inability to perform direct comparisons between different techniques. Lastly, the follow-up time is short, which prevents further observation and analysis of recurrence or oncological benefits in this procedure. Although large prospective studies with longer follow-ups are needed for conclusive benefits, our study revealed that the extraperitoneal laparoscopic extended retroperitoneal lymph node dissection at the time of retroperitoneoscopic nephroureterectomy for UTUC could be performed effectively and safely.

CONCLUSIONS

The present study showed that extraperitoneal laparoscopic extended retroperitoneal lymph node dissection at the time of retroperitoneoscopic nephroureterectomy for UTUC was feasible and safe with acceptable morbidities. Larger prospective trials are needed to conclusively address its potential therapeutic benefit.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article is accessible under reasonable requests.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethics Committee of Shanghai Renji Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JW Huang, study design, manuscript draft. HY Qian, study design, manuscript draft. YC Yuan, manuscript draft, data collection. XY Cai, statistical analysis, data collection. YH Chen, trial conduction. J Zhang, trial conduction. W Kong, trial conduction. XR Wu, trial conduction. M Cao, trial conduction. YR Huang, trial conduction. HG Chen, study supervision, manuscript revision. W Xue, study supervision, manuscript revision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.791140/full#supplementary-material

Supplementary Figure 1 | Illustration of trocar disposition.

Supplementary Figure 2 | (A) Locations and frequency of lymph node metastasis for primary tumors of right renal pelvis. **(B)** Locations and frequency of lymph node metastasis for primary tumors of right middle ureter. **(C)** Locations and frequency of lymph node metastasis for primary tumors of left renal pelvis.

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The Evolution of Clinicopathological Diagnostic Features of Upper Tract Urothelial Carcinoma in China: A Summary of 2561 Cases in the Last 20 Years

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Objectives: To summarize the clinicopathological diagnostic features and evolutionary trends of upper tract urothelial carcinoma (UTUC) in China over the past 20 years.

Methods: All patients diagnosed with upper tract urothelial carcinoma in the Peking University First Hospital from 2001 to 2020 were retrospectively collected. Data were divided into two groups (2001-2010 and 2011-2020) according to the date of diagnosis. Statistical analysis was done with the SPSS V22.0. Chi-square analysis and t-test were adopted to analyze depending on the data type. Subgroup analysis based on 5 years was used for visualization to present trends. Both Kaplan-Meier curve and Cox regression were used for univariate and multivariate survival analysis.

Results: The study included 2561 cases diagnosed with upper tract urothelial carcinoma in total. Compared with the first decade (2001-2010), patients of the second decades (2011-2020) had elder mean age (66.65 versus 67.59, years, p=0.025), higher male proportion (43.5% versus 49.0%, p=0.034), lower incidence of renal pelvic tumors (53.4% versus 45.8%, p<0.001) and multifocality (18.6% versus 12.0%, p<0.001), higher incidence of ureteral tumors (52.2% versus 60.9%, p<0.001).In recent ten years, the incidence of muscle-invasive urothelial carcinoma (pT2+) decreased significantly (64.4% versus 54.9%, p<0.001),and the mean size of renal pelvic tumors increased(3.46 versus 3.73, cm, p=0.043). The size of the ureteral tumor, the histopathologic grade showed no significant change. The prognostic analysis based on 709 patients regularly followed at our center revealed that the male gender and G3 histopathological grade were independent risk factors for poorer prognosis in patients with UTUC.

Conclusion: In the past 20 years, the clinicopathological diagnostic features of upper tract urothelial carcinoma in the Chinese population has changed significantly, suggesting

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an increased risk of a poorer prognosis for UTUC. This trend may be related to updating diagnostic techniques and self-monitoring awareness. However, we need more high-grade, multicenter trials to verify it in the future.

Keywords: evolution, clinicopathological diagnostic features, upper tract urothelial carcinoma (UTUC), Chinese population, 20 years

INTRODUCTION

Upper tract urothelial carcinoma (UTUC) is an uncommon malignant disease that occurs in the pyelocaliceal cavity and ureter, accounting for only 5% to 10% of the urothelial carcinomas (UCs) (1). UTUCs usually were multicentric and prone to recurrence, and have a higher grade and stage at diagnosis (2). Therefore, the early diagnosis and management of UTUC have been a hot issue of clinical concern.

A recent study based on the SEER database showed that the incidence of UTUC in the United States gradually declined over the last 30 years and that the disease was significantly more prevalent in people over 70 years of age, in men, and renal pelvis (3). However, presumably due to the widespread application of aristolochic acid drugs, UTUC in the Chinese populations has different epidemiological characteristics from those of Western populations. Compared to Western people, the Chinese UTUC population has a higher tumor grade, relatively lower tumor stage, and lower malignancy in female patients compared to the males. And there were more female patients than their counterparts (4). To our knowledge, there was no study on the trend of evolution of the pathological characteristics of the UTUC of the Chinese population in recent years.

This study aimed to summarize the clinical and pathological characteristics of UTUC of the Chinese population based on large sample size and to explore the association between pathological features and clinical characteristics of the UTUC. We further analyzed and elucidated the evolution of the distribution of pathological and clinical features of UTUC in the Chinese population over the past 20 years.

PATIENTS AND METHODS

Patient Selection

All consecutive UTUC patients who underwent radical nephroureterectomy or segmental ureterectomy or only ureteroscopy in Peking University First Hospital, one of the largest urological clinical centers in China with a large number of UTUC patients from all over China and the world each year, between January 2001 and December 2020, were included in the first screening process. The HIS (Hospital Information System) was the help in collecting basic patient information and pathology information. Cases with a pathological diagnosis of non-UTUC, cytological findings only, history of neoadjuvant chemotherapy, and duplicates were filtered and excluded by two researchers (CR Xu and CW Yuan). The protocol of this study was approved by the Ethics Committee of Peking University First Hospital. Approval reference number. 2021[130].

Pathological Diagnosis

Tumors were staged by the 2002 TNM classification system (5) and graded as G1, G2, and G3 according to the 1973 WHO classification system (6) (Considering that some pre-2004 cases have not yet adopted 2004 WHO updated grade criteria). Other pathological information such as tumor multifocality, primary location (renal pelvic or ureter), and the maximum diameter of the mass were analyzed as well. When masses are found in the renal pelvis and ureter separately at the same time, the primary sites were recorded separately. Each pathological diagnosis was made by two pathologists who independently reviewed and agreed on their conclusions, and in the event of disagreement between the two physicians, the diagnosis was reviewed by another higher-level pathologist.

Prognostic Information and Analysis

To further investigate the association of pathological features with patients' prognosis and quality of survival, we collected a total of 709 unselected patients with UTUC who had previously received regular follow-up from 2001 to 2021 at our center and for whom complete prognostic survival data were available (all included in the overall sample of this study). Basic patient information and pathological characteristics such as gender, tumor location, multifocality or not, presence of muscle invasion, and pathological grade were included in the analysis. Cancer-specific survival (CSS) was adopted as the primary prognostic endpoint.

Statistical Analysis

All data were divided into two groups according to the time of diagnosis at every ten years (2001-2010 and 2011-2020). To better show the trends in pathological characteristics over time, subgroup analyses made senses according to 5 years (Shown in **Tables 2-B**, **3-B**). For categorical variables, we applied the R*C columnar combined chi-square method to analyze the differences in the comparison rates. The t-test, on the other hand, was suitable for the comparison of sample means of continuous type variables. The results of Fisher's exact test were adopted when the expectation value <5 appeared in the results of the R*C column table. Kaplan-Meier curve and Cox regression were used for prognostic analyses, and variables satisfying $p < 0.2$ after univariate analysis were selected for inclusion in the multivariate regression test. All statistical analysis processes were done with the help of SPSS V22.0 (IBM, Armonk, NY). P values are 2-sided, with statistical

significance defined as P<0.05. Graphpad Prism version 8.0.1 (GraphPad, San Diego) was used to visualize data. Two authors (CR Xu and CW Yuan) separately completed the data input and statistical analysis, with the entire process supervised by a third author (XS Li).

RESULTS

From 2001 to 2020, a total of 3269 patients recorded were found in the His system. After removing duplicate records, records of non-surgical treatments, and data with cytological pathology results only, 2561 cases of UTUC were finally recruited in this study. The clinicopathological characteristics of these patients, including 1214 (47.4%) males and 1347 (52.6%) females, with 1228 (48.0%) tumors of renal pelvic, 1496 (58.4%) ureteral tumors, and 356 (13.9%) multifocal tumors were summarized in **Table 1**.

TABLE 1 | Clinicopathological characteristics of included patients.

Variables	Patients, n	Median (IQR) or %
Age, years	2561	68 (20-93)
<55	272	10.6%
55-60	1352	52.8%
≥70	937	36.6%
Gender		
Male	1214	47.4%
Female	1347	52.6%
Location		
Renal Pelvic	1228	48.0%
Ureter	1496	58.4%
Multifocality	356	13.9%
With Cis	69	2.7%
T Stage of all cases		
T Not clear	20	0.8%
Cis only	2	0.1%
T1+Ta	1063	41.5%
T2	780	30.5%
T3	647	25.3%
T4	49	1.9%
Grading of all cases		
G1	58	2.3%
G2	1369	53.5%
G3	1134	44.3%
Tumor of Renal Pelvic		
T Not clear	6	0.5%
Cis only	1	0.1%
T1+Ta	517	42.1%
T2	287	23.4%
T3	379	30.9%
T4	37	3.0%
G1	14	1.1%
G2	759	61.9%
G3	455	37.1%
Tumor Size (maximum, cm)		3.2 (0.1-27.0)
<2.0cm	192	15.6%
2-5cm	763	62.1%
≥5.0cm	273	22.2%
Tumor of Ureter		
T Not clear	18	1.2%
Cis only	3	0.2%
T1+Ta	609	40.7%
T2	532	35.6%
T3	322	21.5%
T4	12	0.8%
G1	46	3.1%
G2	696	46.5%
G3	754	50.4%
Tumor Size (maximum, cm)		2.5 (0.1-27.0)
<2.0cm	373	24.9%
2-5cm	954	63.8%
≥5.0cm	169	11.3%

Cis, carcinoma in situ.

Age, Gender and Primary Site of Tumors

Table 2-A demonstrated the changes in age, gender composition, and primary site of the UTUC patients in the last 20 years. Comparing the 1st decade (2001 to 2010, abbreviated as 1stD) versus the 2nd decade (2011 to 2020, abbreviated as 2ndD), the age of UTUC patients showed a significant increasing trend (p=0.025), but both the proportion of the young patients (age<55 years old) and elderly patients group (age≥70 years old) did not change significantly (p=0.088; p=0.853). In terms of gender composition, there were more female patients than male patients in the last 20 years (1347 versus 1214, 1.11:1). The difference in sexual ratio decreased over the past ten years, while the proportion of male patients significantly rise (p=0.034).

There was also a significant difference in the proportion of the primary site of tumors between 1stD and 2ndD. Renal pelvic tumors were found less than the last time (p<0.001), while the incidence of ureteral tumors has gradually increased (p<0.001). Specifically, the incidence of multifocal tumors decreased significantly in the recent ten years (p<0.001).

To better show trends in change, we further completed a five-year-based subgroup analysis. The results were broadly consistent with the ten-year-based results, with the difference that the incidence of elderly patients (≥70 years old) showed significant between-group variability (p=0.002), but the overall trend showed fluctuations. The results and trends were shown in **Table 2-B** and **Figure 1**.

Tumor Stage and Histological Grading

We detailed the trends in tumor histologic T staging and grading in **Tables 3-A, B**.

First, we performed a subgroup analysis to investigate whether the pathological features of MICUs (muscle-invasive urothelial carcinomas, pT2+) and high-grade (G3) UTUC were associated with the clinical features of tumors. It was found that compared with the female, a higher proportion of male patients had MIUCs (54.5% versus 61.1%, p=0.001). Young patients (<55 years old, 37.9% *vs* 45.0%, p<0.001), patients with UTUC primarily sited in the renal pelvis (37.1% *vs* 50.9%, p<0.001) had a lower incidence of high-grade (G3) tumors compared with the elder patients (≥55 years old) and patients with no renal pelvic tumor. Patients with primary ureteral tumors had a higher incidence of high-grade tumors compared to non-ureteral tumors (50.7% versus 35.2%, p<0.001).

Comparing to the 1stD, significant variations in the composition of different pT-stages were seen in our study (p<0.001). To better understand the specific trends, we

TABLE 2-A | Changes in age, gender composition, and primary site of the UTUC patients in the last 20 years.

Variables	2001-2010	2011-2020	P Value
Cases	734	1827	
Gender			
Male	319 (43.5%)	895 (49.0%)	0.034*
Female	415 (56.5%)	932 (51.0%)	
Age,years	66.65±10.11	67.59±10.09	0.025*
<55	90 (12.3%)	182 (10.0%)	0.088
≥70	324 (44.1%)	815 (44.6%)	0.83
Primary Site			
Renal pelvic	390 (53.1%)	836 (45.8%)	<0.001**
Ureter	381 (53.0%)	1113 (60.9%)	<0.001**
Multifocality	136 (18.6%)	220 (12.0%)	<0.001**

*P<0.05; **P<0.01.

TABLE 2-B | Five-year-based subgroup analysis of changes in age, gender composition, and primary site of the UTUC.

Variables	2001-2005	2006-2010	2011-2015	2016-2020	P Value
Cases	284	450	869	958	
Gender					
Male	128 (45.1%)	191 (42.4%)	415 (47.8%)	480 (50.1%)	0.048*
Female	156 (54.9%)	259 (57.6%)	454 (52.2%)	478 (49.9%)	
Age,years	66.32±9.63	66.86±10.40	67.51±10.39	67.65±9.82	0.168
<55	37 (13.0%)	53 (11.8%)	93 (10.7%)	89 (9.3%)	0.245
≥70	118 (41.5%)	206 (45.8%)	426 (49.0%)	389 (40.6%)	0.002**
Primary Site					
Renal pelvic	137 (48.2%)	255 (56.7%)	424 (48.8%)	412 (43.0%)	<0.001**
Ureter	156 (54.9%)	227 (50.4%)	511 (58.8%)	602 (62.8%)	<0.001**
Multifocality	61 (21.6%)	75 (16.7%)	120 (13.8%)	100 (10.4%)	<0.001**

*P<0.05; **P<0.01.

separately analyzed the incidence of MIUCs which has significantly decreased in the last 10 years (p<0.001). However, both the incidence of various histopathological grading of the renal pelvic tumors (p=0.385) and ureteral tumors (p=0.195) remained unchanged. Especially, the incidence of G3 tumors seemed not to change significantly between two decades (p=0.187). The evolutionary trend of pathological staging and grading depending on time were shown in **Figure 2**.

Tumor Size

Compared to 1stD, all UTUC patients in 2ndD had an increase in the mean maximum tumor diameter before the surgery. There was a significant difference in the increase in size of renal pelvic tumors (3.46 versus 3.73, cm, p=0.043, shown in **Figure 3**), however, ureteral tumors remained almost unchanged (p=0.609) (Detailed change about the tumor size was shown in **Tables 3-A, B**).

Correlation Between Pathological Features and Prognosis

The outcome of the univariate regression analysis suggested that male gender (p<0.001), muscle invasion (pT2+, p=0.048), and G3 histopathological grade (p=0.030) significantly predicted poorer CSS. In contrast, the tumor location and multifocality did not seem to affect the UTUC patients' survival outcome significantly. After multifactorial Cox regression analysis, both male gender (p<0.001, HR: 0.57, 95%CI: 0.43-0.76), G3 histopathological grade (p=0.013, HR:1.43, 95%CI:1.08-1.91) might be two independent risk factors for predicting worse CSS in UTUC patients (shown in **Figure 4**) This finding indicated that though the histological grade of tumor did not changed significantly between past two decades, the increased incidence in male patients would suggest a relatively worse survival in UTUC than before.

DISCUSSION

Upper tract urothelial carcinoma (UTUC) is a sort of urological malignant tumor which performs relatively low incidence but poor prognosis. Because UTUC is often insidious, multicentric, and aggressive in origin, there may be a high risk of recurrence even after timely radical surgical treatment (7). Among the many influencing factors associated with prognosis, high TNM stage of the tumor, high histological grade (G3), lymph node metastasis, multifocality, male patients, and renal pelvis tumor are notably associated with a high mortality or recurrence rate (4, 8). What is more, the pathological features of the tumors also make sense to the protocol of treatment of the patients. Although radical nephroureterectomy (RNU) remains the current gold standard procedure for localized UTUC, a proportion of patients will lose the opportunity to receive adjuvant chemotherapy due to postoperative renal insufficiency (9). Patients with low-risk UTUC (unifocal disease, non-muscle invasion, pathologic

TABLE 3-A | Changes in pathological characteristics of UTUC in the last 20 years.

Variables	2001-2010	2011-2020	P Value
Tumor of Renal Pelvic			
T Not clear	5 (1.3%)	0 (-)	<0.001**
Cis	1 (0.3%)	0 (-)	
T1+Ta	125 (32.1%)	392 (46.9%)	
T2	130 (33.3%)	157 (18.8%)	
T3	120 (30.8%)	259 (31.0%)	
T4	9 (2.3%)	28 (3.3%)	
G1	6 (1.5%)	6 (0.7%)	0.385
G2	242 (62.1%)	517 (61.8%)	
G3	142 (36.4%)	313 (37.4%)	
Tumor Size (maximum,cm)	3.46±1.97	3.73±2.15	0.043*
Tumor of Ureter			
T Not clear	13 (3.4%)	6 (0.5%)	0.018*
Cis	0 (-)	3 (0.3%)	
T1+Ta	136 (35.7%)	473 (42.5%)	
T2	149 (39.1%)	381 (34.2%)	
T3	80 (21.0%)	242 (21.7%)	
T4	3 (0.8%)	8 (0.7%)	
G0	2 (0.5%)	0 (-)	
G1	10 (2.6%)	34 (3.1%)	0.195
G2	188 (49.3%)	506 (45.5%)	
G3	181 (47.5%)	573 (51.5%)	
Tumor Size (maximum,cm)	3.00±2.47	3.08±2.29	0.609
MIUC[a]	470 (64.0%)	1001 (54.8%)	<0.001**
High-grade(G3)	310 (42.2%)	824 (45.1%)	0.187

**P<0.05; **P<0.01; a MIUC, muscle-invasive urothelial carcinoma (pT2+).*

TABLE 3-B | Five-year-based subgroup analysis of changes in pathological characteristics of UTUC.

Variables	2001-2005	2006-2010	2011-2015	2016-2020	P Value
Tumor of Renal Pelvic					
T Not clear	3 (2.2%)	3 (1,2%)	0 (-)	0 (-)	<0.001**
Cis	0 (-)	1 (0.4%)	0 (-)	0 (-)	
T1+Ta	33 (24.1%)	92 (36.1%)	200 (47.1%)	192 (46.6%)	
T2	45 (32.8%)	85 (33.3%)	85 (20.0%)	72 (17.5%)	
T3	52 (42.4%)	68 (26.7%)	128 (30.2%)	131 (31.8%)	
T4	4 (2.9%)	6 (2.4%)	11 (2.6%)	17 (4.1%)	
G1	7	1	4	2	<0.001**
G2	80	162	285	232	
G3	50	92	135	178	
Tumor Size (maximum,cm)	3.49±2.03	3.45±1.95	3.86±1.98	3.60±2.30	0.068
Tumor of Ureter					
T Not clear	3 (1.9%)	9 (4.0%)	2 (0.4%)	4 (0.7%)	<0.001**
Cis	0 (-)	0 (-)	1 (0.2%)	2 (0.3%)	
T1+Ta	37 (23.7%)	99 (43.6%)	222 (43.4%)	251 (41.7%)	
T2	86 (55.1%)	65 (28.6%)	171 (33.5%)	210 (34.9%)	
T3	26 (16.7%)	54 (23.8%)	112 (21.9%)	130 (21.6%)	
T4	4 (2.6%)	0 (-)	3 (0.6%)	5 (0.8%)	
G1	9 (5.8%)	3 (1.3%)	24 (4.7%)	10 (1.7%)	<0.001**
G2	76 (48.7%)	114 (50.2%)	250 (48.9%)	256 (42.5%)	
G3	71 (45.5%)	110 (48.5%)	237 (46.4%)	336 (55.8%)	
Tumor Size (maximum,cm)	3.09±2.16	2.95±2.67	3.07±2.04	3.09±2.48	0.901
MIUC[a]	214 (75.4%)	259 (57.6%)	475 (54.7%)	528 (55.1%)	<0.001**
High-grade(G3)	121 (42.6%)	189 (42.0%)	339 (39.0%)	485 (50.6%)	<0.001**

P<0.05; **P<0.01; [a]MIUC, muscle-invasive urothelial carcinoma (pT2+) in* ***Table 2–A**.*

biopsy, and urine cytology suggesting low histologic grading, maximum diameter <2 cm, and with no evidence of metastasis) have the opportunity to undergo the kidney-sparing surgery, which means they could have a comparable prognosis and better quality of life (1, 10, 11). Therefore, understanding the characteristics of the natural history and pathology plays a crucial role in predicting the prognosis of patients with UTUC. Unlike the Western population, the Chinese patients of UTUC have distinctive characteristics (12). Thus, clinical treatment guidelines and assessment of prognosis for Chinese UTUC patients are always difficultly obtained by totally copy the experience of European and American countries, which

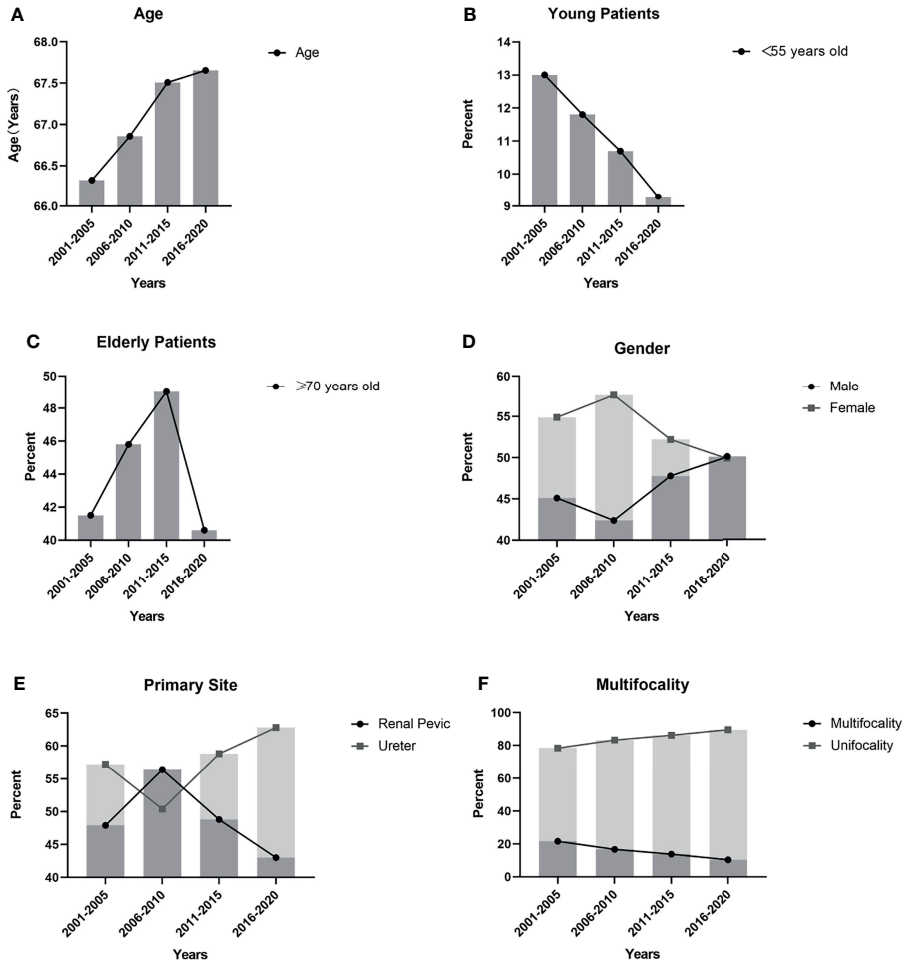


FIGURE 1 | Trends in clinical characteristics based on 5 years: **(A)** The mean age of the UTUC patients gradually increased and the difference between two decades was significant (p=0.025); **(B)** The incidence of young patients (<55 years old) showed a gradual decrease, but there was no significant difference (p=0.245); **(C)** There was a significant difference (p=0.002) in the incidence rate of elderly patients (≥70 years old), but the trend fluctuated dramatically; **(D)** The proportion of male patients gradually declined, and the proportion of female patients gradually increased. The difference between two decades was significant (p=0.034); **(E)** The incidence of renal pelvic tumors and ureteral tumors and the difference between two decades was significant (p<0.001); **(F)** The trend showed the incidence of multifocal tumors gradually decreased and the difference between two decades was significant (p<0.001).

deserves exploration in the future. After analysis, our study suggested that the morbidity characteristics of Chinese UTUC patients were predominantly in the middle to high age group (55-70 years old), female, with ureteral tumors, muscle invasion (pT2+), and high-grade pathology (G2, G3), which was consistent with the outcome of Singla's (13). With the advancement of science and technology, the treatment paradigm of UTUC has been updated (14), but as far as we know, there were no studies have been conducted on the evolution of the natural history and pathological features of this disease over the past 20 years. Munoz (15) once analyzed the evolution of the incidence and survival of UTUC in the United States from 1973 to 1996, according to the National Cancer Institute Surveillance, Epidemiology and End Results (SEER) database, and concluded that the incidence of UTUC has shown a mild increase and a gradual increase in 5-disease specific survival, and hypothesized that this trend might be related to the widespread use of ureteroscopy. Raman et al. (16) similarly found a slow increase in the overall incidence of UTUC. Unlike the above two researchers, Wu (3) suggested a decreasing trend in the incidence of UTUC in the United States and a predominance of male patients, renal pelvic tumors, and patients older than 70 years. And this conclusion revealed a gradual change in the trend of UTUC pathogenesis.

In the present study, we found a significant decrease in the incidence of renal pelvic tumors, muscle-invasive and multifocal UTUC in the last 10 years, but the histological grading of the tumors remained unchanged, however, the incidence of the ureter and the size of the renal pelvic tumors showed an increasing trend. It was reported that patients with UTUC who were aware of active self-monitoring and intervene in the disease before the onset of symptoms tended to have relatively less

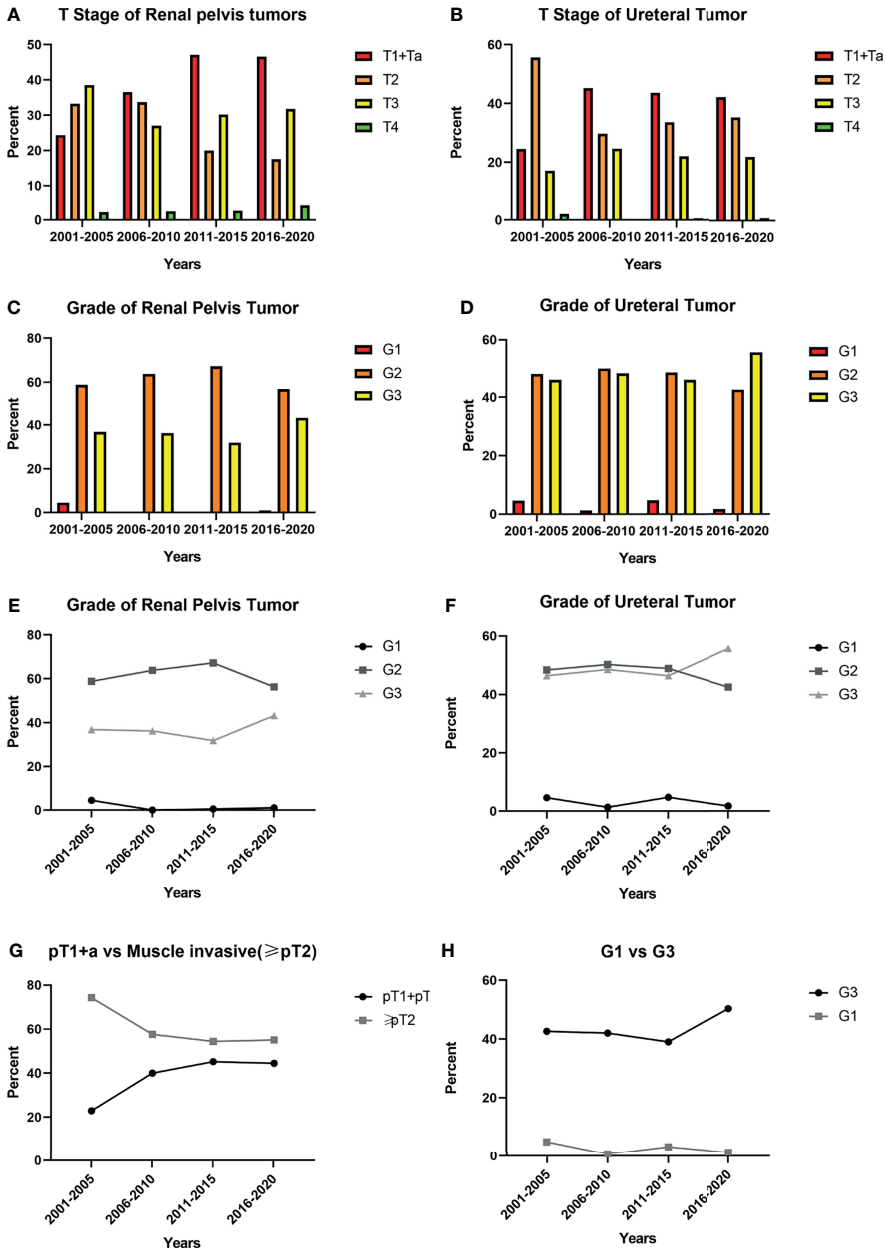


FIGURE 2 | Trends in pathological characteristics based on 5 years: **(A, B)**. The incidence of different pT stage of renal pelvic tumors and ureteral tumors in previous 20 years; **(C, D)**: The incidence of different histological grading stage of renal pelvic tumors and ureteral tumors in previous 20 years; **(E, F)** The trend of incidence of different histological grading stage of renal pelvic tumors and ureteral tumors in previous 20 years; **(G)** The trend showed the incidence of muscle invasive urothelial carcinomas (MIUCs) gradually declined and the difference between two decades was significant ($p<0.001$); **(H)** The trend showed the incidence of high-grade(G3) UTUC gradually increased, but the difference between two decades was not significant ($p=0.187$).

malignant pathological features and better prognosis (17). Thus, we speculated that the increase in the proportion of early-stage tumors may be related to the increased use of imaging examination technology such as CTU and the increased awareness of the population for self-monitoring. The evolution of tumor unifocal onset, histological grade, and tumor size may be related to the daily lifestyle habits of the Chinese population such as the application of aristolochic acid drugs or the changing disease spectrum of some chronic diseases such as lithanguria and chronic kidney disease (18–20). Further trials are needed to validate the conjecture.

To evaluate whether there are any changes in the age and gender composition of current Chinese UTUC patients, we explored the trends in the proportion of different genders and the evolution of the patients into young patients group (<55 years old) and elderly patients (≥70 years old) group according to

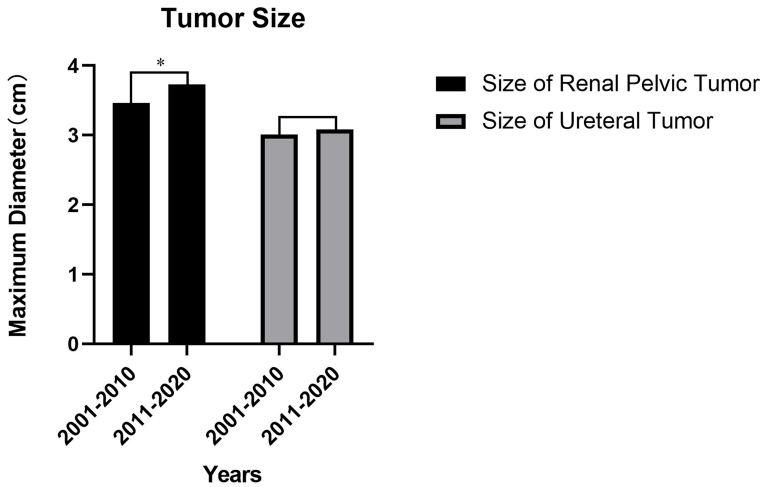
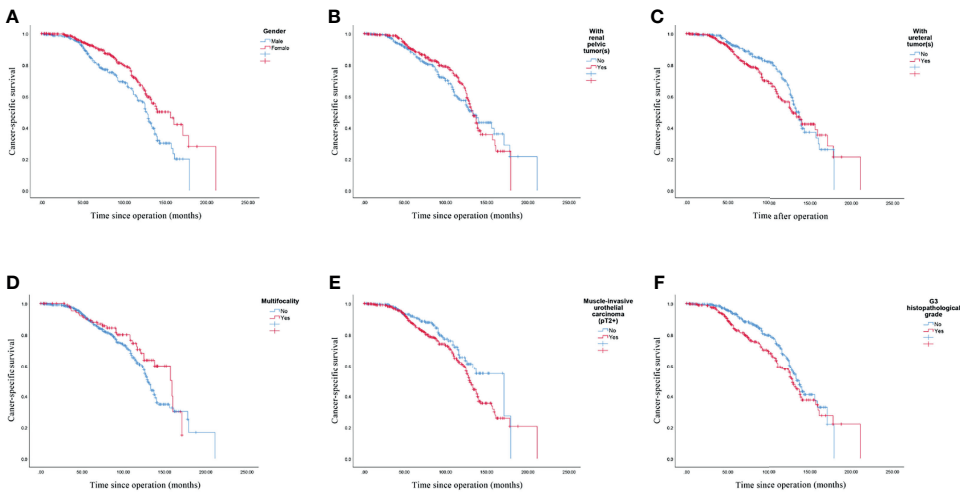


FIGURE 3 | Tumor size of the UTUC in the 1st decade vs 2nd decade. *p=0.043.



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	CSS			
	Univariate		Multivariate	
Variables	p	HR (95% CI)	p	HR (95% CI)
Gender	<0.001**	0.60 [0.45-0.80]	<0.001**	0.57 [0.43-0.76]
With renal pelvic tumor(s)	0.433	0.89 [0.68-1.18]		
With ureteral tumor(s)	0.095	1.27 [0.96-1.69]	0.097	
Multifocality	0.098	0.72 [0.49-1.06]	0.176	
Muscle-invasive UTUC (pT2+)	0.048*	1.39 [1.00-1.93]	0.405	
G3 histopathological grade	0.030*	1.37 [1.03-1.81]	0.013*	1.43 [1.08-1.91]

FIGURE 4 | Prognostic analysis of the UTUC patients: the endpoint is cancer-specific survival, and the risk factor is: **(A)** Gender; **(B)** With renal pelvic tumor(s); **(C)** With ureteral tumor(s); **(D)** Multifocality; **(E)** Muscle-invasive urothelial carcinoma (pT2+); **(F)** G3 histopathological grade; **(G)** Univariate and multivariate analysis of all included factors. *p<0.05, **p<0.01.

the previous studies (21). The result revealed that the proportion of male patients and the average age gradually increased over the past 20 years, but the proportion of young and elderly patients did not change significantly. Since previous studies have shown a female majority in the Chinese UTUC population, unlike in Western countries, this may be associated with the prevalence of aristolochic acid-based herbs. The current decrease in the proportion of female patients may be related to the reduction in the addition of aristolochic acid component drugs by Chinese pharmaceutical companies. At the same time, the risk factor of smoking makes the proportion of male patients appear relatively higher. Notably, we also found that male UTUC patients had

significantly poorer cancer-specific survival females. This increasing-proportion trend of the male gender implies that the potential health and economic burden of UTUC on society are also increasing, which warrants early attention and preventive measures.

Based on the pathological features, the molecular characteristics of UTUC are a current research hotspot in the field of diagnosis and treatment. Despite sharing a similar histological type, it is still controversial whether the molecular features of UTUC are equivalent to bladder cancer due to the variability in clinical presentation and genomics (22). From an early understanding of the molecular mechanism of Lynch-related UTUC with mutations in MMR genes, the second-generation sequencing technology has immensely improved the efficiency of exploration now (22, 23). The discovery of molecules such as FGFR-3 has led to advances in molecular diagnosis and immunotherapy of UTUC (24). The maturation of urine-based methylation detection technology also marks the upcoming era of molecular non-invasive detection in UTUC (25). Recently, Fujii successfully classified UTUC into five subtypes based on mutation type (26). This subtype classification system may be combined with pathological diagnosis prospectively to better assist in foreseeing the prognosis of UTUC patients and selecting appropriate strategies of chemotherapy or immunotherapy. However, most of the conclusions of molecular studies still need to be validated by further clinical trials. We also expect a discovery of molecules which with higher selectivity for UTUC.

Our study still has some limits. It was a retrospective analysis, so the selective bias was relatively inevitable. And we could not reasonably infer the causes of this evolutionary trend based on the current conclusion. Secondly, the data analysis stemmed from a single-center database, one of the largest urologic oncology clinical centers in China, which meant the representativeness of the findings still needs to be validated by further refinement of the multicenter study. Finally, we selected all patients with well-established follow-up outcomes for prognostic analysis, which meant the representativeness would not be satisfied. However, the missed visits were because the patients treated were from various regions all over China, which makes it hard to return to our center and finish the follow-up. However, this situation also demonstrated that the selection bias of our study was relatively low.

CONCLUSION

In conclusion, UTUC in the Chinese population changed significantly in the last 20 years in patients' age, gender composition, primary site, and multifocality. The proportion of high-T-stage UTUC was gradually decreasing and might imply an improved prognosis in general, but the size of the renal pelvic tumors seemed bigger, while there was no significant change in pathological grading. This trend may be related to the update of diagnostic techniques and self-monitoring awareness, which still needs multi-center trials to verify in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Peking University First Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CX, CY, and CZ contributed equally to this article. CX was the first author and CY and CZ were the co-first author. JL, LQZ, and XL were the equally correspondent author of this article. All authors are accountable for all aspects of the work. Conceptualization: CX, CY, and DF. Formal analysis: CX, CY, and CZ. Investigation: CY, CX, ZL, and YW. Methodology: CX, CY, DF, XW, and XL. Project administration: LQZ, XL, CZ, and ZH. Resources: CY, YY, QT, GX, and LZ. Supervision: JL, XL, LQZ, and ZH. Visualization: CX, CY, and CZ. Writing – original draft: CX, CY, and CZ. Writing – review & editing: CX, CY, CZ, JL, LQZ, and XL. All authors approved the final article.

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The Prognostic Impact of Tumor Location in pT3N0M0 Upper Urinary Tract Urothelial Carcinoma: A Retrospective Cohort Study

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Background: We aimed to evaluate the impact of tumor location on cancer outcomes in patients with pT3N0M0 upper tract urothelial carcinoma (UTUC) treated with radical nephroureterectomy (RNU) with bladder cuff excision.

Materials and Methods: We retrospectively reviewed 302 patients with pT3N0M0 UTUC who underwent RNU with bladder cuff excision at our institution between 2005 and 2019, including 191 renal pelvis tumors and 111 ureteral tumors. Clinicopathologic characteristics were compared between renal pelvis and ureter urothelial carcinomas. Multivariate Cox proportional hazard regression was used to assess the association between outcomes and clinical factors. Outcomes of interest included intravesical recurrence-free survival (IVRFS), local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), and cancer-specific survival (CSS), which were measured using the Kaplan–Meier curve with a log-rank test.

Results: A total of 302 patients underwent RNU with bladder cuff excision. During the median follow-up of 42.7 months, 70 (23.2%), 95 (31.5%), and 99 (32.8%) patients experienced intravesical recurrence, local recurrence, and distant metastasis, respectively. Seventy (23.2%) patients died from UTUC. Multivariate Cox regression analysis showed that tumor location was an independent predictor of local recurrence (HR = 2.05, p = 0.001), with borderline independent significance in intravesical recurrence (HR = 1.54, p = 0.074) and distant metastasis (HR = 1.45, p = 0.08). Kaplan–Meier analysis showed that ureter tumors had a worse 5-year local recurrence (log-rank p < 0.001) and borderline worse 5-year intravesical recurrence (log-rank p = 0.055) and 5-year distant metastasis (log-rank p = 0.073).

Conclusion: Ureter tumors seem to be associated with worse oncological outcomes, especially with local recurrence in UTUC. Further large and long-term studies are warranted for investigating biological differences based on tumor location.

Keywords: tumor location, upper urinary tract urothelial carcinoma, oncological outcomes, pT3N0M0, radical nephroureterectomy

INTRODUCTION

Urothelial carcinoma (UC), the fourth most common malignancy worldwide, can be located in the upper (pyelocaliceal cavity and ureter) or lower (bladder and urethra) urinary tract. Upper urinary tract urothelial carcinoma (UTUC) is uncommon and accounts for only 5% of all urothelial tumors (1). The estimated annual incidence of UTUC is 2/100000 in Western countries. Most cases occur in the renal pelvis and are approximately twice as common as ureteral tumors. For decades, radical nephroureterectomy (RNU) with bladder cuff excision has been regarded as the standard treatment for patients with UTUC. Furthermore, segmental resection and endoscopic management can also be considered in selected patients based on tumor location, tumor size, and histological characteristics (2).

In Taiwan, UTUC has an unusually high incidence and female predominance. It also accounts for >30% of UC cases in the country according to the Taiwan Cancer Registry Annual Report in 2017. Previous reports have demonstrated that cigarette smoking, arsenic exposure, and occupational carcinogens lead to this phenomenon (3, 4). Due to scant symptoms and delayed diagnosis, UTUC presented at least a muscle-invasive appearance (56%), which results in worse outcomes than those of bladder cancer (5). Patients with advanced UTUC tend to have a poor prognosis and high recurrence rates after RNU (1, 6). Therefore, identifying potential risk factors for UTUC is an important public health issue.

Tumor stage, grade, and lymphovascular invasion are the most significant prognostic factors in patients with UTUC (2). However, whether tumor location is an independent predictor of oncologic outcomes remains controversial. Several studies have reported that ureteral lesions are significantly associated with worse recurrence-free survival (RFS). Moreover, ureteral tumor is an independent risk factor that may result in worse cancer-specific death, recurrence, and metastasis in patients with UTUC (7, 8). Tumors invading the peripelvic or periureteral fat are associated with an approximately 3.5 times higher risk of cancer-specific death than tumors invading the renal parenchyma (9). Nevertheless, some studies have shown no difference in oncologic outcomes between renal pelvic and ureteral tumors (10, 11).

Compared with renal pelvic tumors, ureteral tumors are associated with local recurrence of surgical bed recurrence (12). Adjuvant radiotherapy has been reported to decrease locoregional recurrence rates in patients with locally advanced UTUC (13). In addition, the POUT trial reported that adjuvant platinum-based chemotherapy administered within 90 days after RNU can significantly contribute to survival benefits in patients with UTUC (5). Thus, in our study, we aimed to investigate the prognostic impact of tumor location in patients with pT3N0M0 UTUC who may respond to adjuvant therapy after RNU.

MATERIALS AND METHODS

Study Population

This study included patients with clinical T3N0M0 UTUC who underwent RNU at our institution between January 2005 and August 2019 and excluded patients who underwent nephron-sparing surgery and those with non-urothelial carcinoma histology. Finally, 302 patients with clinical and pathological data were included for the analysis. Among them, 136 (45.0%) and 166 (55.0%) patients underwent open and laparoscopic RNU. Clinical T3N0 was defined as image nodal negative UTUC. We selected the patients with clinically T3N0 UTUC. The lymph node (LN) dissection rate was 16.9% in this cohort because template LN dissection was not routinely performed in our institute. All patients underwent preoperative cystoscopy or computed tomography (CT) to determine the presence of a concurrent bladder tumor or distant metastasis. Demographic data such as age, sex, smoking history, concurrent bladder cancer, adjuvant therapy, disease recurrence outcome, and death were obtained using chart review. This study was approved by the institutional review board of our hospital (IRB number: 202000185B0), and written privacy consent was obtained from all participants.

Pathological Evaluation

The diagnosis of UC was confirmed by histological analysis, and variant histology was also included in this study. Genitourinary pathologists reviewed all slides according to strict identical criteria and were blinded to the clinical outcomes. Tumor grading was evaluated according to the 2004 and 2016 World Health Organization classifications (14, 15). Tumors were staged according to the Eighth American Joint Committee on Cancer (AJCC) tumor–node–metastasis (TNM) classification. We excluded pT4 tumors of the renal pelvis that also infiltrated beyond the renal capsule into the perinephric fat. Renal pelvis tumors which invades through the basement membrane and into the renal parenchyma was staged as pT3. In addition, pagetoid spread of tumor within collecting ducts and renal tubules were excluded due to its CIS nature (16). Lymphovascular invasion, grade, variant histology, and concomitant carcinoma *in situ* were also assessed in each representative slide.

Follow-Up Protocol and Definition of an Oncological Event

Our institutional follow-up protocol included postoperative fiber-cystoscopy every 3 months and renal ultrasonography to assess the contralateral urinary tract every 6 months during the first 2 years, every 6 months during the third year, and then annually thereafter. Abdominal CT was performed either annually or depending on the patient's condition to assess lymph node status and local or regional recurrence of the tumor. Bone scanning, chest CT, and magnetic resonance imaging were performed when clinically indicated. Intravesical recurrence was defined as post-nephroureterectomy urinary bladder tumor recurrence. Local recurrence was defined as locoregional recurrence at the ipsilateral surgical field, and distant metastasis was defined as disease recurrence outside the urinary tract and out of the locoregional surgical field. Disease in the urinary bladder or contralateral upper urinary tract was not considered to indicate metastasis. Cancer-specific mortality was defined as local recurrence or distant metastasis at the time of death.

Statistical Analysis

SPSS v.17 software was used for statistical analysis. Chi-square or two-sample t-tests were used to understand the distribution of these two groups and for intergroup comparisons. The Kaplan–Meier method with log-rank test was used to compare intravesical recurrence-free survival (IVRFS), local recurrence-free survival (LRFS), distant metastasis-free survival (DMFS), and cancer-specific survival (CSS) between the groups. Multivariate Cox regression analysis was used to identify independent prognostic factors upon oncologic outcome; only the parameters with p-value < 0.1 (borderline significance) in the univariate analysis were included in the multivariate analysis. Statistical significance was set at $p < 0.05$, as shown in **Table 1**, and an independent association was defined as p-value < 0.05, as shown in **Table 2**.

RESULTS

Patient clinicopathological characteristics stratified by tumor location are summarized in **Table 1**. Our cohort included 155 men (51.3%) and 147 women (48.7%). The median age of all patients was 68 years (interquartile range [IQR]: 62–76 years). The total median follow-up duration was 42.7 months (IQR: 13.4–62.9 months). Overall, 191 (63.2%) patients with renal pelvic tumors and 111 (36.8%) with ureteral urothelial carcinoma were analyzed. Renal pelvic tumors were more likely to be associated with papillary architecture than ureteral tumors (71.7% and 45.9%, respectively; $p < 0.001$). The presence of variant histology was more frequent in renal pelvic tumors (48.2%) than in ureteral tumors (27.0%) ($p < 0.001$). 35 (19.3%) patients with renal pelvic tumors and 16 (14.4%) patients with ureteral tumors received LN dissection during RNU (p= 0.475). No significant difference was found in the rest of the clinical features between the two groups (**Table 1**).

Overall, 70 patients (23.2%) experienced intravesical recurrence. Kaplan–Meier survival curves with a log-rank test stratified by tumor location showed that pT3 ureteral tumors were associated with borderline significantly lower IVRFS than were renal pelvic tumors (**Figure 1**; 5-year intravesical recurrence rates: 19.4 *vs.* 29.7%, p = 0.055). On univariate and multivariate analyses, concurrent bladder cancer and smoking were associated with intravesical recurrence (**Table 2**). Tumor location was a borderline risk factor for intravesical recurrence (adjusted hazard ratio [HR]: 1.54, ureter *vs.* renal pelvis; 95% confidence interval [CI], 0.96–2.46; p = 0.074).

In this cohort, 95 patients (31.5%) presented with local recurrence, including 43 (22.5%) and 52 (46.8%) patients with

TABLE 1 | Clinicopathologic Characteristics for pT3N0M0 UTUC.

Variables	Renal pelvic UC n = 191	Ureteral UC n = 111	*p* value
Follow up (Months)	45.1 ± 41.0	38.8 ± 34.0	0.156
Age (years)	68.2 ± 10.7	67.6 ± 10.0	0.625
Sex, n (%)			0.806
Male	97 (50.8%)	58 (52.3%)	
Female	94 (49.2%)	53 (47.7%)	
Nodal status, n (%)			0.475
Nx	156 (81.7%)	95 (85.6%)	
N0	35 (19.3%)	16 (14.4%)	
Smoking, n (%)	25 (13.1%)	15 (13.5%)	0.916
Concurrent Bladder cancer, n (%)	21 (11.0%)	15 (13.5%)	0.515
High grade, n (%)	190 (99.5%)	111 (100%)	0.445
Lymphovascular invasion, n (%)	105 (55.0%)	53 (47.7%)	0.225
Papillary, n (%)	137 (71.7%)	51 (45.9%)	<0.001
Carcinoma in situ, n (%)	86 (45.0%)	51 (45.9%)	0.877
Variant histology, n (%)	92 (48.2%)	30 (27.0%)	<0.001
Adjuvant chemotherapy, n (%)	21 (11.0%)	21 (18.9%)	0.055
5-year Intravesical recurrence, n (%)	37 (19.4%)	33 (29.7%)	0.055*
5-year Local recurrence, n (%)	43 (22.5%)	52 (46.8%)	<0.001*
5-year Distant metastasis, n (%)	54 (28.3%)	45 (40.5%)	0.073*
5-year Cancer death, n (%)	39 (20.4%)	31 (27.9%)	0.153*

*, *Kaplan Meier analysis.*

TABLE 2 | Multivariate Cox proportional hazard regression analyses to predict oncological outcome from upper tract urothelial carcinoma after radical nephroureterectomy.

Variable	Intravesical Recurrence			Local Recurrence			Distant Metastasis			Cancer Specific Death		
	Uni	Multi	HR (95%CI)	Uni	Multi	HR (95%CI)	Uni	Multi	HR (95%CI)	Uni	Multi	HR (95%CI)
U *vs*. RP	0.055	0.074	1.54 (0.96-2.46)	<0.001	0.001	2.05 (1.33-3.15)	0.073	0.08	1.45 (0.96-2.20)	0.153		
Con. BCa	0.021	0.046	1.86 (1.01-3.41)	0.286			0.804			0.044	0.055	1.81 (0.99-3.30)
LVI	0.055	0.086	0.66 (0.41-1.06)	0.032	0.031	1.58 (1.04-2.38)	0.001	0.001	2.05 (1.35-3.09)	0.193		
LND	0.1			0.434			0.52			0.481		
PA	0.197			0.003	0.130	0.72 (0.47-1.10)	0.062	0.189	0.76 (0.50-1.15)	0.405		
CIS	0.770			0.343			0.671			0.239		
Variant	0.255			0.389			0.193			0.158		
HG	0.579			0.509			0.507			0.576		
Adj. CT	0.190			0.556			0.928			0.739		
Smoking	0.003	0.036	1.87 (1.04-3.37)	0.997			0.014	0.004	2.04 (1.25-3.33)	0.002	0.001	2.46 (1.43-4.24)
Female	0.293			0.908			0.498			0.311		
Age>68	0.731			0.993			0.014	0.003	1.84 (1.23-2.77)	0.047	0.020	1.77 (1.10-2.86)

Uni, Univariate; Multi, Multivariate; HR, Hazard ratio; CI, Confidence interval; U, ureter; RP, renal pelvis; BCa, Bladder cancer; LVI, Lymphovascular invasion; LND, Lymph node dissection; PA, Papillary; CIS, Carcinoma in situ; HG, High grade; Adj. CT, Adjuvant chemotherapy.

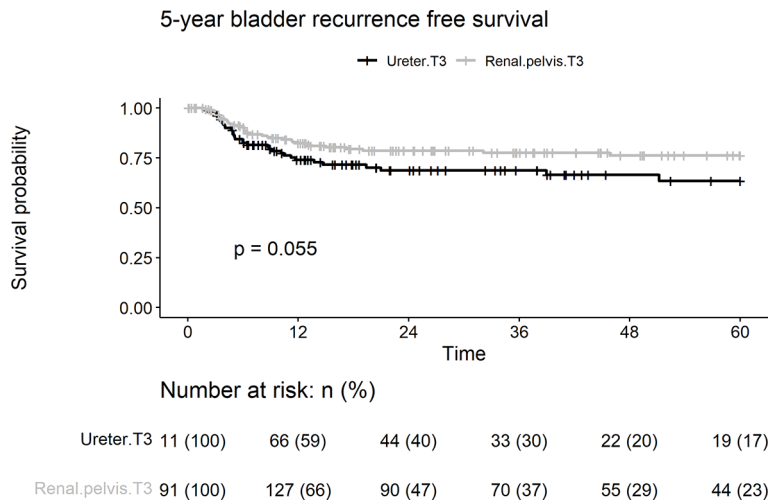

FIGURE 1 | Kaplan-Meier analyses for 5-year intravesical recurrence-free survival stratified by tumor stage among patients with pT3N0M0 upper tract urothelial carcinoma following radical nephroureterectomy.

renal pelvic and ureteral UC, respectively. Patients with pT3 ureteral tumors had a significantly shorter LRFS duration than those with renal pelvic tumors (log-rank test, $p < 0.001$; **Figure 2**). Univariate analyses showed that tumor location, lymphovascular invasion, and papillary architecture ($p < 0.001$, $p = 0.032$, and $p = 0.003$, respectively) were significantly associated with local recurrence (**Table 2**). Multivariate analysis revealed that tumor location (HR = 2.05, $p = 0.001$) and lymphovascular invasion (HR = 1.58, $p = 0.031$) were independent risk factors for predicting local recurrence of UTUC.

During follow-up, distant metastases were observed in 99 (32.8%) patients. The 5-year distant metastasis rate in the ureteral group (40.5%) was significantly higher than that in the renal pelvic group (28.3%; $p = 0.073$) (**Figure 3**). Lymphovascular invasion, smoking, and age > 68 years were associated with distant metastasis of UC on univariate and multivariate analyses (**Table 2**). Tumor location was a borderline independent risk factor for distant metastasis (adjusted hazard ratio [HR]: 1.45, ureter *vs*. renal pelvis; 95% confidence interval [CI], 0.96–2.20; $p = 0.08$).

Cancer-specific death from pT3 UTUC occurred in 70 (23.2%), 39 (20.4%), and 31 patients (27.9%) in the renal pelvic and ureteral groups, respectively. There were no differences between the two groups in terms of 5-year cancer death rates ($p = 0.153$) (**Figure 4**). On univariate analyses, age > 68 years, concurrent bladder cancer, and smoking were associated with cancer death (**Table 2**). On multivariable analysis, smoking and age > 68 years remained associated with worse cancer survival. Tumor location was not a risk factor for cancer death.

DISCUSSION

Radical nephroureterectomy with bladder cuff excision is the standard treatment for UTUC. Despite surgical management, the

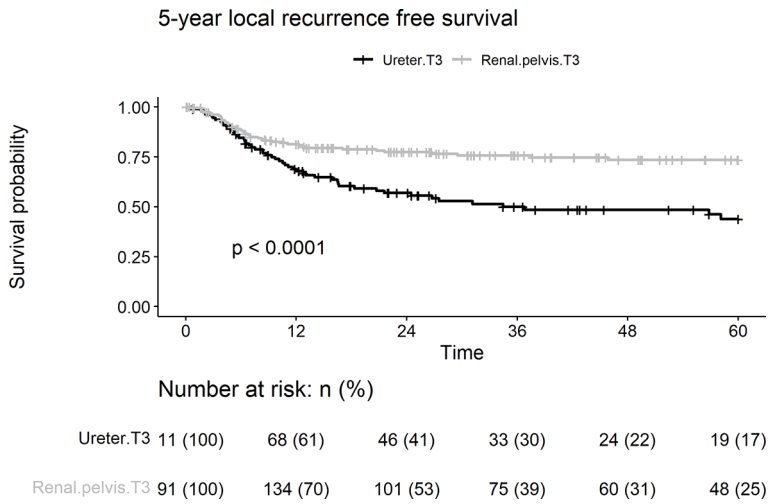

FIGURE 2 | Kaplan-Meier analyses for 5-year local recurrence-free survival stratified by tumor stage among patients with pT3N0M0 upper tract urothelial carcinoma following radical nephroureterectomy.

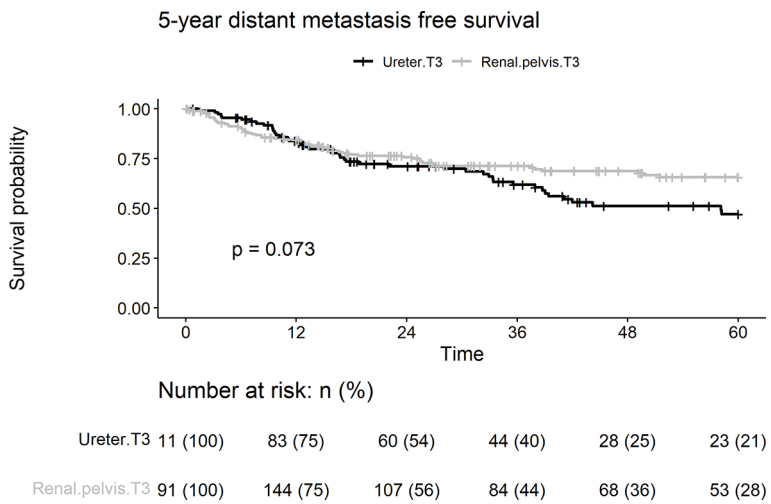

FIGURE 3 | Kaplan-Meier analyses for 5-year distant metastasis-free survival stratified by tumor stage among patients with pT3N0M0 upper tract urothelial carcinoma following radical nephroureterectomy.

prognosis of UTUC remains poor. The 5-year cancer specific survival of advanced UTUC is 50% for T3 tumors and <20% for T4 tumors (17). Tumor stage, tumor architecture (18), tumor grade, and lymphovascular invasion have been identified as prognostic factors for UTUC. In addition to these well-established predictors, tumor location has been reported as a potential risk factor for oncological outcomes (2). Several studies have described that patients with ureteral tumors seem to be associated with a worse prognosis than patients with renal pelvis tumors. However, there remains a paucity of detailed research analyses of oncological outcomes in the literature. Although they were large population-based analyses, the authors of the aforementioned studies evaluated the impact of tumor location without focusing on locally advanced stage-specific and node-negative UTUC (7, 8, 19).

Although the recurrence rate of locally advanced UTUC is relatively high, variations in clinical course are often observed in clinical practice (20). Adjuvant therapy is a common treatment strategy used to improve survival rates in this population (5, 21). According to the reviewed literature, there is a scarcity of data focused on the prognostic impact of tumor location in patients with locally advanced UTUC. In the present study, we demonstrated that pT3 ureteral tumor location was independently associated with local recurrence and borderline associated with intravesical recurrence and distant metastasis.

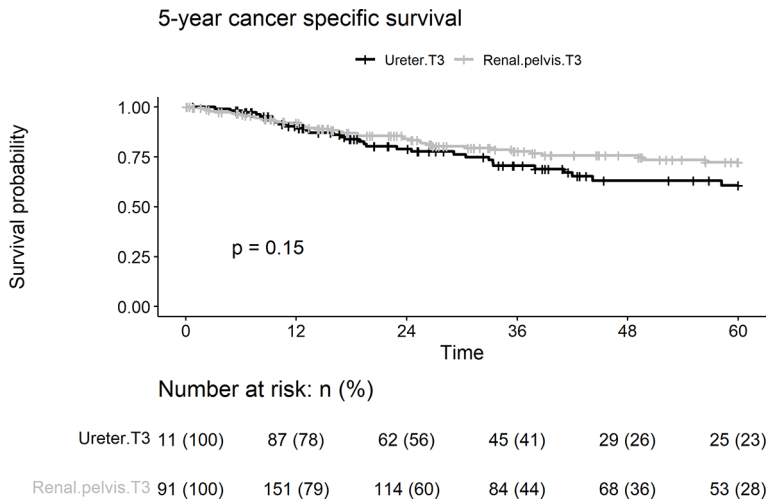

FIGURE 4 | Kaplan-Meier analyses for 5-year cancer specific survival stratified by tumor stage among patients with pT3N0M0 upper tract urothelial carcinoma following radical nephroureterectomy.

The disease recurrence of UTUC include bladder, local, and distant recurrence, with the majority of relapses occurring within the first 3 years after RNU (22). Early studies have reported that anatomical reasons may result in prognostic differences. Park et al. found that ureter UC was associated with significantly higher disease recurrence and cancer death. They supposed that the renal pelvis is located within the perinephric fat, which may serve as a barrier to resist metastatic spreading. However, the ureter is surrounded by weak adipose tissues surrounded by thinner adventitia that are rich in blood plexus and lymphatic ducts, facilitating tumor invasion (23). An additional anatomical difference is regarding the thinness of the muscularis of propria. Muscularis propria may be entirely absent in the renal pelvis with only a thin layer of fibrous connective tissue between the urothelium and the kidney. Therefore, pT3 tumors in renal pelvis may occur earlier compared to the ureter tumors (16). Thus, it is a challenge for surgeons to completely excise ureteral tumors and adjacent tissues, which may contain micro-metastases. Among renal pelvic tumors with peripelvic adipose tissues invasion, the distinction between T3 and T4 depends on the presence of Gerota's fascia. Ureter tumor located in periureteral fat is regarded as T3 disease unless it invades adjacent organs which occurs very rarely (pT4). Given that the limitation of distinction between T3 and T4 by surgical resection and ureter anatomy, surgical margins may help to identify tumors with high risk of local recurrence. Yoo et al. found that tumor location was associated with local recurrence of UTUC after RNU, and ureteral UC was a significant risk factor for surgical bed recurrence among 353 patients with UTUC. However, this study was not stage-specific. In addition, most patients had T2 or less (75.9%) and low-grade UTUC (55%) (12). In addition, local recurrence may be related to tumor spillage during surgery and lymph node status. In our study, we selected patients with clinically T3N0 UTUC and performed RNU according to oncological principles preventing tumor seeding. Over time, minimally invasive approaches are increasingly being utilized with less perioperative complications and faster recovery (24). Lenis et al. concluded that compared with open RNU, robot-assisted RNU does not compromise performance of lymph node dissection and may be associated with LN yield (25).

Adjuvant treatment such as radiotherapy may be considered for patients at high risk of local recurrence. A Korean multicenter study demonstrated that adjuvant radiation therapy significantly reduced the local recurrence in patients with advanced-stage, nonmetastatic UTUC (21). In this cohort, we found that tumor location had a more significant impact on local recurrence than lymphovascular invasion. The high local recur rate of ureter cancer was the clinical unmet need that we observed. Adjuvant radiation therapy may be considered according to recurrence pattern to improve the local regional control rate. Furthermore, well-known risk factors for local recurrence can help clinicians in risk stratification, patient selection for adjuvant radiotherapy, and active surveillance.

The incidence of intravesical recurrence in patients with UTUC after RNU ranges from 22% to 47% (26–28). Tumor location was also a controversial risk factor for intravesical recurrence in previous studies. In this cohort, ureteral UC presented with borderline independent significance for intravesical recurrence. In a propensity score-matched case-control study published in 2020, Jiang et al. evaluated 229 patients with UTUC and demonstrated that tumor location was not an independent predictor for intravesical recurrence, where tumors were classified as located in either the renal pelvis or the ureter (29). However, Otsuka et al. reported that a lower ureteral lesion served as an independent risk factor for intravesical recurrence, where a lower ureter lesion was defined as the lowest cancer component within 5 cm from the lower end of the ureter (30). This difference in oncologic outcomes may be related to the different classification methods of tumor position. Delayed detection of intravesical recurrence might cause the

development of muscle invasive disease and impaired quality of life in patients with UTUC. Therefore, intensive surveillance of intravesical recurrence is necessary.

Smoking is a modifiable risk factor of bladder cancer (31). Furthermore, Rink et al. reported that smoking may increase the risk of disease recurrence and cancer-specific mortality in patients with UTUC (32). We found that old age, smoking, and lymphovascular invasion were independent prognostic factors for UTUC in distant metastasis. However, tumor location was found to be a borderline independent risk factor for distant metastasis. For locally advanced UTUC, the most recent and largest randomized controlled study (the POUT trial) indicated that adjuvant chemotherapy improved survival after RNU in patients in whom systemic chemotherapy was not contraindicated (5). Nevertheless, adjuvant chemotherapy was not associated with prognosis in this cohort. This result may be attributed to the fact that not all patients were eligible for chemotherapy or an incomplete treatment cycle.

To our knowledge, there are a number of limitations to the present study. First, this was a retrospective study conducted in a single institution, which may have some intrinsic bias. Second, the prognostic impact was found for the first intravesical recurrence, local recurrence, and distant metastasis, rather than cancer-specific mortality. However, this method was the most objective way to observe cancer behavior due to the presence of confounding factors such as a sequential treatment strategy, which depends on each patient's socioeconomic status, hospice care choice, and general health condition. Third, patients who received adjuvant chemotherapy did not always fulfill the standard treatment cycles due to adverse effects and patient preferences (5). In addition, follow-up images such as abdominal/chest CT, MRI or bone scanning were arranged based on patient's condition rather than a standard protocol. Disease recurrence may be undetected. The low rate of LN dissection (16.9%) also could be a bias and influenced our results independently of surgical approach, since local recurrence could be related to LN recurrence in the ipsilateral surgical field. We showed that ureteral T3 tumors were independently associated with local regional recurrence after RNU. This result could help guide decision-making about more intensive postoperative adjuvant chemotherapy or even radiation to improve local regional disease control and might prevent further distant metastasis.

In conclusion, ureteral tumor location was an independent risk factor for local recurrence and borderline independent risk for intravesical recurrence and distant metastasis in patients with pT3N0M0 UTUC following RNU. Local recurrence differences could be influenced by different level of surrounding tissues invasion classified as pT3 such as Gerota's fascia and renal parenchyma that are absent in ureteral tumors. In addition, regular postoperative follow-up and feasible adjuvant therapies should be considered to improve the oncological outcomes. Further studies are necessary to validate the tumor location-specific clinical cancer course. According to our observation, a prospective adjuvant trial focused on local regional control and prevention of subsequent distant metastasis for ureter T3 tumors is worthy of investigation to improve the oncological outcome.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

TC: manuscript draft and analysis. HLL: study design, analysis and supervision. HW, PC, WY, YCC, WChinL, YTChen, YTCheng, CK, WChiaL, CC, YCS, YL, HYL, YC, YLS, and CH: data collection, administrative, technical, and material support. All authors contributed to the article and approved the submitted version.

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frontiers in Oncology

ORIGINAL RESEARCH
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Identification of Germline Mutations in Upper Tract Urothelial Carcinoma With Suspected Lynch Syndrome

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Objective: Whole-exon sequencing (WES) is a commercially available tool for hereditary disease testing. However, little is known about hereditary upper-tract urothelial carcinoma (UTUC) in the Chinese population. This study aims to investigate the prevalence of Lynch syndrome (LS) in UTUC patients with high-risk features and identify the germline mutations of genetic predisposition gene mutations in those patients.

Methods: In total, 354 consecutive UTUC patients undergoing surgery were universally recruited, of whom 108 patients under 60 years old or with a personal/family history of cancer underwent universal immunohistochemistry staining to detect the expression of mismatch repair (MMR) proteins (MLH1, MSH2, MSH6 and PMS2). Patients with deficient or weak MMR protein staining or meeting the Amsterdam II criterion were defined as suspected LS patients, who further experienced microsatellite instability (MSI) (BAT25, BAT26, BAT40, D2S123, D5S346, D17S250) detection and performed WES analysis to explore germline pathogenic/likely pathogenic (P/LP) alterations.

Results: Of 108 patients, 90 (83.3%) cases were included due to younger than 60 years, and 18 cases due to personal/family history. IHC staining identified 21 patients with deficient MMR protein staining and 15 cases with weak MMR protein staining. Three cases met the Amsterdam II criterion but with proficient MMR protein staining. Finally, WES analysis was performed in 38 suspected LS patients and P/LP germline mutations were identified in 22 individuals. Genetic testing confirmed 5 LS cases, including 3 cases with novel mutations. MSI-harboring tumor was discovered in 4 LS cases, one of whom had weak MMR protein staining. Germline P/LP variants in DNA damage repair genes were found in 11 cases. In addition, we found that 11 patients had high- or moderate-penetrance P/LP mutations other than MMR genes. The common P/LP variants in high- or moderate-penetrance genes were 4 in ATM, 3 in MSH6 and KIT, and 2 in APC, NF1 and DICER.

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Conclusions: We identified approximately 11% of UTUC cases as suspected LS and at least 1.4% patients with confirmed LS-associated UTUC. In addition, broader germline genetic testing could be considered to screen for cancer severity in hereditary UTUC patients.

Keywords: DNA mismatch repair, upper tract urothelial carcinoma, inherited cancer, Lynch syndrome, whole-exon sequence

INTRODUCTION

Hereditary diseases are typically correlated with an advanced risk of various symptoms or malignancies. Determining a specific genetic cancer susceptibility is essential to provide patients and their families with an opportunity for disease surveillance and potential guidance for preventive treatment. One of the most prevalent hereditary malignancies is Lynch syndrome (LS), which results from pathogenic alterations in one of the mismatch repair (MMR) genes.

As an autosomal dominant genetic susceptibility syndrome, LS is prone to early-onset colorectal cancer and other related cancers, especially upper tract urothelial carcinoma (UTUC) (1). The approximate incidence rate of UTUC in individuals with LS ranges from 1% to 28% (2). Routine screening of LS-related UTUC has recently been brought into clinical practice for a decade, yet current studies have reported that the prevalence of LS in UTUC is 3%-21% (3–7). The classical inherited cancer risk evaluation method includes identifying subjects whose medical history meets the clinical diagnostic standard for a particular genetic disease and then performing targeted sequencing only on the genes related to the disease (8). Although clinical criteria and prediction tools can help guide genetic testing for LS, approximately 30% to 50% of families who meet strict clinical diagnostic standards for LS will eventually fail to detect pathogenic germline MMR gene mutations (9). In addition, it is pyramidally recognized that the broad phenotypic extent of LS-related tumors may overlap with other inherited diseases (9). Therefore, the traditional diagnostic standard is probably not the ideal inherited disease hazard evaluation tactic in suspected LS patients.

Next-generation sequencing (NGS) technology can analyze a large number of genetic susceptibility genes simultaneously, possessing the strength of high efficiency and affordability, and is convenient for use in exploring other potential genetic diseases (8, 10). However, few studies on LS-related UTUC identified by genetic testing have been reported, and whole exon sequencing (WES) analysis of germline DNA has also been scarce. Thus, we proposed a selective screening process for LS in high-risk UTUC patients using immunohistochemistry (IHC), followed by microsatellite instability (MSI) analysis andWES, and further identified the frequency of genetic predisposition gene mutations among suspected LS patients.

MATERIALS AND METHODS

Patient Selection

In total, 354 consecutive UTUCs without a history of LS undergoing surgery from Peking University First Hospital between Jan 01, 2016 and Dec 31, 2017 were collected. The patients were selected according to three inclusion criteria (one of any): 1) diagnosis of UTUC <60 years of age; 2) personal history of malignant tumors; and 3) family history of malignant tumors. Finally, 108 patients who met above criteria were performed MMR protein staining. The flowchart of patient selection was showed in **Figure 1**. Personal/Family history information is collected from medical archives and during follow-up. The LS-associated tumor includes colorectal cancer, endometrial cancer, intestine cancer, gastric cancer, pancreatic cancer, ovarian cancer, urothelial carcinoma, biliary tract carcinoma, sebaceous adenomas/cancer, and cerebral tumors. This study was approved by the Peking University First Hospital Ethics Committee. All patient-derived samples and clinicopathological information were collected after verbal informed consent was obtained.

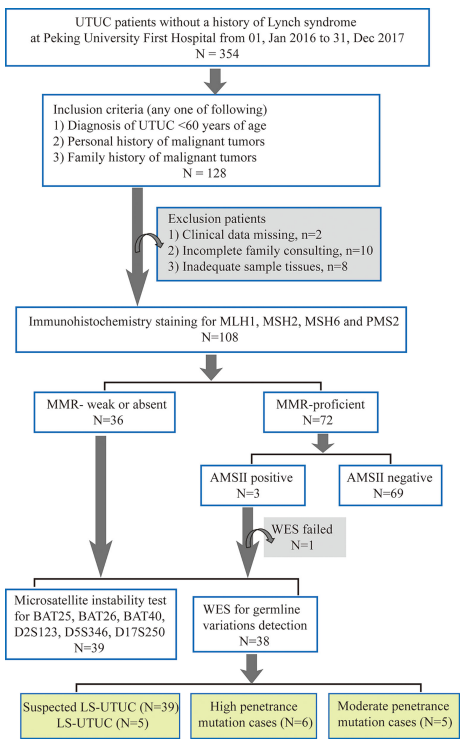

FIGURE 1 | Flowchart of Lynch Syndrome screening. UTUC, upper tract urothelial carcinoma; MMR, mismatch repair; AMS, Amsterdam; WES, whole exon sequencing; LS-UTUC, Lynch Syndrome related UTUC.

Immunohistochemical Staining

Immunohistochemical staining of the MMR proteins MLH1, MSH2, MSH6 and PMS2 was performed on paraffin-embedded tissue sections of 108 patients (**Supplementary Material**). Four μm thick FFPE tissue sections were stained with primary monoclonal antibodies against MSH2 (Abcam, UK, ab52266, mouse), MSH6 (Abcam, UK, ab92471, rabbit), MLH1 (Abcam, UK, ab92312, rabbit), and PMS2 (Abcam, UK, ab110638, rabbit). The proficient expression of MMR proteins in tumor cells was determined by the presence of nuclear staining. Loss of staining in cancer cells with positive nuclei in the positive control in parallel indicates a loss of protein expression (**Supplementary Figure 1**). Weak protein staining was defined by the absence of nuclear staining in more than half of urothelial carcinoma cells and weak staining of the remaining tumor cells (**Supplementary Figure 2**). The processed IHC slides were blindly evaluated by 2 pathologists.

MSI Analysis

DNA from 39 suspected LS patients, including 36 patients with deficient or weak MMR protein staining and 3 patients who met Amsterdam II criteria, were extracted (**Supplementary Methods**). A panel of 6 microsatellites (BAT25, BAT26, BAT40, D2S123, D5S346, and D17S250) was used to determine the MSI status of 39 matched samples. We defined the MSI status as stable (no allele altered), low (1 allele altered) and high (≥2 altered markers).

Germline Sequencing and Interpretation

Because the tumor from a patient meeting Amsterdam II criteria was unable to build library and perform WES, whole exon sequencing analysis were only carried out on 38 patients with suspected LS (**Supplementary Methods**). The genes analyzed in this study were summarized in **Supplementary Table 1**. Pathogenicity was identified in accordance with the American College of Medical Genetics standard (11). Alteration types in inherited susceptible genes were judged by PathoMAN (https://pathoman.mskcc.org/) (10, 12), which is an automated hereditary mutation evaluation software. Consistent with prior studies (10, 13), all sequence variants were classified into the following tiers: pathogenic, likely pathogenic, uncertain clinical significance, likely benign, benign, and polymorphism.

Statistical Analysis

Parameters were compared by the chi-squared test, Fisher's exact test, Wilcoxon rank test or Kruskal-Wallis test as indicated. P-values < 0.05 were considered statistically significant, and all tests were two-sided. All statistical analyses were performed in SPSS version 15.0 (IBM Corporation, America).

RESULTS

Patient Characteristics

Ninety patients (83.3%) were recruited due to ages younger than 60 years, and 18 patients (16.7%) were recruited due to personal or family history of malignant tumors (**Supplementary Table 2**). The median age of 108 patients was 55 years (range, 29-84 years), and 47 patients (43.5%) were female. IHC staining showed 21 (19.4%) patients with deficient MMR protein staining and 15 patients (13.9%) with MMR proteins weak staining. In total, 12 patients were deficient MSH6 or MSH6/MSH2 protein staining, while 7 patients had deficient MLH1 protein staining and no patient had deficient PMS2 protein staining(**Supplementary Table 2**). Suspected LS patients were significantly associated with no history of bladder cancer or concurrent bladder cancer, but were not associated with gender, pathological variables or the presence of personal or first-degree relatives LS-related cancer (**Supplementary Table 3**).

Germline Findings

A total of 38 suspected LS patients underwent germline genetic testing. WES analysis showed that pathogenic/likely pathogenic (P/LP) germline variants were identified in 22 of 38 (57.9%) individuals (**Figure 2**). Five cases (5/354, 1.4%) were LS cases, including 3 cases with novel mutations. Of the 5 LS patients, 1 patient presented weak MMR protein staining, 4 patients deficient MMR protein staining, and MSI-high/low tumors were found in 4 patients (**Table 1**). Besides, patients with MMR variants of uncertain significance were identified in 7 cases, and MSI-high/low tumors occurred in 5 cases (**Table 2**). Among the 12 patients with variant of uncertain significance or P/LP variant, 5 had muscle-invasive tumors and none of them experienced lymph node metastasis or had G3 tumors (**Tables 1**, **2** and **Supplementary Table 3**). Moreover, the published literature were systematically

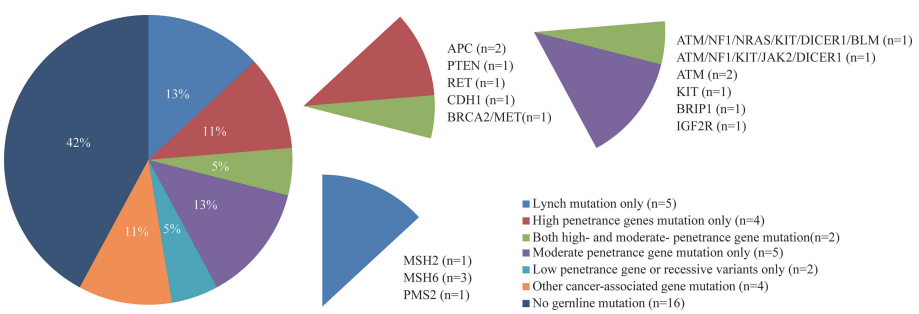

FIGURE 2 | Frequency and penetrance of germline pathogenic/likely pathogenic variants in patients with suspected LS-UTUC.

TABLE 1 | Germline likely pathogenic/pathogenic MMR mutations in 38 patients who underwent whole exon sequencing.

ID	Gender	Age	Multifocal	Pathological feature	MMR staining	MSI staining	MMR mutation	Genotype	Mutation types	Function	History of personal history/FDR	First description
P010	female	81	yes	T3G2N0	MSH6/ MSH2 -/-	high	MSH2: exon2: c.295A>T: p.Arg99*	Het	nonsense	pathogenic	colon cancer (herself, 45 years); sarcoma (herself, 70 years); bladder cancer (herself, 84 years)	this report
P075	male	41	no	T1G2N0	MSH6/ MLH1 -/w	low	MSH6: exon8: c.3699dupA: p.Glu1234fs	Het	frameshift	pathogenic	none	this report
P084	male	48	no	T1G2N0	MSH6/ MLH1/ PMS2 w/w/w	low	MSH6: exon9: c.3880delT: p.Cys1294fs	Het	frameshift	pathogenic	Lymphoma (his father, 45 years); thyroid cancer (his sister, 40 years); thyroid cancer (his son, 19 years)	this report
P094	female	50	no	T3G2N0	MSH6/ MLH1 -/-	low	PMS2: exon12: c.2012C>T: p.Thr671Met	Het	missense	likely pathogenic	none	rs587780046
P100	female	83	no	T1G2N0	MSH6 -	stable	MSH6: exon5: c.3261delC: p.Phe1088fs	Het	frameshift	pathogenic	Rectal cancer (himself, 55years)	rs267608078

MMR, mismatch repair; MSI, microsatellite instability; FDR, first-degree relative; Het, heterozygote.

reviewed and we found that the median age of LS-associated UTUC patients was 61 years (range 36-86 years), 73% (109 of 180) of patients had MSH2 LP/P mutation, 54% (96 of 179) of patients were female, and 36.6% (49 of 134) of patients had muscle-invasive tumors (**Supplementary Table 4** and **Figure 3**).

WES testing identified 6 cases with P/LP mutations in high-penetrance genes other than MMR genes, and 5 cases had mutations in moderate-penetrance genes only (**Figure 2**). The common P/LP variants in high- or moderate-penetrance genes were 4 in ATM, 3 in MSH6 and KIT, and 2 in APC, NF1 and

TABLE 2 | Germline MMR mutations with variant of uncertain significance in 38 patients who underwent whole exon sequencing.

ID	Gender	Age	Multifocal	Pathological feature	MMR staining	MSI status	Genotype	MMR mutation	Mutation types	History of personal history/FDR	First description
P003	male	55	no	T1G1N0	positive	stable	Het	MSH2:exon9: c.1470G>C: p.Lys490Asn	missense	Colon cancer (himself, 58ys; his brother, 45ys; his sister, 47ys); Rectal cancer (his brother, 53ys; his nephew, 35ys)	this report
P006	male	59	no	T1G2N0	positive	stable	Het	MSH6:exon10: c.4068_4071dupGATT: p.Lys1358fs	frameshift	Colon cancer (his uncle, 49ys; his grandmother, 45ys)	rs55740729
P009	male	56	no	T2G2N0	MSH6/ MSH2 weak/-	high	Het	MSH6:exon7: c.3587A>T: p.Glu1196Val	missense	none	this report
P024	male	57	no	T3G2N0	PMS2/ MSH6 weak/ weak	low	Het	MSH6:exon4: c.1501C>T: p.His501Tyr	missense	none	rs779411998
P030	male	41	no	T1G2N0	MSH6/ MSH2 -/-	high	Het	MSH2:exon3: c.489_494delTGGGTA	frameshift	endometrial cancer (his mother, 56ys)	this report
P066	female	57	no	T2G2N0	MSH6/ MLH1 -/weak	high	Het	MSH6:exon2: c.449C>T:p.Pro150Leu	missense	none	this report
P073	male	59	no	T1G2N0	MSH6/ MLH1 -/-	low	Het	MSH6:exon6: c.3529C>G: p.Leu1177Val	missense	none	rs748398941
P094	female	50	no	T3G2N0	MSH6/ MLH1 -/-	low	Het	MLH1:exon8: c.649C>T: p.Arg217Cys;	missense	none	rs4986984

MMR, mismatch repair; MSI, microsatellite instability; FDR, first-degree relative; Het, heterozygote.

DICER (**Figure 2** and **Supplementary Table 5**). Additionally, the most prevalent variants of uncertain significance in MMR, high- or moderate-penetrance genes were 8 in PKD1, 6 in FAT1, and 5 in MSH6 (**Supplementary Figure 3, 4** and **Supplementary Table 6**). Surprisingly, no LP/P or VUS mutation of TP53 gene was found in 38 patients. In total, 11 patients had germline P/LP variants in DNA damage repair (DDR) genes, and 19 patients had one or more DDR with variant of uncertain significance (**Figure 2** and **Supplementary Table 6**).

Clinical Characteristics Associated With P/LP Variants

In 38 patients who underwent WES, 16 patients had moderate- or high-penetrance P/LP variants (**Figure 2**). About 80% (4 out of 5) of patients with multi-organic cancer had moderate- or high-penetrance P/LP variant, and 62.5% (10 out of 16) of patients with moderate- or high-penetrance P/LP variant had a history of personal second malignancy or cancer in the first-degree relatives (**Figure 2** and **Supplementary Table 5**). Both probands (P003 and P006) met the Amsterdam II criteria, but they had positive MMR protein staining and a stable microsatellite status (**Supplementary Figure 5**), however, LP/P variants in MMR genes were not identified (**Supplementary Table 5**). Further germline mutation analysis showed that they all carried multiple other high- and moderate-penetrance LP/P variants (**Supplementary Table 5**). One case had one high-penetrance variant (CDH1) and 6 moderate-penetrance variants (ATM, NF1, NRAS, KIT, DICER1, and BLM), and another case had 2 high-risk variants (BRCA2 and MET) and 5 moderate-risk variants (ATM, NF1, KIT, DICER1 and JAK2). (**Supplementary Table 5**).

MSI Analysis

MSI was significantly associated with deficient MMR protein staining (p=0.018, **Supplementary Table 2**). In 21 patients with deficient MMR protein staining, approximately 81% of tumors were harboured MSI, but only 46.7% were harboured MSI in weak MMR protein staining by IHC (**Supplementary Figure 6**). In 5 LS cases, tumors of 4 patients were harboured MSI-H/L. In 7 patients with the MMR variants of uncertain significance, 5 patients had MSI-harboring tumors. Moreover, MSI-harboring tumors were found in 76.5% (13 out of 17) of patients with personal second cancer or with cancer in first-degree relatives (**Supplementary Table 2**).

DISCUSSION

In this study, we roughly identified the morbidity of LS-related UTUC and carriers with LP/P germline mutations in suspected LS patients. On account of recall bias, only 3 patients met the Amsterdam II criteria in our cohort, but were not identified as LS-associated UTUC. Evidently, a series of studies identified that approximately 90% of patients with LS-related UTUC present MSI-harboring tumors (4–6, 14, 15). Consistent with previous findings, our results showed that 80% of LS cases demonstrated MSI-harboring tumors and 71.4% of patients with the MMR variants of uncertain significance present MSI-harboring lesions (**Table 1**). In contrast, for patients with urothelial carcinoma (including pelvis, ureter and bladder tumors), Alicia Latham et al. (16) reported that only 3.6% of non-LS cases demonstrated MSI, and 37.5% of patients with MSI-harboring urothelial tumors were LS. Strict selection for MSI detection is probably the most likely reason for this discrepancy.

In our study, a total of 19% (4 out of 21) of patients with deficient MMR protein staining and 6.7% (1 out of 15) of patients with weak MMR protein staining were identified as LS-related UTUC. According to rigorous molecular diagnosis criteria, all tumor cells without any MMR protein staining could be considered a loss of MMR protein expression, which was then followed by genetic testing for LS. One patient (ID: P084) had an MSI-L-harboring tumor and weak MSH2 protein staining by IHC but was still identified as LS by genetic testing (**Table 1**). The IHC staining image of P084 is demonstrated in **Supplementary Figure 2**, which shows that the top of the tumor away from normal tissue showed deficient MSH2 protein staining, whereas the proximal tumor showed weak MMR protein staining. Although pathogenetic mutations in the MMR gene could lead to tumorigenesis in LS patients, the regulation of MMR gene expression in separate tumor areas is influenced by the temporal and spatial heterogeneity of tumor growth. Besides, it is very possible that tumor mosaicism which partly expresses MMR

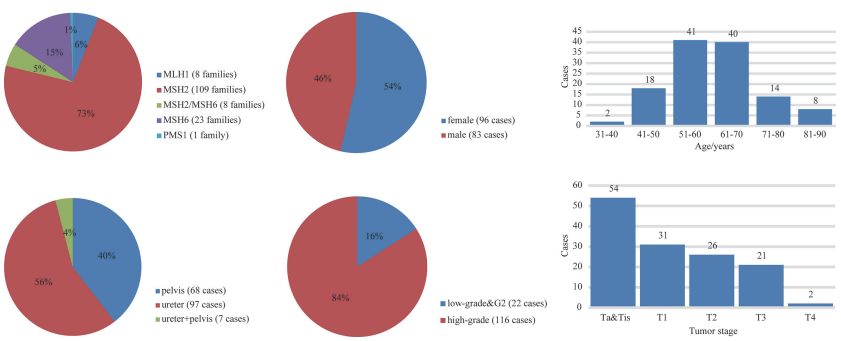

FIGURE 3 | The germline variants distribution and clinicopathological features of LS-UTUC by literature review.

protein contributes to several cells with stained. As a consequence, screening LS patients by IHC staining method remains further improved.

The prevalence of LS among consecutive UTUC patients was at least 1.4% (5 out of 354) in our cohort. Previous studies found that the incidence rate of LS-related UTUC ranged from 1.4% to 21.3% (5–7, 17). In our study, 3 novel LP/P MMR mutations were found, and 3 out of 5 LS-UTUCs were carriers of the MSH6 variant, but the major variant of previous studies was MSH2 (**Table 1** and **Figure 3**). Despite relatively small sample size and single-center nature, these data imply that the primary reason leading to this difference is probably LS diagnosis pathway and geographic distribution. In fact, it has been reported that aristolochic acid intake not only damages kidney tubules, resulting in renal insufficiency, but is also confirmed to be a carcinogen leading to urothelial carcinoma, especially in Asian regions (18, 19). Although LS patients were at higher risk for colorectal cancer, endometrial cancer and other LS-related tumors over their lifetimes, 5 LS-related UTUCs in our study were alive and no metastasis occurred within a median follow-up of 45 months. The Kaplan-Meier plot showed that LS patients tended to have a favorable prognosis (**Supplementary Figure 7**). Certainly, García-Tello et al. (20) discovered that MMR gene expression was associated with a favorable prognosis and Hollande et al. (21) also reported that adjuvant chemotherapy could improve survival rate of advanced UTUC patients with hereditary-like tumors compared with those with sporadic tumors. Accordingly, good surveillance annually to prevent disease recurrence and suitable treatment for surgical management or chemotherapy would be key to acquiring a good outcome.

In addition, we surprisingly confirmed unsuspected germline mutations in high- and moderate- susceptibility genes in 16 of 38 individuals (**Figure 2**). A multicenter study performed germline analysis with a 25-gene targeting sequencing panel from 1260 individuals who had a history of LS-associated tumor, and they identified 9.0% probands with LS mutations and 5.6% probands with mutations in non-LS cancer predisposition genes (9). In addition, a single-center retrospective study investigated the proportion of young colorectal cancer cases associated with genetic predisposition, and found that approximately 20% of individuals carried one or more cancer susceptibility gene mutations (13). Our study found 5 (13.2%) cases with LS mutations and 11 (28.9%) cases with high- and moderate-penetrance gene mutations in 38 suspected LS patients. Even a more rigorous screening criteria was performed in our study, performing NGS may be of great necessity in patients with young age of onset or a history of family LS-related cancer.

As previous studies reported, Yurgelun et al. (9) found that the common LP/P high or moderate genetic susceptibility genes in suspected LS patients included BRCA1/2, ATM, CHEK2 and APC. Moreover, Carlo et al. (10) discovered the most common germline P/LP variants were BRCA1/2, APC, CHEK2, ERCC3 and ATM. In our study, there was only one patient with a BRCA2 mutation, and none carried the CHEK2 variant (**Figure 2** and **Supplementary Table 5**). Two patients (P003 and P006) who met Amsterdam criteria, whose families suffered from colorectal cancer, lung cancer and bladder cancer, were not identified as MMR mutation carriers, but they carried many other tumor-associated genes, such as BRCA2, CDH1, MET, ATM, NF1. To date, BRCA1/2 has been very well studied in breast cancer and gynecological oncology, and BRCA2 mutation carriers have been described to be significantly associated with an elevated risk for prostate cancer, colorectal cancer and urothelial carcinoma (22–25). It has been reported that CDH1 and ATM germline mutation was associated with colorectal cancer (26, 27), and MET mutation plays an important role in lung cancer and colorectal cancer development and progression (28). However, we failed to acquire the medical record of neurofibroma, NF1 germline mutation associated tumor, in their families. In addition, APC germline mutations were found in some patients who had not a second cancer and family history of cancer (**Supplementary Table 5**). Recall bias and incomplete medical examination is the potential reason leading to disagreement between genotype and phenotype correspondences.

Recently, DDR somatic and inherited mutations have been found to independently guide immune checkpoint inhibitor therapy for individuals with advanced urothelial carcinoma (29), so we analyzed the prevalence of DDR mutations in suspected LS-related UTUC patients. We found that 11 patients carried germline P/LP variants in DDR genes, and 19 patients carried one or more variants of uncertain significance (**Supplementary Tables 5, 6** and **Supplementary Figure 3**). To date, the efficacy of chemotherapy for advanced/metastatic cancer in individuals with LS or MSI-H has not yet been clarified, but the efficacy of PD-1 inhibition in metastatic deficient MMR protein staining or MSI-H solid tumors has been demonstrated (30–32). Therefore, germline DDR mutation testing should actually be considered when evaluating its association with therapeutic benefit. Accordingly, whether germline variants of uncertain significance in these genes are also related to treatment and survival need further exploration.

Our research has several limitations. First, single-center and small sample retrospective studies are the main shortcomings. Clinical data concerning personal/family histories and prognosis information were retrospectively acquired by medical reports and telephone interviews, so we could not confirm its accuracy and integrity. Next, we were unable to detect MLH1 promoter hypermethylation in patients with MSI or deficient MMR protein staining, resulting in underestimation of the morbidity of LS. Finally, approximately 10% of LS-related colorectal patients showed intact MMR protein staining on IHC (2), but we only assessed genetic susceptibility gene mutations of suspected LS based on MMR protein staining or clinical criteria. As a result, we cannot fully identify potential germline variants of high-risk patients, such as in the younger patients.

Despite these limitations, our study's main strength provides a promising direction regarding the hereditary risk assessment of suspected LS patients in the age of NGS. The advantage of such NGS diagnosis approach has been broadly discussed, and moreover, NGS testing strategies based on the phenotypes of

probands have been performed in secondary analyses of germline mutation evaluation, and many potential non-LS pathogenic germline variants have been identified (9, 10, 13). **Supplementary Figure 8** provides a feasible genetic mutation analysis pathway for possible hereditary UTUC individuals. However, extensive use of NGS will undoubtedly bring about a dilemma in which increased subjects will be identified as variants of uncertain significance or other inherited variants with vague clinical significance. How patients with unexpected mutations identified by NGS are properly managed probably becomes a growingly prevalent issue for genetic clinicians.

CONCLUSION

We identified approximately 11% of UTUC patients as suspected LS and at least 1.4% of patients as LS-related UTUC. In addition, in individuals with suspected LS, NGS identified many unexpected high- and moderate- penetrance mutations in genetic predisposition genes. Therefore, broader germline genetic testing, particularly NGS, could be considered to screen for cancer severity in hereditary UTUC patients.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Peking University First Hospital Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements. Written informed consent was not obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LZ supervised all the studies. LZ, XL, YG, and SH designed the study. BG, JW, QT, and LL performed the acquisition of the data and sequencing. BG and JW analyzed and interpreted the data. BG, QT, and LL performed the statistical analysis. BG and JW drafted the manuscript. BG, DF, SH, YG, XL, and LZ revised the manuscript. WK, CT, and JL contributed material support. KY, GH, YW, and YH contributed the sample collection and clinical information. QT, LL, and YP contributed material support. YY and QH contributed administrative and technical support. All authors critically commented on and approved the final submitted version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.774202/full#supplementary-material

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Risk Factors for Unfavorable Pathological Types of Intravesical Recurrence in Patients With Upper Urinary Tract Urothelial Carcinoma Following Radical Nephroureterectomy

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Background: Numerous studies have investigated the risk factors of intravesical recurrence (IVR) after radical nephroureterectomy (RNU) in patients with upper urinary tract urothelial carcinoma (UTUC). However, few studies explore the predictors for unfavorable pathological types of IVR following RNU.

Methods: We retrospectively reviewed 155 patients diagnosed with bladder cancer (BC) following RNU. Binary logistic regression was used for the univariable and multivariable analyses. Nomograms were developed based on the multivariable analysis. The concordance index (C-index) was used to assess the performance of the nomograms. We performed internal validation by generating calibration plots.

Results: Muscle-invasive BC (MIBC) was significantly correlated with operation interval ($p = 0.004$) and UTUC T-stage ($p = 0.016$). Operation interval ($p = 0.002$) and UTUC T-stage ($p = 0.028$) were also risk factors for BC > 3 cm. UTUC grade ($p = 0.015$), operation interval ($p = 0.003$), and hydronephrosis ($p = 0.049$) were independent predictors for high-grade BC (HGBC). MIBC ($p = 0.018$) and surgical approach ($p = 0.003$) were associated with multifocal IVR. Besides, MIBC and HGBC were associated with UTUC grade ($p = 0.009$), operation interval ($p = 0.001$), and hydronephrosis ($p = 0.023$). Moreover, only operation interval ($p = 0.036$) was a predictor for BC with at least one unfavorable pathological type. We developed nomograms for MIBC, HGBC, BC > 3 cm, and MIBC and/or HGBC. The calibration curves of the nomograms showed good agreement between the observation and prediction cases. The C-indexes of the nomograms were 0.820 (95% CI, 0.747–0.894), 0.728 (95% CI, 0.649–0.809), 0.770 (95% CI, 0.679–0.861), and 0.749 (95% CI, 0.671–0.827), respectively.

Conclusions: The current study found that operation interval, UTUC T-stage, UTUC grade, surgical approach, and hydronephrosis are independent predictors for unfavorable pathological types of IVR following RNU. Nomograms based on these predictors were developed and internally validated to assess the risk of developing unfavorable pathological types of IVR. Furthermore, patients at high risk of developing unfavorable pathological types BC may benefit from more active follow-up 1 year after RNU by early detection of IVR.

Keywords: upper urinary tract, urothelial carcinoma, nephroureterectomy, bladder recurrence, risk factors

INTRODUCTION

Upper urinary tract urothelial carcinoma (UTUC) is a relatively uncommon disease and accounts for 5%–10% of urothelial carcinomas (1). About 60% of UTUCs are invasive at primary diagnosis. Radical nephroureterectomy (RNU) with the removal of bladder cuff excision is the gold standard treatment. Approximately 22%–47% of UTUC patients develop an intravesical recurrence (IVR) after RNU (2–4). Therefore, IVR monitoring following RNU is of great importance.

Although a few studies have looked into the risk factors for IVR after RNU for UTUC, few have tried to look into predictors for the malignant degree of secondary bladder cancer (BC) (5–7). Presently, IVR after RNU is managed according to the treatment guidelines for primary BC. Transurethral resection of the bladder tumor is recommended for favorable pathological types of IVR, while radical cystectomy (RC) is a curative treatment for most unfavorable pathological types of IVR (8, 9). Considering that advanced IVR after RNU predicts a worse prognosis and necessitates more aggressive treatment, it is critical to find predictors for unfavorable pathological types BC after RNU, such as muscle-invasive, high-grade, >3-cm-diameter, and multifocal IVR.

This study aimed to identify the independent risk factors and develop prediction models based on the predictors for unfavorable pathological types of IVR after RNU.

MATERIALS AND METHODS

Patients

Our institutional review board approved this study. A review of the archived medical records of our hospital identified 169 patients who had IVR after undergoing RNU with curative intent for primary UTUC between January 2002 and September 2021 in Peaking University First Hospital, Beijing, China. We excluded three patients who had previous bladder tumors, two patients with a history of renal transplantation, and nine patients with incomplete follow-up data. Thus, this study included a total of 155 patients. Routine postoperative follow-up and cystoscopy were performed, and no bladder instillation treatment and adjuvant therapy were performed after RNU. Urologic ultrasound, CT, MRI, and ureteroscopy with or without biopsy were used to diagnose all the cases. Before surgery, cystoscopy and chest X-ray were performed to exclude concomitant bladder tumor and metastasis. Further, surgical methods include open and laparoscopic RNU. The distal ureter and bladder cuff excision was performed using the open extravesical technique and the intramural portion within the bladder wall through an open Gibson incision. Postoperative bladder instillation was not adapted after surgery.

Clinical Variables and Tumor-Related Variables

The collected clinical data included age, sex, body mass index (BMI), smoking and drinking status, history of taking aristolochic acid (AA), preoperative ureteroscopic biopsy, hydronephrosis, surgical approach, neutrophil–lymphocyte ratio (NLR), operation interval (time between RNU and management of BC), and pathological features (multifocality, location, stage, grade, and maximum diameter). All tumors were staged by the Union for International Cancer Control TNM classification of malignant tumors in 2002 and graded according to the WHO classification of 2004. Besides, patients were grouped based on muscle-invasive BC (MIBC) and/or high-grade BC (HGBC) after RNU since patients must receive cystectomy based on the guideline. We also grouped patients by BC with at least one of the above four unfavorable pathological types of IVR to identify predictors for unfavorable pathological types of BC following RNU without dissecting around the kidney. The definition of smoking status was not homogeneous in different studies (10). Our study divided smoking status into three groups, non-smoker, former smoker, and current smoker, based on our data. All medical records were reviewed by two researchers independently.

Statistical Analysis

All analyses were performed using R version 3.6.1 (The R Foundation) and SPSS (version 24.0, IBM, Armonk, NY, USA). Univariate binary logistic regression was performed for each variable to explore the independent predictors for unfavorable pathological types of IVR. Factors with a p-value <0.2 were included for stepwise multivariate binary logistic analysis. The

Abbreviations: UTUC, upper urinary tract urothelial carcinoma; RNU, radical nephroureterectomy; IVR, intravesical recurrence; BC, bladder cancer; BMI, body mass index; AA, aristolochic acid; NLR, neutrophil–lymphocyte ratio; MIBC, muscle-invasive bladder cancer; HGBC, high-grade bladder cancer; OR, odds ratio; C-index, concordance index; IQR, interquartile range.

calculation was done based on odds ratios (ORs) and 95% CI, and a value of $p < 0.05$ was considered statistically significant. Based on the multivariate binary logistic regression analysis results, nomograms were developed using R's rms package version 3.0. The performance of the prediction model was measured using the concordance index (C-index). We conducted internal validation by constructing calibration plots and validated them with 500 bootstrap samples to reduce bias.

RESULTS

Patients and Tumor Characteristics

Table 1 summarizes the demographic information of included patients. The median patient age was 66 years (range: 31–87 years), and the median operation interval was 25.8 months (interquartile range (IQR), 7–33.5 months). Within 1 year of the primary procedure, 67 patients out of 155 (43.2%) were diagnosed with BC.

Independent Risk Factors for Unfavorable Pathological Types of Intravesical Recurrence

Tables 2, **3**, **4**, **5**, **6**, **7** present the results of univariate and multivariate analyses. For multivariate analysis, risk factors with a p-value of less than 0.2 were included. The operation interval ($p = 0.004$) and stage of primary UTUC ($p = 0.016$) were significantly correlated with MIBC after RNU. BC > 3 cm after RNU was also associated with operation interval ($p = 0.002$) and stage of primary UTUC ($p = 0.028$). High-grade IVR was predicted independently by UTUC grade ($p = 0.015$), operation interval ($p = 0.003$) and hydronephrosis ($p = 0.049$). MIBC ($p = 0.018$) and surgical approach ($p = 0.003$) were associated with multifocal IVR. Besides, following RNU, MIBC and HG-BC were linked to primary tumor grade ($p = 0.009$), operation interval ($p = 0.001$), and hydronephrosis ($p = 0.023$). Furthermore, only the operation interval ($p = 0.036$) was a predictor of BC with at least one unfavorable pathological type. Finally, we divided our research group into two groups based on the time between operations, with a 1-year cutoff. Operation interval was associated with all unfavorable pathological types of IVR except for the multifocal type in the more than 1 year group, while it was only correlated with MIBC in the other group (**Supplementary Tables 1–6**).

Prediction Models for Unfavorable Pathological Types of Intravesical Recurrence

These independent risk factors were pooled to develop the prediction models. We developed nomograms for MIBC, HGBC, BC > 3 cm, and MIBC and/or HGBC. The nomograms are presented in **Figure 1**. The calibration curves of the prediction models demonstrated good agreement between the observation and prediction cases in our cohort (**Figure 2**). Moreover, the C-indexes of the above nomograms were 0.820 (95% CI, 0.747–0.894), 0.728 (95% CI, 0.649–0.809), 0.770 (95% CI, 0.679–0.861), and 0.749 (95% CI, 0.671–0.827).

TABLE 1 | Clinicopathologic characteristics of patients.

Characteristics	Number of patients No. (%) (n = 154)
Median age	68 (range, 31–87)
Mean operation interval	25.8 (IQR, 7–33.5 months)
Operation interval ≤12 months	67 (43.2)
Operation interval >12 months	88 (56.8)
Gender	
Male	80 (51.6)
Female	75 (48.4)
Median BMI	24.2 (IQR, 21.9–26.7)
BMI	
<25	96 (61.9)
≥25	59 (38.1)
Stage of UTUC	
<T2	64 (41.3)
≥T2	91 (58.7)
Grade of UTUC	
Low grade	49 (31.6)
High grade	106 (68.4)
Multifocal tumor	
Yes	33 (21.3)
No	122 (78.7)
Diameter of UTUC	
≤3 cm	97 (62.6)
>3 cm	58 (37.4)
Location	
Renal pelvis	68 (43.8)
Ureter	77 (49.7)
Both	10 (6.5)
Preoperative ureteroscopic biopsy	
Yes	25 (16.1)
No	130 (83.9)
Hydronephrosis	
Yes	92 (59.4)
No	63 (40.6)
AA	
Yes	10 (6.5)
No	145 (93.5)
Smoking	
Never	121 (78.1)
Former	9 (5.8)
Current	25 (16.1)
Drinking	
Yes	10 (6.5)
No	142 (93.5)
Surgical approach	
Open	59 (38.1)
Laparoscopic	96 (61.9)
Median NLR	2.6 (IQR, 1.9–3.9)
NLR ≥ 2.5	
Yes	82 (52.9)
No	73 (47.1)
MIBC	
Yes	16 (10.3)
No	139 (89.7)
HGBC	
Yes	69 (44.5)
No	86 (55.5)
Multifocal BC	
Yes	97 (62.6)
No	58 (37.4)
Diameter of BC	
≤3 cm	136 (87.7)
>3 cm	19 (12.3)

(Continued)

TABLE 1 | Continued

Characteristics	Number of patients No. (%) (n = 154)
MIBC and/or HGBC	
Yes	71 (45.8)
No	84 (54.2)
BC with at least one unfavorable pathological type	
Yes	120 (77.4)
No	35 (22.6)

IQR, interquartile range; BMI, body mass index; UTUC, upper urinary tract urothelial carcinoma; NLR, neutrophil–lymphocyte ratio; MIBC, muscle-invasive bladder cancer; HGBC, high-grade bladder cancer; BC, bladder cancer.

DISCUSSION

UTUC is a relatively rare malignancy, accounting for 5%–10% of urothelial carcinomas. Despite significant advances in diagnosis and management, 22%–47% of patients still develop IVR after RNU (3, 11). Many studies have identified risk factors for IVR following RNU, including T-stage, multifocality, diagnostic preoperative ureteroscopic biopsy, male sex, previous BC, smoking, chronic kidney disease, necrosis, tumor location, transurethral resection of the bladder cuff, positive resection margin, and surgical approach (7, 12–16). However, limited data are available about unfavorable pathological types of BC that develop after RNU. Our study explores the predictors and

TABLE 2 | Univariate and multivariate analyses for factors associated with MIBC after RNU.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Operation Interval	1.017 (1.003–1.032)	0.016	1.027 (1.009–1.450)	0.004
Stage of UTUC (<T2 *vs.*≥T2)	5.636 (1.234–25.742)	0.026	8.992 (1.499–53.108)	0.016
AA	0.963 (0.114–8.136)	0.972		
Age	1.038 (0.983–1.097)	0.180		
BMI	1.104 (0.95–1.284)	0.197		
BMI (<25 *vs.*≥25)	2.289 (0.803–6.518)	0.121	2.679 (0.855–8.401)	0.091
Diameter of UTUC (≤3 cm *vs.*>3 *cm*)	0.525 (0.161–1.711)	0.285		
Drinking	0.706 (0.086–5.814)	0.746		
Gender	0.703 (0.248–1.994)	0.508		
Grade of UTUC	3.576 (0.780–16.395)	0.101		
Hydronephrosis	3.291 (0.897–12.07)	0.072	3.710 (0.899–15.311)	0.070
Multifocal tumor	0.223 (0.028–1.753)	0.154		
Surgical approach	1.027 (0.353–2.990)	0.961		
NLR	1.079 (0.980–1.188)	0.121		
NLR (<2.5 *vs.*≥2.5)	0.878 (0.312–2.473)	0.806		
Smoking	1.161 (0.350–3.856)	0.807		
Preoperative ureteroscopic biopsy	0.720 (0.153–3.387)	0.678		
Location		0.272		
(Ureter *vs.*renal pelvis)	2.667 (0.807–8.809)	0.108		
(Both *vs.*renal pelvis)	1.778 (0.178–17.727)	0.624		

MIBC, muscle-invasive bladder cancer; RNU, radical nephroureterectomy; OR, odds ratio; UTUC, upper urinary tract urothelial carcinoma; AA, aristolochic acid; BMI, body mass index; NLR, neutrophil–lymphocyte ratio.

TABLE 3 | Univariate and multivariate analyses for factors associated with HGBC after RNU.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-Value	OR (95% CI)	p-Value
Operation Interval	1.023 (1.009–1.038)	0.034	1.022 (1.008–1.037)	0.003
Stage of UTUC (<T2 *vs.*≥T2)	2.039 (1.054–3.944)	0.316		
AA	1.952 (0.528–7.215)	0.253		
Age	1.018 (0.987–1.050)	0.826		
BMI	1.011 (0.921–1.109)	0.564		
BMI (<25 *vs.*≥25)	1.212 (0.631–2.325)	0.952		
Diameter of UTUC (≤3 cm *vs.*>3 *cm*)	1.020 (0.530–1.963)	0.304		
Drinking	0.526 (0.155–1.789)	0.602		
Gender	0.845 (0.448–1.593)	0.008		
Grade of UTUC	2.696 (1.302–5.581)	0.098	2.636 (1.206–5.761)	0.015
Hydronephrosis	1.739 (0.903–3.351)	0.034	2.079 (1.005–4.303)	0.049
Multifocal tumor	0.766 (0.350–1.678)	0.505		
Surgical approach	0.422 (0.217–0.819)	0.011	0.511 (0.246–1.059)	0.071
NLR	1.062 (0.966–1.168)	0.212		
NLR (<2.5 *vs.*≥2.5)	0.769 (0.407–1.452)	0.418		

(Continued)

TABLE 3 | Continued

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	**p-Value**	**OR (95% CI)**	**p-Value**
Smoking	1.433 (0.673–3.049)	0.351		
Preoperative ureteroscopic biopsy	0.532 (0.215–1.32)	0.174		
Location		0.163		
(Ureter *vs.*renal pelvis)	0.302 (1.418–0.731)	0.302		
(Both *vs.*renal pelvis)	3.769 (0.895–15.88)	0.071		

HGBC, high-grade bladder cancer; RNU, radical nephroureterectomy; OR, odds ratio; UTUC, upper urinary tract urothelial carcinoma; AA, aristolochic acid; BMI, body mass index; NLR, neutrophil–lymphocyte ratio.

TABLE 4 | Univariate and multivariate analyses for factors associated with multifocal BC after RNU.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	**p-Value**	**OR (95% CI)**	**p-Value**
Operation Interval	1.009 (0.996–1.022)	0.167		
Stage of UTUC (<T2 *vs.*≥T2)	0.499 (0.252–0.989)	0.046	0.416 (0.201–0.860)	0.018
AA	2.517 (0.516–12.282)	0.254		
Age	0.981 (0.950–1.012)	0.227		
BMI	0.965 (0.877–1.061)	0.459		
BMI (<25 *vs.*≥25)	1.135 (0.579–2.224)	0.713		
Diameter of UTUC (≤3 cm *vs.*>3 *cm*)	1.224 (0.621–2.410)	0.559		
Drinking	0.674 (0.215–2.113)	0.499		
Gender	1.238 (0.645–2.375)	0.521		
Grade of UTUC	1.088 (0.542–2.185)	0.813		
Hydronephrosis	0.520 (0.262–1.031)	0.061	0.593 (0.288–1.220)	0.155
Multifocal tumor	1.059 (0.477–2.353)	0.888		
Surgical approach	0.368 (0.179–0.757)	0.007	0.322 (0.151–0.686)	0.003
NLR	0.979 (0.901–1.065)	0.624		
NLR (<2.5 *vs.*≥2.5)	0.551 (0.284–1.070)	0.078	0.560 (0.280–1.121)	0.102
Smoking	0.868 (0.401–1.878)	0.720		
Preoperative ureteroscopic biopsy	1.328 (0.534–3.305)	0.542		
Location		0.379		
(Ureter *vs.*renal pelvis)	0.638 (0.323–1.258)	0.194		
(Both *vs.*renal pelvis)	1.116 (0.263–4.733)	0.882		

BC, bladder cancer; RNU, radical nephroureterectomy; OR, odds ratio; UTUC, upper urinary tract urothelial carcinoma; AA, aristolochic acid; BMI, body mass index; NLR, neutrophil–lymphocyte ratio.

TABLE 5 | Univariate and multivariate analyses for factors associated with BC greater than 3 cm after RNU.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	**p-Value**	**OR (95% CI)**	**p-Value**
Operation Interval	1.019 (1.006–1.033)	0.005	1.024 (1.009–1.039)	0.002
Stage of UTUC (<T2 *vs.*≥T2)	2.961 (0.934–9.385)	0.065	4.286 (1.166–15.756)	0.028
Age	1.002 (0.957–1.049)	0.922		
BMI	1.101 (0.957–1.266)	0.180		
BMI (<25 *vs.*≥25)	1.973 (0.751–5.184)	0.168		
Diameter of UTUC (≤3 cm *vs.*>3 *cm*)	1.251 (0.472–3.317)	0.653		
Drinking	0.574 (0.070–4.683)	0.604		
Gender	1.048 (0.401–2.740)	0.924		
Grade of UTUC	1.854 (0.582–5.911)	0.296		
Hydronephrosis	1.200 (0.445–3.238)	0.719		
Multifocal tumor	1.258 (0.704–2.249)	0.534		
Surgical approach	0.646 (0.246–1.697)	0.375		
NLR	0.663 (0.181–2.426)	0.196		
NLR (<2.5 *vs.*≥2.5)	1.064 (0.968–1.170)	0.607		
Smoking	0.777 (0.297–2.032)	0.678		
Preoperative ureteroscopic biopsy	1.262 (0.421–3.786)	0.483		

BC, bladder cancer; RNU, radical nephroureterectomy; OR, odds ratio; UTUC, upper urinary tract urothelial carcinoma; AA, aristolochic acid; BMI, body mass index; NLR, neutrophil–lymphocyte ratio.

TABLE 6 | Univariate and multivariate analyses for factors associated with MIBC and/or HGBC after RNU.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	**p-Value**	**OR (95% CI)**	**p-Value**
Operation Interval	1.026 (1.011–1.042)	0.001	1.025 (1.010–1.041)	0.001
Stage of UTUC (<T2 *vs.*≥T2)	2.227 (1.151–4.310)	0.017		
AA	1.846 (0.500–6.820)	0.358		
Age	1.024 (0.993–1.056)	0.134		
BMI	0.995 (0.906–1.091)	0.907		
BMI (<25 *vs.*≥25)	1.113 (0.581–2.133)	0.746		
Diameter of UTUC (≤3 cm *vs.*>3 *cm*)	1.172 (0.610–2.251)	0.633		
Drinking	0.498 (0.146–1.690)	0.263		
Gender	0.759 (0.403–1.430)	0.394		
Grade of UTUC	2.908 (1.404–6.022)	0.004	2.889 (1.307–6.386)	0.009
Hydronephrosis	1.897 (0.984–3.656)	0.056	2.360 (1.125–4.953)	0.023
Multifocal tumor	0.717 (0.328–1.570)	0.406		
Surgical approach	0.411 (0.212–0.799)	0.009	0.496 (0.236–1.042)	0.064
NLR	1.057 (0.962–1.161)	0.248		
NLR (<2.5 *vs.*≥2.5)	0.765 (0.406–1.443)	0.408		
Smoking	1.339 (0.629–2.846)	0.449		
Preoperative ureteroscopic biopsy	2.462 (1.247–4.859)	0.009		
Location		0.189		
(Ureter *vs.*renal pelvis)	1.405 (0.726–2.718)	0.313		
(Both *vs.*renal pelvis)	3.543 (0.842–14.911)	0.084		

MIBC, muscle-invasive bladder cancer; HGBC, high-grade bladder cancer; RNU, radical nephroureterectomy; OR, odd ratio; UTUC, upper urinary tract urothelial carcinoma; AA, aristolochic acid; BMI, body mass index; NLR, neutrophil–lymphocyte ratio.

TABLE 7 | Univariate and multivariate analyses for factors associated with BC with at least one unfavorable pathological type after RNU.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	**p-Value**	**OR (95% CI)**	**p-Value**
Operation Interval	1.031 (1.006–1.058)	0.016	1.027 (1.002–1.053)	0.036
Stage of UTUC (<T2 *vs.*≥T2)	0.933 (0.433–2.011)	0.860		
AA	2.757 (0.337–22.544)	0.344		
Age	0.999 (0.963–1.035)	0.940		
BMI	1.016 (0.909–1.135)	0.777		
BMI (<25 *vs.*≥25)	1.725 (0.761–3.912)	0.192		
Diameter of UTUC (≤3 cm *vs.*>3 *cm*)	1.015 (0.466–2.213)	0.969		
Drinking	0.429 (0.131–1.406)	0.162		
Gender	0.871 (0.409–1.853)	0.719		
Grade of UTUC	1.619 (0.740–3.541)	0.227		
Hydronephrosis	0.966 (0.448–2.082)	0.930		
Multifocal tumor	0.722 (0.300–1.741)	0.469		
Surgical approach	0.327 (0.132–0.807)	0.015	0.412 (0.163–1.042)	0.061
NLR	1.029 (0.916–1.156)	0.633		
NLR (<2.5 *vs.*≥2.5)	0.591 (0.273–1.281)	0.182		
Smoking	0.799 (0.334–1.914)	0.615		
Preoperative ureteroscopic biopsy	0.909 (0.332–2.488)	0.853		
Location		0.959		
(Ureter *vs.*renal pelvis)	1.086 (0.499–2.362)	0.835		
(Both *vs.*renal pelvis)	1.231 (0.237–6.394)	0.805		

BC, bladder cancer; RNU, radical nephroureterectomy; OR, odds ratio; UTUC, upper urinary tract urothelial carcinoma; AA, aristolochic acid; BMI, body mass index; NLR, neutrophil–lymphocyte ratio.

establishes prediction models for unfavorable pathological types of IVR following RNU.

The present study showed that operation interval and T-stage of primary UTUC were risk factors for MIBC and BC > 3 cm after RNU, consistent with Kim's results (17). We first found that grade of UTUC (p = 0.015), hydronephrosis (p = 0.049), and operation interval (p = 0.003) were independent risk factors for HGBC. Besides, MIBC and HGBC following RNU were associated with UTUC grade (p = 0.009), operation interval (p = 0.001), and hydronephrosis (p = 0.023). These findings support the

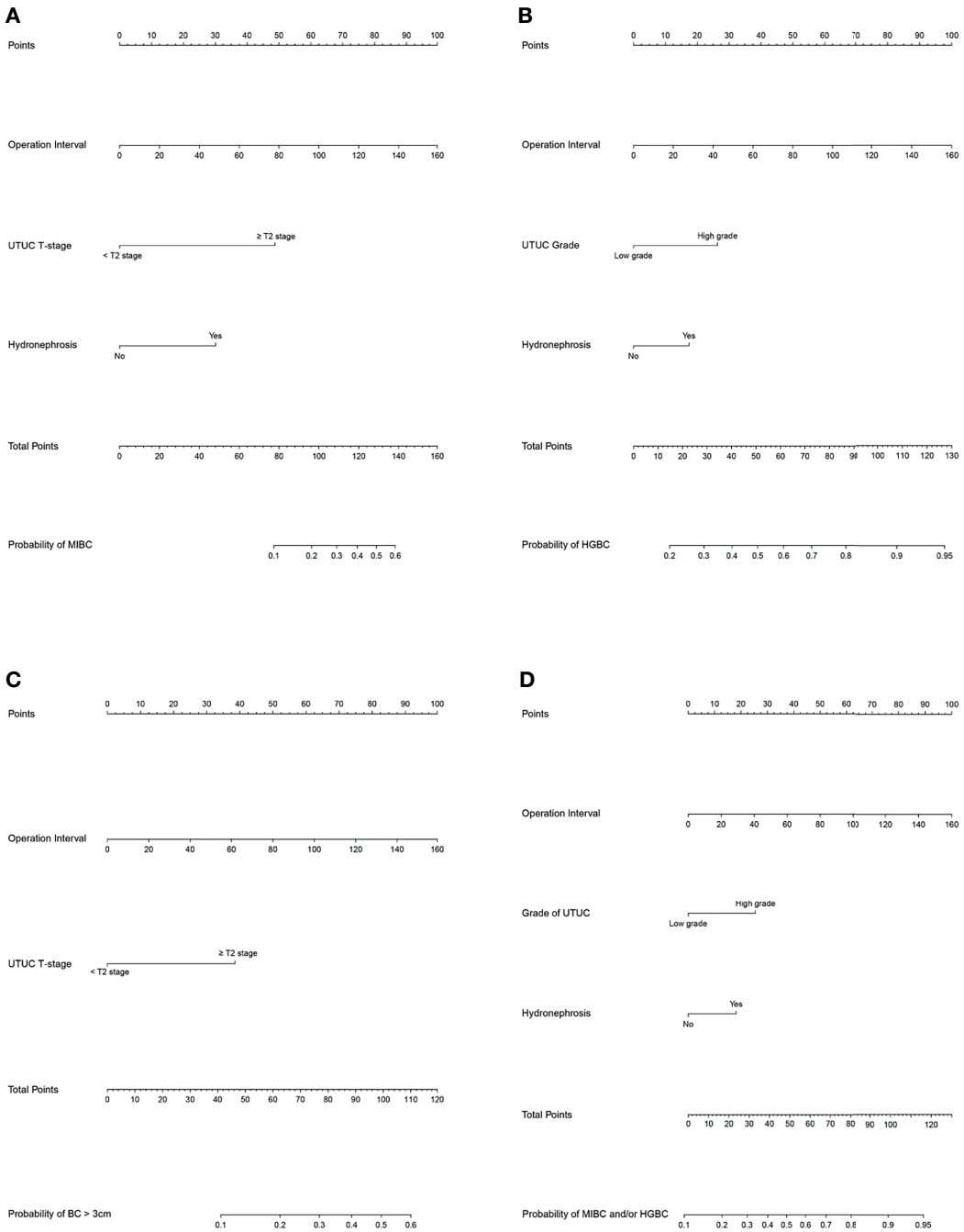

FIGURE 1 | Nomograms for the prediction of unfavorable pathological types intravesical recurrence (IVR) following radical nephroureterectomy (RNU). **(A)** Nomogram to predict the risk of muscle-invasive bladder cancer (MIBC) after RNU. **(A)** Bomogram to predict the risk of high-grade bladder cancer (HGBC) after RNU. **(C)** Nomogram to predict the risk of bladder cancer >3 cm after RNU. **(D)** Nomogram to predict the risk of MIBC and/or HGBC after RNU. To use the nomogram, first, assign the points of each variable of the patient by drawing a line straight up to the top line labeled "Points". Sum up the points of the risk factors as total points. Then draw a vertical line down from the axis labeled "Total Points" to the bottom line to get the predicted possibility of unfavorable pathological types intravesical recurrence (IVR) following RNU.

intraluminal seeding hypothesis as a predominant mechanism of IVR following RNU (7, 18). Moreover, MIBC ($p = 0.018$) and surgical approach ($p = 0.003$) were associated with multifocal BC after RNU. Open surgery is a risk factor for multifocal IVR, which may cause a poor prognosis, consistent with a randomized prospective study (15). Only operation interval ($p = 0.036$) was a predictor for BC with at least one unfavorable pathological type, suggesting that follow-up after RNU is of great importance.

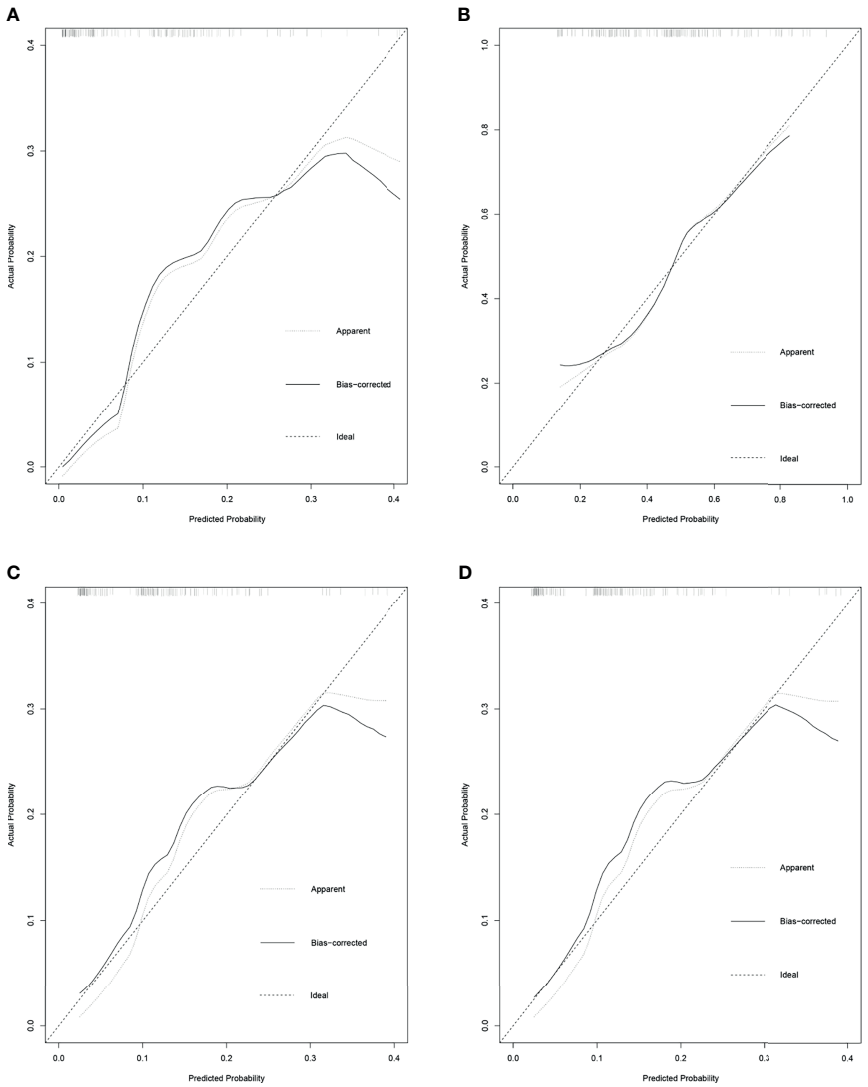

FIGURE 2 | The calibration curves for the prediction nomograms of unfavorable pathological types intravesical recurrence following radical nephroureterectomy (RNU). **(A)** Calibration curves for the prediction nomograms of muscle-invasive bladder cancer (MIBC) after RNU. **(B)** Calibration curves for the prediction nomograms of high-grade bladder cancer (HGBC) after RNU. **(C)** Calibration curves for the prediction nomograms of bladder cancer >3 cm after RNU. **(D)** Calibration curves for the prediction nomograms of MIBC and/or HGBC after RNU.

Smoking at the time of RC is significantly correlated with increased risk for postoperative infections, complications, and perioperative mortality.

We chose the time between two procedures as an indicator to explore the relationship between time after RNU and the malignant degree of IVR, considering it is an objective variable. Operation interval was a predictor for all unfavorable pathological types of IVR except multifocal type. We further investigated the influence of operation interval on the malignant degree of IVR by dividing the cohort into two groups with a cutoff of 1 year. Operation interval was correlated with MIBC, HGBC, BC > 3 cm, and MIBC and/or HGBC in the more than 1 year group. However, operation interval was only associated with MIBC in the other group. There are currently different opinions on an optimal surveillance regimen in UTUC patients after RNU (19–21). It is widely accepted that patients with low-risk tumors are recommended to receive cystoscopy at 3 months. If negative, perform cystoscopy after 9 months and then yearly for 5 years. For patients with high-risk tumors, both cystoscopy and urinary cytology should be performed at 3 months. If negative, perform repeat cystoscopy and cytology every 3 months for 2 years, every 6 months after that until 5 years, and then yearly. Further, CT urography and chest CT should be performed every 6 months for 2 years, and then once a year. Most studies took 2 years as a cutoff when exploring the follow-up protocol (4, 22–25). However, operation interval was an independent risk factor for unfavorable pathological types of IVR except for the multifocal type in the more than 1 year group, while only associated with MIBC in the

other group. Moreover, previous studies have shown that the risk of recurrence and death increases during the follow-up (26). Hence, patients with a high risk of developing unfavorable pathological types BC may benefit from more active follow-up 1 year after RNU by early detection of recurrence. We also established prediction models for unfavorable pathological types BC, which can help identify patients with a high risk of developing unfavorable pathological types of IVR.

Notably, the present study has several limitations because it was a retrospective study that may have caused inherent selection bias and was conducted on a single hospital. Besides, the sample size was relatively small. Moreover, the predictive models were internally validated to exhibit good performance; thus, external validation by long-term follow-up in multiple centers is still required to verify the utility of the nomograms.

CONCLUSION

To summarize, the current study identified independent risk factors for unfavorable pathological types of BC developing after RNU, such as operation interval, UTUC T-stage, UTUC grade, surgical approach, and hydronephrosis. To assess the risk of developing unfavorable pathological types BC after RNU, accurate prediction models based on these predictors were developed and internally validated. Furthermore, patients at high risk of developing unfavorable pathological types BC may benefit from more active follow-up 1 year after RNU by early diagnosis of IVR.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Peking University First Hospital Institutional Review Board. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GX and ZZ conceived the study. JZ and XZ extracted all the data and performed the analyses. LZ, ZH, XL, and WY supervised the project and provided guidance throughout the preparation of this manuscript. JZ and XZ prepared the tables and figures. JZ wrote the manuscript. GX provided funding. All authors contributed to and revised the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.834692/full#supplementary-material

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Impact of Adjuvant Chemotherapy on Variant Histology of Upper Tract Urothelial Carcinoma: A Propensity Score-Matched Cohort Analysis

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Background: The advantage of adjuvant chemotherapy for upper urinary tract urothelial cancer (UTUC) has been reported, whereas its impact on upper tract cancer with variant histology remains unclear. We aimed to answer the abovementioned question with our real-world data.

Design, Setting, and Participants: Patients who underwent radical nephroureterectomy (RNU) and were confirmed to have variant UTUC were retrospectively evaluated for eligibility of analysis. In the Taiwan UTUC Collaboration database, we identified 245 patients with variant UTUC among 3,109 patients with UTUC who underwent RNU after excluding patients with missing clinicopathological information.

Intervention: Those patients with variant UTUC were grouped based on their history of receiving adjuvant chemotherapy or not.

Outcome Measurements and Statistical Analysis: Propensity score matching was used to reduce the treatment assignment bias. Multivariable Cox regression model was used for the analysis of overall, cancer-specific, and disease-free survival.

Results and Limitations: For the patients with variant UTUC who underwent adjuvant chemotherapy compared with those without chemotherapy, survival benefit was identified in overall survival in univariate analysis (hazard ratio (HR), 0.527; 95% confidence interval (CI), 0.285–0.973; $p = 0.041$). In addition, in multivariate analysis, patients with adjuvant chemotherapy demonstrated significant survival benefits in cancer-specific survival (OS; HR, 0.454; CI, 0.208–0.988; $p = 0.047$), and disease-free survival (DFS; HR, 0.324; 95% CI, 0.155–0.677; ($p = 0.003$). The main limitations of the current study were its retrospective design and limited case number.

Conclusions: Adjuvant chemotherapy following RNU significantly improved cancer-related survivals in patients with UTUC with variant histology.

Keywords: adjuvant chemotherapy, nephroureterectomy, upper urinary tract urothelial cancer, variant histology, UTUC

INTRODUCTION

Upper urinary tract urothelial carcinoma (UTUC) is a rare cancer and accounts for only 5%–10% of genitourinary urothelial cancers (UC) in Western countries (1). UTUC with nontransitional-cell variant histopathology (vUTUC) is an even rarer situation, accounting for 8%~24% among historical UTUC series where the patients were managed with radical nephroureterectomy (RNU) (1–9). vUTUC is commonly associated with more adverse pathological features and advanced disease status at presentation when compared with those of UTUC with pure urothelial pathology (pUTUC) (1–4, 6–9). In addition, patients with vUTUC are usually associated with worse outcomes in overall (OS), cancer-specific (CSS), and disease-free survival (DFS) (1). Therefore, a variant histopathologic feature in UTUC is an important prognostic factor that should be recognized for subsequent treatment planning and disease surveillance.

Bladder UC shares several similar histopathological and prognostic features with UTUC. Variant histology in bladder UC is also associated with adverse pathological features and poor outcomes (10–12). The poor outcomes of variant histology bladder UC raised the speculation of whether perioperative chemotherapy will improve outcomes in this distinct subset of bladder UC. Neoadjuvant chemotherapy has been reported to improve survival outcomes with variant histologic features in bladder UC (10, 13). A recent meta-analysis revealed that good outcomes are associated with chemotherapy for small-cell and micropapillary variants, while chemotherapy has a potential role in squamous cell and adenocarcinoma variants (13).

The success of adjuvant chemotherapy in variant bladder UC certainly led to the speculation whether vUTUC would benefit from adjuvant chemotherapy, but the study of the more aggressive vUTUC is usually limited by its even rare presentation. Although the safety and efficacy of adjuvant chemotherapy for UTUC had been confirmed in the Perioperative Chemotherapy Versus Surveillance in Upper Tract urothelial cancer) trial, the outcomes of adjuvant chemotherapy for patients with vUTUC remain scarce (14). There is no randomized controlled trials (RCTs) or comparative trial focusing on the even rarer and more aggressive vUTUC. Therefore, we conducted a propensity score-matched study to examine the impact of adjuvant chemotherapy on vUTUC.

MATERIAL AND METHODS

Data Source

This UTUC registry database was conducted by the Taiwan UTUC Collaboration Group, a multicenter Internet-based

registry partially sponsored by the Taiwan Urological Association. This retrospective study was reviewed and approved by the institutional review board (IRB No.: 063-X34-105), and the requirement of informed consent was waived due to its anonymous nature without any identifiable information in the database. The study protocols and methods were carried out in accordance with relevant guidelines and regulations.

Patient Selection

Patients who underwent RNU and were confirmed to have UTUC with variant histology were retrospectively evaluated for eligibility of analysis. Those patients with postoperative adjuvant chemotherapy were defined as systemic chemotherapy given within 4 months after RNU (15). There were 3,109 patients examined for eligibility, with 245 cases enrolled for final analysis (**Figure 1**). vUTUC was defined as those patients with upper urinary tract carcinoma who exhibited a nontransitional-cell histopathology type under pathological evaluation. The groups were categorized by vUTUC with adjuvant chemotherapy (vUTUC+C/T) and vUTUC without adjuvant chemotherapy (vUTUC–C/T). Those cases with neoadjuvant chemotherapy or missing histology/staging information, chemotherapeutic agents, and survival information were excluded.

Pathological Evaluation

The histopathological diagnosis and staging of RNU specimens were reviewed by local genitourinary pathologists in each institution according to the 2010 American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging system, and histopathologic grading was made according to the 2015 WHO/ISUP recommendation grading system. The histopathological diagnosis of variant histology has been well accepted by the uropathological community, and the diagnostic criteria were described in the WHO classification of tumors (16).

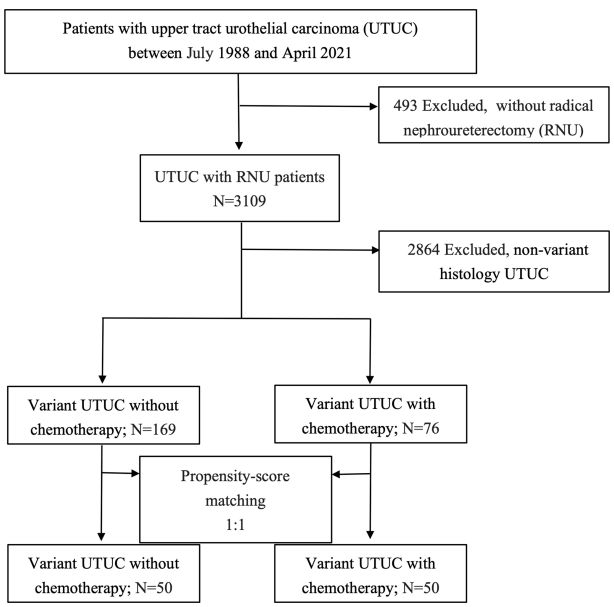


FIGURE 1 | Flow diagram of case recruitment process.

Follow-Up

The follow-up schedule for patients was every 3–6 months in the 1st year after RNU and every 6–12 months thereafter. Cross-sectional imaging (computed tomography (CT) or/and magnetic resonance images (MRI)) was used to determine recurrence/progression-free statuses. Cystoscopy examination was used to determine urinary bladder recurrence. UTUC recurrence or metastasis was defined as local recurrence of tumor bed, regional lymph nodes, or distant metastasis.

Matching Methods

To address the imbalance of potential confounders between the control and treatment adjuvant groups, we matched treatment "adjuvant" groups using propensity scores. In the statistical analysis of observational data, propensity score matching (PSM) is a statistical matching technique that attempts to estimate the effect of a treatment, policy, or other intervention by accounting for the covariates that predict receiving the treatment. In each simulated dataset, we estimated the propensity score using a logistic regression model to regress treatment status on the baseline covariates. The propensity score model included lymphovascular invasion, surgical margin, and pathological stage. We then formed matched pairs between the control group managed by watch and wait and those who had treatment adjuvant using nearest-neighbor matching gender (tolerance levels: 0), age (tolerance levels: 5), and propensity score (tolerance levels: 0.01). Our study on propensity score matching is one-to-one or pair matching, in which pairs of control and treated subjects are formed, such that matched subjects have similar values of the propensity score.

Statistical Analysis

Demographic and clinicopathological differences between groups were compared using Pearson Chi-square with Bonferroni correction for categorical variables. The Kaplan–Meier estimator was used to estimate the rates of survival outcomes, and the survival curves were compared using the stratified log-rank test. Cox proportional hazard model was selected to assess the effect of the treatment groups on the survival outcomes, alone and after adjusting for potential confounders. Those clinicopathological variables were selected with stepwise regression then included in the multivariate analysis. All the univariate significant and nonsignificant relevant covariates should be put on the variable list to be selected. The significance levels for entry (SLE) and stay (SLS) are suggested to be set to 0.05 and 0.1, respectively. The best regression model is then identified manually by reducing the significant levels to 0:05, corresponding to the chosen level. All statistical assessments were two-tailed and considered statistically significant at $p < 0.05$. Statistical analyses were carried out with IBM SPSS statistical software version 22. The description of statistical methods was based on the standard format of statistical analysis of the Taiwan UTUC collaboration group.

RESULTS

Study Cohort and Baseline Characteristics

In total, 245 patients who underwent RNU with variant histology were enrolled for final analysis. The recorded variant histopathological types included sarcomatoid differentiation (16.3%), squamous cell carcinoma (53.1%), adenocarcinoma (7.7%), neuroendocrine differentiation (4.1%), and mixed variant histology (7.7%). In total, 75 of 245 (30.6%) patients with vUTUC underwent adjuvant chemotherapy. **Table 1** summarizes the baseline clinical characteristics of the 169 cases in the vUTUC-C/T group and 75 cases in the vUTUC+C/T group before matching. Significant bias was observed in baseline characteristics in gender, age, comorbidity, tumor multiplicity, lymphovascular invasion status, pT/N stage, and preoperative renal function status before matching. After PSM, we derived 1:1 50 paired cohorts for vUTUC with and without adjuvant chemotherapy. These two groups were well-matched for all confounding variables (**Table 2**). In addition, there was no significant difference in the distribution of variant histology subtypes. The median cycles of adjuvant chemotherapy for the vUTUC+C/T group were 4 (interquartile range: 3–5). The most

TABLE 1 | Baseline demographic characteristics of patients with variant upper tract urothelial cancer (UTUC) undergoing radical nephroureterectomy (RNU) before matching.

Variables	UC with variants/no C/T (*N* = 169) *N* (%)	UC with variants/C/T (*N* = 75) *N* (%)	*p*-value[a]
Gender			
Men	56 (33.1)	45 (60.0)	<0.001**
Women	113 (66.9)	30 (40.0)	
Age			
<70	65 (38.5)	51 (68.0)	<0.001**
≥70	104 (61.5)	24 (32.0)	
Comorbidity			
Coronary artery disease	14 (8.3)	6 (7.0)	0.918
Arrhythmia	9 (5.3)	2 (2.0)	0.346
Hypertension	97 (57.4)	28 (36.0)	0.003**
Diabetes	51 (30.2)	18 (23.0)	0.296
Gouty arthritis	3 (1.8)	3 (3.0)	0.309
Gastrointestinal disorder	21 (12.4)	8 (10.0)	0.670
2nd malignancy (not urothelial cancer)	27 (16.0)	11 (14.0)	0.764
Tumor location			
Renal pelvis	87 (51.5)	35 (46.0)	0.593
Ureter	47 (27.8)	21 (27.0)	
Renal pelvis + ureter	35 (20.7)	20 (26.0)	
Tumor size			
<3 cm	56 (33.1)	26 (34.0)	0.869
≥3 cm	113 (66.9)	50 (65.0)	
RNU histology			
Low grade	5 (3.0)	0 (0.0)	0.499
High grade	158 (93.5)	73 (97.0)	
Gx	4 (2.4)	1 (1.0)	
G2	1 (0.6)	1 (1.0)	
Well-differentiated	1 (0.6)	0 (0.0)	
Multiplicity			
Not available	0 (0.0)	1 (1.0)	0.018*
No	115 (68.9)	39 (52.0)	
Yes	52 (31.1)	35 (46.0)	
CIS			
No	133 (78.7)	58 (76.0)	0.677
Yes	36 (21.3)	18 (23.0)	
Lymphovascular invasion			
No	112 (66.3)	38 (50.0)	0.016*
Yes	57 (33.7)	38 (50.0)	
Surgical margin			
Free	150 (88.8)	67 (88.0)	0.891
Positive	19 (11.2)	9 (11.0)	
Tumor necrosis			
No	108 (64.3)	44 (58.0)	0.403
Yes	60 (35.7)	31 (41.0)	
Synchronous bladder tumor			
No	142 (84.5)	61 (80.0)	0.684

(Continued)

TABLE 1 | Continued

Variables	UC with variants/no C/T (*N* = 169) *N* (%)	UC with variants/C/T (*N* = 75) *N* (%)	*p*-value[a]
Yes	26 (15.4)	15 (19.0)	
Pathological stage T			
pTis/pTa/pT0/pT1/pT2	58 (34.3)	10 (13.0)	0.001**
pT3/pT4	111 (65.7)	66 (86.0)	
Pathological stage N			
pN0	36 (21.3)	24 (31.0)	0.007**
pN+	20 (11.8)	17 (22.0)	
pNx	113 (66.9)	35 (46.0)	
eGFR			
≧60	44 (27.2)	33 (45.8)	0.005**
<60	118 (72.8)	39 (54.2)	
Post-OP eGFR			
≧60	13 (10.3)	13 (25.0)	0.012*
<60	113 (89.7)	39 (75.0)	
Histologic			
Sarcomatoid differentiation	27 (16.0)	13 (17.0)	0.687
Squamous cell carcinoma	86 (50.9)	44 (57.0)	
Adenocarcinoma	15 (8.9)	4 (5.0)	
Neuroendocrine tumors	6 (3.6)	4 (5.0)	
Mixed-cell type	13 (7.7)	6 (7.0)	
Missing	21 (12.4)	5 (6.0)	
Regimen of chemotherapy			
Gemcitabine and cisplatin	8 (38.1)	31 (44.0)	0.776
MVAC	0 (0.0)	3 (4.0)	
Taxane-based	0 (0.0)	1 (1.0)	
Carboplatin-based	7 (33.3)	19 (27.0)	
Others	6 (28.6)	16 (22.0)	
Bladder UC after RNU			
No	136 (81.9)	64 (84.0)	0.663
Yes	30 (18.1)	12 (15.0)	
Lymphadenectomy			
No	116 (68.6)	36 (47.7)	0.002**
Yes	53 (31.4)	40 (52.6)	
Follow-up (months)[b] (median (IQR))	22.2 (6.2–54.1)	29.1 (11.1–5.9)	0.313

[a]*Chi-squared test calculated for the different variables.*

[b]*Wilcoxon rank-sum test calculated for the difference in medians.*

**p < 0.05; **p < 0.01.*

RNU, radical nephroureterectomy; CIS, carcinoma in situ; MVAC, methotrexate, vinblastine, doxorubicin (Adriamycin), cisplatin.

used chemotherapeutic regimen was gemcitabine plus cisplatin, followed by gemcitabine plus carboplatin, and then MVAC regimen (methotrexate, vinblastine, doxorubicin plus cisplatin). At the end of our follow-up, forty-three of 100 cases had an event in overall survival and 38 of 100 cases had at least one event in disease progression.

Survival Analyses: Variant UTUC Without Chemotherapy vs. Variant UTUC With Chemotherapy

The median follow-up period was comparable between the vUTUC −C/T and vUTUC+C/T groups (22.2 and 29.1 months, respectively) (**Table 1**). In the univariate analyses, survival difference was only identified in overall survival (hazard ratio (HR), 0.527; 95% confidence interval (CI), 0.285–0.973; p = 0.041) (**Table 3**). However, in multivariate analyses, after adjusting with confounding factors selected with stepwise selection, significant survival benefit was found in disease-free survival (DFS) and cancer-specific survival (CSS) for the vUTUC+C/T patients (**Tables 4** and **5**). In brief, patients with variant histology who underwent adjuvant chemotherapy was associated with significant survival benefit in DFS (HR, 0.324; 95% CI, 0.155–0.677; p = 0.003) and CSS (HR, 0.454; 95% CI, 0.208–0.988; p = 0.047). LVI was the common independent risk factor for CSS and DFS. Positive surgical margin status is the independent risk factor for DFS (HR, 6.047; 95% CI, 1.554–23.53; p = 0.009).

Comparison of Kaplan–Meier estimated survival curves between vUTUC with and without adjuvant chemotherapy in localized vUTUC (pT0–T2) and advanced vUTUC (pT3–T4) disease after PSM. The survival benefit was observed in OS and DFS for advanced vUTUC disease with adjuvant chemotherapy (**Figures 2**–**4**).

DISCUSSION

UTUC is an uncommon malignancy in Western countries, and UTUC associated with variant histology is even rarer; with an

TABLE 2 | Baseline demographic characteristics of patients with variant upper tract urothelial cancer (UTUC) undergoing radical nephroureterectomy (RNU) after matching.

Variables	UC with variants/No C/T (*N* = 50) *N* (%)	UC with variants/C/T (*N* = 50) *N* (%)	*p*-value[a]
Gender			
Men	23 (46.0)	23 (46.0)	1.000
Women	27 (54.0)	27 (54.0)	
Age			
<70	27 (54.0)	31 (62.0)	0.418
≥70	23 (46.0)	19 (38.0)	
Comorbidity			
Coronary artery disease	4 (8.0)	4 (8.0)	1.000
Arrhythmia	4 (8.0)	2 (4.0)	0.400
Hypertension	29 (58.0)	19 (38.0)	0.045*
Diabetes	15 (30.0)	9 (18.0)	0.160
Gouty arthritis	1 (2.0)	3 (6.0)	0.307
Gastrointestinal disorder	4 (8.0)	6 (12.0)	0.505
2nd malignancy (not urothelial cancer)	9 (18.0)	8 (16.0)	0.790
Tumor location			
Renal pelvis	26 (52.0)	19 (38.0)	0.369
Ureter	12 (24.0)	16 (32.0)	
Renal pelvis + ureter	12 (24.0)	15 (30.0)	
Tumor size			
<3 cm	12 (24.0)	20 (40.0)	0.086
≥3cm	38 (76.0)	30 (60.0)	
RNU histology			
Low grade	1 (2.0)	0 (0.0)	0.169
High grade	46 (92.0)	48 (98.0)	
Gx	3 (6.0)	0 (0.0)	
G2	0 (0.0)	1 (2.0)	
Multiplicity			
Not available	0 (0.0)	1 (2.0)	0.158
No	33 (67.3)	25 (50.0)	
Yes	16 (32.7)	24 (48.0)	
CIS			
No	36 (72.0)	38 (76.0)	0.648
Yes	14 (28.0)	12 (24.0)	
Lymphovascular invasion			
No	30 (60.0)	28 (56.0)	0.685
Yes	20 (40.0)	22 (44.0)	
Surgical margin			
Free	47 (94.0)	48 (96.0)	0.646
Positive	3 (6.0)	2 (4.0)	
Tumor necrosis			
No	29 (59.2)	28 (56.0)	0.749
Yes	20 (40.8)	22 (44.0)	
Synchronous bladder tumor			
No	40 (81.6)	41 (82.0)	0.999
Yes	9 (18.4)	9 (18.0)	
Pathological stage T			
pTis/pTa/pT0/pT1/pT2	7 (14.0)	7 (14.0)	1.000
pT3/pT4	43 (86.0)	43 (86.0)	
Pathological stage N			
pN0	15 (30.0)	13 (26.0)	0.842
pN+	9 (18.0)	11 (22.0)	
pNx	26 (52.0)	26 (52.0)	
eGFR			
≧60	15 (31.9)	21 (44.7)	0.203
<60	32 (68.1)	26 (55.3)	
Post-OP eGFR			
≧60	4 (9.1)	7 (22.6)	0.104
<60	40 (90.9)	24 (77.4)	
Histologic			
Sarcomatoid differentiation	8 (16.0)	10 (20.0)	0.692
Squamous cell carcinoma	19 (38.0)	25 (50.0)	

(Continued)

TABLE 2 | Continued

Variables	UC with variants/No C/T (*N* = 50) *N* (%)	UC with variants/C/T (*N* = 50) *N* (%)	*p*-value[a]
Adenocarcinoma	5 (10.0)	3 (6.0)	
Neuroendocrine tumors	3 (6.0)	3 (6.0)	
Mixed-cell type	7 (14.0)	4 (8.0)	
Missing	8 (16.0)	5 (10.0)	
Regimen of chemotherapy			
Gemcitabine and cisplatin	2 (20.0)	18 (40.0)	0.623
MVAC	0 (0.0)	2 (4.0)	
Taxane-based	0 (0.0)	1 (2.0)	
Carboplatin-based	4 (40.0)	13 (28.0)	
Others	4 (40.0)	11 (24.0)	
Bladder UC after RNU			
No	42 (84.0)	41 (82.0)	0.790
Yes	8 (16.0)	9 (18.0)	
Lymphadenectomy			
No	27 (54.0)	26 (52.0)	0.841
Yes	23 (46.0)	24 (48.0)	

[a]*Chi-squared test calculated for the different variables.*
[b]*Wilcoxon rank-sum test calculated for the difference in medians.*
*$p < 0.05$.
RNU, radical nephroureterectomy; CIS, carcinoma in situ; MVAC, methotrexate, vinblastine, doxorubicin (Adriamycin), cisplatin.

overall incidence of 13.4% among the worldwide UTUC cohort (1). The variant histology UTUC is an important adverse prognostic factor affecting most survival domains of UTUC (1, 17, 18). The squamous or/and glandular variant histology was associated with an even worse CSS compared with the other UTUC variants (1). Therefore, vUTUC is a warning and prognostic indicator that could require upfront systemic therapy which usually means chemotherapy before or after RNU for UTUC. Based on recent evidence, perioperative chemotherapy (neoadjuvant or adjuvant) is beneficial in prolonging survivals of UTUC (19–21). Although perioperative chemotherapy had been proved beneficial in bladder UC variants, currently available literature failed to reveal its benefit in vUTUC (3, 4, 8, 22).

Current evidence regarding the efficacy of systemic chemotherapy on vUTUC remained scarce in the literature. As UTUC and bladder UC shared several histological and prognostic features, the regimen of chemotherapy for UTUC commonly follows the classic regimen for bladder urothelial carcinoma. Hence, most urologists/oncologists adapt classic chemotherapy regimens of bladder UC for vUTUC. Hajiran et al. conducted a cohort study to compare the result of cystectomy followed by neoadjuvant chemotherapy. The patients with variant histology (micropapillary, plasmacytoid, nested variant, sarcomatoid, or neuroendocrine variant histology), had a comparable response rate as the pure urothelial carcinoma cohort (23). A National Cancer Database study showed neoadjuvant chemotherapy was associated with significant pathological downstaging for all histological variants (sarcomatoid, micropapillary, squamous, neuroendocrine, and adenocarcinoma) and overall survival improvement for patients with variant bladder UC (24). In addition, MIBC with micropapillary, plasmacytoid variant, or squamous/glandular differentiation should be treated with neoadjuvant chemotherapy according to the recommendation of the 2020 EAU-ESMO Consensus Statements (25). Therefore, there is a clear role of chemotherapy in bladder UC with histological variants. Hence, the current cisplatin-based chemotherapy regimen is potentially effective in urothelial carcinoma with variant histology, as UTUC deserved a prospective randomized trial to confirm our speculation.

There was a national cancer database study that analyzed survivals of the UTUC with variant histology in the renal pelvis after surgery (26). Being restricted to the limited clinicopathological information in the cancer database, it only revealed the survival benefit of adjuvant chemotherapy in patients with pure UTUC but not for the patients with UTUC variants. Indeed, several limitations would hamper the power of cancer database study, such as significant variations in regimen and cycles of chemotherapy, comorbidity of patients, and intergroup bias of adverse histologic factors. Based on our findings, several important clinicopathological factors were independently associated with the survivals of the variant UTUC cohort (**Tables 3–5**). The important clinicopathological data that include comorbidities, surgical margin status, tumor size, tumor location, and lymphovascular invasion were generally not available in the cancer database study, therefore, limited the power of a national cancer database study. Hence, a comprehensive clinicopathological database could help in clarifying the real-world strategies of cancer treatment.

Murakami and their colleagues reported a multi-institutional study where those patients with UTUC with variant histology is an independent risk factor for recurrence-free survival but not for CSS (6). In addition, those vUTUC with pT3 or higher T stage and/or positive lymph node status were indicated for adjuvant chemotherapy. However, due to its retrospective study design and very-limited case number (37 UTUC cases with variant histology), significant selection bias and low power to reveal the real impact of adjuvant chemotherapy. To the best of our knowledge, our study is currently the limited PSM cohort study for the impact of chemotherapy on vUTUC. The current

TABLE 3 | Univariate and multivariate regression overall survival (OS) analyses in patients with variant upper tract urothelial cancer (UTUC) undergoing radical nephroureterctomy (RNU).

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
OS group				
UTUC with variants/no C/T	1	0.041*	1	0.070
UTUC with variants/C/T	0.527 (0.285, 0.973)		0.532 (0.301, 1.048)	
Hypertension	2.093 (1.128, 3.882)	0.019*	2.165 (1.152, 4.069)	0.016*

CI, confidence interval; HR, hazard ratio; OS, overall survival; C/T, chemotherapy.
**p < 0.05.*

TABLE 4 | Univariate and multivariate regression cancer-specific survival (CSS) analyses in patients with variant upper tract urothelial cancer (UTUC) undergoing radical nephroureterctomy (RNU).

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
CSS group				
UTUC with variants/no C/T	1	0.090	1	0.047*
UTUC with variants/C/T	0.522 (0.247, 1.106)		0.454 (0.208, 0.988)	
Sex				
Men	1		1	
Women	0.546 (0.255, 1.166)	0.118	0.329 (0.329, 0.137)	0.013*
Lymphovascular invasion	2.482 (1.171, 5.259)	0.018*	3.761 (1.667, 8.485)	0.001**
Surgical margin	3.542 (1.061, 11.825)	0.040*	6.047 (1.554, 23.53)	0.009**
Hypertension	2.306 (1.077, 4.939)	0.031*		

CI, confidence interval; HR, hazard ratio; CSS, cancer-specific survival; C/T, chemotherapy.
**p < 0.05; **p < 0.01.*

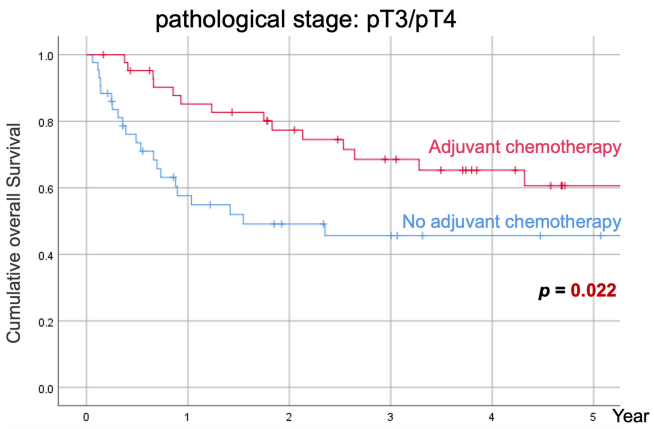
TABLE 5 | Univariate and multivariate regression disease-free survival (DFS) analyses in patients with variant upper tract urothelial cancer (UTUC) undergoing radical nephroureterctomy (RNU).

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
DFS group				
UTUC with variants/no C/T	1	0.072	1	0.003**
UTUC with variants/C/T	0.555 (0.292, 1.054)		0.324 (0.155, 0.677)	
Tumor size				
<3 cm	1	0.025*		
≥3 cm	2.565 (1.129, 5.828)			
Lymphovascular invasion	2.556 (1.339, 4.878)	0.004**	2.665 (1.188, 5.975)	0.017*
Pathological stage N				
pN0	1		1	
pN+	3.118 (1.291, 7.528)	0.011*	3.374 (1.284, 8.864)	0.014*
pNx	1.187 (0.512, 2.752)	0.689	1.598 (0.652, 3.918)	0.306
Hypertension	1.975 (1.036, 3.768)	0.039*		

CI, confidence interval; HR, hazard ratio; DFS, disease-free survival; C/T, chemotherapy.
**p < 0.05; **p < 0.01.*

study did not identify the beneficial effect of adjuvant chemotherapy in CSS and DFS for vUTUC (**Tables 4** and **5**). In addition, the presence of LVI, positive lymph node status, or a positive surgical margin are independent risk factors for poor survival outcomes, and adjuvant chemotherapy should be considered in this vUTUC after RNU.

The impact of the subtypes of variant histology has been reported in the literature without a definite conclusion. The micropapillary, squamous, and/or glandular subtypes were potentially associated with a worse CSS among patients with UTUC in a prior meta-analysis (1). However, the variant histology of adenocarcinoma had been reported to be associated with a better OS compared with pure UTUC in a cancer database study (26). This inconclusive result could be derived from excluding metastatic disease during case enrollment, therefore, excluding advanced variant adenocarcinoma subtype, which is commonly an important factor of lethal disease (27). According to our analysis,



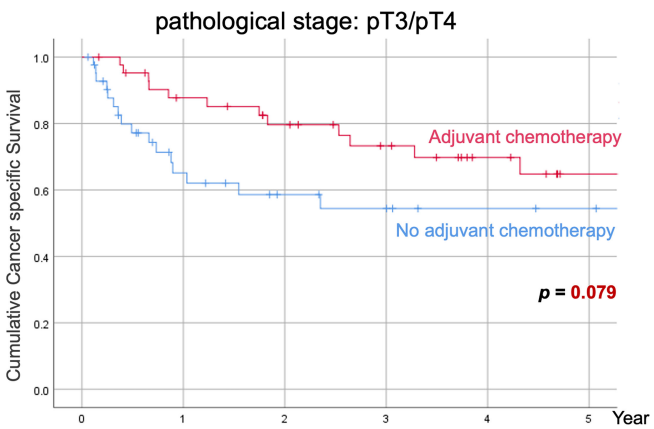
Adjuvant C/T	Time(year)	0	1	2	3	4	5
No	N	43	21	15	13	10	9
	%	1.00	0.58	0.49	0.46	0.46	0.46
Yes	N	43	34	28	22	15	9
	%	1.00	0.86	0.78	0.69	0.65	0.60

FIGURE 2 | Kaplan–Meier analyses of overall survival in patients with advanced vUTUC with or without adjuvant chemotherapy.

different variant subtypes had comparable survival outcomes (**Supplementary Figure S1**). However, limited by the small case number in each subtype, the real impact of vUTUC subtypes should be clarified with a large-scale prospective study.

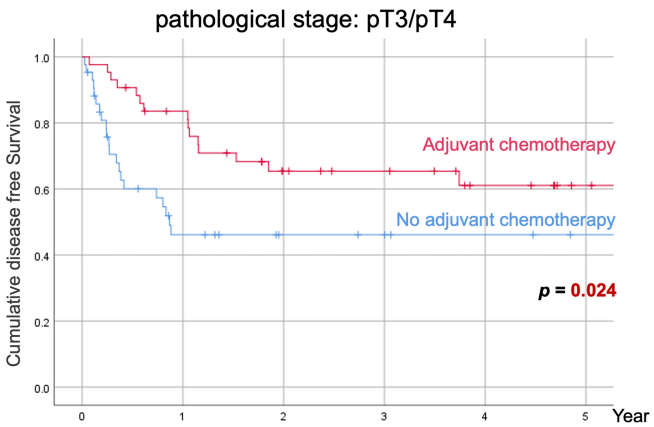
Lymphadenectomy has been recommended in RNU for UTUC for its potential survival benefit. In our matched cohort, 23 (46%) of 50 patients without adjuvant chemotherapy and 24 (48%) of 50 patients with chemotherapy underwent lymphadenectomy during RNU. As the role of extended lymphadenectomy remained controversial in UTUC treatment, extended or regional lymphadenectomy was only performed in selected cases who were clinically suspected of having advanced or nodal diseases; otherwise, lymphadenectomy was not performed in low-risk diseases. According to a large population cohort of 16,619 UTUCs, only 15.4% of cases underwent LND; therefore, the proportion of lymphadenectomy in our cohort is clearly not low when compared with the historical series (28).

Our cohort was extracted from the Taiwan UTUC registry which is a multicenter UTUC cohort that enrolled more than 4,000 UTUC cases in Taiwan. In the currently enrolled cohort, we identified 245 (7.2%) vUTUC among 3,043 Taiwan UTUC patients from 16 centers. The incidence of variant UTUC varied



Adjuvant C/T	Time(year)	0	1	2	3	4	5
No	N	43	21	15	13	10	9
	%	1.00	0.66	0.59	0.55	0.55	0.55
Yes	N	43	34	28	22	15	9
	%	1.00	0.88	0.80	0.74	0.70	0.64

FIGURE 3 | Kaplan–Meier analyses of cancer-specific survival in patients with advanced vUTUC with or without adjuvant chemotherapy.



Adjuvant C/T	Time(year)	0	1	2	3	4	5
No	N	43	16	11	10	8	6
	%	1.00	0.48	0.48	0.48	0.48	0.48
Yes	N	43	33	21	18	12	7
	%	1.00	0.83	0.64	0.64	0.60	0.60

FIGURE 4 | Kaplan–Meier analyses of disease-free survival in patients with advanced vUTUC with or without adjuvant chemotherapy.

among centers in Taiwan; it generally ranges from 2% to 13% with only two exceptions (only two centers had a high incidence of 20% which accounted for only 15% of the vUTUC cohort). According to a recent meta-analysis, vUTUC generally accounted for about 13% of all UTUC worldwide, and the incidence varied significantly among different centers (1). Therefore, significant interobserver variation in making the diagnosis of vUTUC is common and clearly inevitable in a multicenter study. Histology review has been recommended for the multicenter study of vUTUC; however, relevant studies remained extremely scarce in the literature. Last year, we randomly enrolled 154 UTUC cases from the Taiwan UTUC registry for histology review (29). Based on our review, 7.8% and 30.5% variant UTUC were identified by the local or the reviewing pathologist, respectively. Only a slight interobserver agreement was achieved with a kappa value of 0.168. However, whether the vUTUC has an impact on disease outcome, according to the univariate analysis, the presentation of variant histology was the only risk factor of DFS in the local pathology reviewed cohort, but not in the review pathology cohort. This could relate to an overdiagnosis of clinically nonsignificant vUTUC in the review pathologist's cohort, therefore, certainly underestimating the impact of vUTUC on disease outcome. In summary, whether histology review of vUTUC is beneficial in disease outcome prediction for multicenter study remained controversial and need a prospective large cohort study in the future.

LIMITATIONS

This study has some limitations. First, the current study is still limited by the small sample size, the lack of the desired power level, and the effect size that does not correspond to the required sample size. Second, the pathology was not centrally reviewed; therefore, interobserver reporting bias was also considered one of the limitations. To minimize the impact of in-concordance of pathology between observers, we used a standardized histological report format which was approved by the Pathology Society of Taiwan based on the AJCC TNM staging system, and the principles of pathology management for urothelial cancer in the NCCN guidelines to ensure a standardized management protocol. In addition, genitourinary pathologists in Taiwan followed the same training program, specimen manipulation protocol, diagnostic criteria, standardized report template, and peer review system in each local institution to minimize the interobserver bias. Third, the retrospective nature of the study design is subject to selection bias. We attempted to account for this by the multi-institution enrollment, PSM, and multivariate Cox regression analyses with adjustment of confounding factors. In addition, cases with incomplete or missing information were excluded, except for some minor variables. Fourth, a lymphadenectomy was mainly performed in patients with clinical suspicious nodal diseases or advanced clinical stages, therefore, not routinely performed during RNU in Taiwan. Hence, the rate of pNx was as high as 45%~76%, which significantly undermined our ability to have further interpretation regarding the influence of the nodal status. Fifth, overall mortality and cancer-specific mortality could be partially overlapped in the primary comparison of matched cohorts. To minimize this effect, multivariate analysis was performed to adjust the confounders that could impact the survival. Finally, the lack of standard templates in reporting specific variant histology inevitably introduced further bias in the reported results (24).

CONCLUSION

In summary, adjuvant chemotherapy following RNU significantly improved CSS and DFS in patients with UTUC with a variant histology in the current propensity score-matched

study. Hence, the effect of adjuvant systemic chemotherapy deserves a further prospective, multi-institutional study to elucidate the optimal care for these rare and challenging patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization: H-YW and C-WL. Data curation: W-ML, H-LK, Y-HC, H-CW, I-HC, J-TL, C-YH, C-HC, J-ST, W-RL, Y-HJ, Y-KL, C-YT, S-DC, TH, AC, Y-CJ, I-SC, Y-TC, J-SC, B-JC, C-CY, WL, C-CW, C-SC, and H-YW. Formal analysis: Y-CT. Methodology: W-ML and H-LK. Supervision: J-TL, AC, and Y-CJ. Writing—original draft: Y-CT. Writing—review and editing: Y-CT. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.843715/full#supplementary-material

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Is Lymph Node Dissection Necessary During Radical Nephroureterectomy for Clinically Node-Negative Upper Tract Urothelial Carcinoma? A Multi-Institutional Study

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Purpose: This study aimed to compare the oncological outcomes of patients with upper tract urothelial carcinoma (UTUC) without clinical lymph node metastasis (cN0) undergoing lymph node dissection (LND) during radical nephroureterectomy (NU).

Methods: From the updated data of the Taiwan UTUC Collaboration Group, a total of 2726 UTUC patients were identified. We only include patients with ≥ pT2 stage and

enrolled 658 patients. The Kaplan–Meier estimator and Cox proportional hazards model were used to analyze overall survival (OS), cancer-specific survival (CSS), disease-free survival (DFS), and bladder recurrence-free survival (BRFS) in LND (+) and LND (–) groups.

Results: A total of 658 patients were included and 463 patients without receiving LND and 195 patients receiving LND. From both univariate and multivariate survival analysis, there are no significant difference between LND (+) and LND (-) group in survival rate. In LND (+) group, 18.5% patients have pathological LN metastasis. After analyzing pN+ subgroup, it revealed worse CSS ($p = 0.010$) and DFS ($p < 0.001$) compared with pN0 patients.

Conclusions: We found no significant survival benefit related to LND in cN0 stage, ≥ pT2 stage UTUC, irrespective of the number of LNs removed, although pN+ affected cancer prognosis. However, from the result of pN (+) subgroup of LND (+) cohort analysis, it may be reasonable to not perform LND in patients with cT2N0 stage due to low positive predictive value of pN (+). In addition, performing LND may be considered for ureter cancer, which tends to cause lymphatic and hematogenous tumor spreading. Further large prospective studies are needed to validate our findings.

Keywords: lymph node dissection, clinical lymph node negative, muscle-invasive stage, pathological lymph node positive, upper tract urothelial carcinoma

INTRODUCTION

Upper tract urothelial carcinoma (UTUC), comprising renal pelvis and ureter cancer, has a higher incidence and female predominance in Taiwan than in other countries. In Taiwan, UTUC accounts for 40% of urothelial carcinomas (UCs), while it accounts for approximately 5–10% of UCs in Western countries (1–3). Radical nephroureterectomy (NU) with bladder cuff excision is the standard treatment for non-metastatic UTUC (3). According to previous studies, approximately 30–40% of lymph node involvement is discovered at the time of surgery (4); however, the percentage of patients receiving lymph node dissection (LND) varies widely. In a large population cohort of 16,619 UTUCs, 15.4% of patients underwent LND (5). Chappidi et al. revealed that the trend of LND increased from 20% (60/295) in 2004 to 33% (106/320) in 2012, which may reflect that LND is gradually becoming more acceptable for surgeons in clinical practice (6). In our recently published study, we also discovered different proportions of LND in different minimally invasive NU approaches, and robot-assisted NU had the highest LND rate (41.1%). The surgical technique and experience of surgeons also affects the rate of LND (7).

Although the benefits of LND are well-established in muscle-invasive bladder cancer (BC), the role of routine concomitant LND in UTUC, which is considered to have a similar histology and phenotype, is still controversial (8). Some physicians hypothesized that performing LND provides more accurate pathological disease staging and potentially better oncological outcomes, especially at higher T stages of UTUC (9, 10). Pathologically, lymph node metastasis is a poor prognostic factor for survival in UTUC, which might strengthen the rationale for performing LND in UTUC (11). However, it is not known if LND is clinically beneficial for patients without lymph node involvement. Nevertheless, LND may result in a higher risk of postoperative complications. A previous study demonstrated that patients receiving LND have a higher rate of hemorrhagic complications because the lymph nodes are near the great vessels (5, 12).

In the present study, based on a large retrospective cohort from multiple institutions in Taiwan, we aimed to resolve the issue of whether LND is necessary for UTUC patients with clinical node-negative status (cN0) on imaging studies before radical surgery.

MATERIALS AND METHODS

Patient Collection

This study was approved by our Institutional Review Board (KMUHIRB-E(I)-20180214). We retrospectively reviewed the updated data from 15 participating hospitals under the Taiwan UTUC Collaboration Group and identified 2767 patients with UTUC. We excluded patients who did not receive NU (n = 480) and those with pathological T1 (pT1) or pTis stage disease (n = 1088). Patients with cN (+) (n = 328) disease or those lacking any variables of interest or who were lost to follow-up (n = 213) were also excluded. Finally, we included 658 patients with clinical N0 status who received NU between July 2001 and February 2021. Patients were divided into groups with and without LND (defined as LND (+) or LND (–), respectively).

In addition to the LND (+) and (–) groups, various variables were collected for analysis, including age, gender, history of BC, preoperative hydronephrosis, tumor location, tumor size, tumor focality, and important pathological features such as tumor

grade, pT stage, histological variant, and lymphovascular invasion (LVI).

Definitions and Endpoints

Pathological tumor staging was based on specimens obtained after NU, with or without LND, according to the 2010 TNM (tumor, node, and metastasis) classification, and the tumor grade was defined according to the 2004 World Health Organization/ International Society of Urologic Pathology consensus classification. Regular follow-up strategies follow standard guidelines. The endpoint was to compare the survival outcomes including overall survival (OS), cancer-specific survival (CSS), disease-free survival (DFS), and bladder recurrence-free survival (BRFS) between the LND (+) and LND (−) groups. The cause of death was determined by the attending doctor or death certificate.

Statistical Analysis

To compare differences between groups, we used Student's t-test for continuous variables and Pearson's chi-squared test for categorical variables. The Kaplan–Meier estimator was used to estimate the rates of prognostic outcomes, and survival curves were compared using the stratified log-rank test. The Cox proportional hazards model was selected to evaluate the impact of LND on prognosis, with or without correction for confounding factors. IBM SPSS Statistics software version 26 was used for the analysis. All statistical analyses were two-tailed, and $p < 0.05$, was considered significant.

RESULTS

We compared the basic clinical and pathological characteristics of patients undergoing RNU between LND (+) and LND (−) groups (**Table 1**). A total of 658 patients were included in this study. Overall, 463 patients did not receive LND, and 195 patients received LND. The median number of LN removed is 4. There were significant differences in age ($p = 0.022$), Eastern Cooperative Oncology Group (ECOG) scores ($p < 0.001$), histological variants ($p = 0.008$), and pT stage ($p < 0.001$).

Survival Outcomes (OS, CSS, DFS)

As can be seen in the univariate survival analysis shown in **Table 2**, LND status was not associated with OS, CSS, or DFS. The overall 5-year OS rate in LND (−) patients was 68% and that of LND (+) patients was 69%. The 5-year CSS rates were 77% for LND (−) and 75% for LND (+) patients. The 5-year DFS rates were 64% for LND (−) and 60% for LND (+) patients. Kaplan–Meier analysis showed that OS ($p = 0.359$), CSS ($p = 0.339$) and DFS ($p = 0.431$) were not significant difference between LND (−) and LND (+) (**Figures 1A-C**). Furthermore, multivariate survival analysis and adjusted 5-year survival rates indicated that LND (+) was not associated with better survival outcomes (OS, $p = 0.672$; CSS, $p = 0.770$; and DFS, $p = 0.489$) (**Table 3**). Kaplan–Meier analysis also showed insignificant impact on survival (OS: $p = 0.623$; CSS: $p = 0.792$; DFS: $p = 0.572$) (**Figures 2A-C**). Regarding OS, age ($p = 0.003$, $p = 0.016$), ECOG status ($p < 0.001$, $p = 0.001$), previous BC ($p = 0.008$, $p = 0.006$), preoperative hydronephrosis ($p < 0.001$, $p < 0.001$), and pT4 stage ($p < 0.001$, $p < 0.001$) were significant in both univariate and multivariate analyses, respectively. Regarding CSS, ECOG ($p < 0.001$, $p = 0.047$), previous BC ($p = 0.018$, $p = 0.008$), concurrent BC ($p = 0.002$, $p = 0.020$), preoperative hydronephrosis ($p = 0.005$, $p < 0.001$), and pT4 stage ($p < 0.001$, $p < 0.001$) were significantly different in both univariate and multivariate analyses, respectively. Regarding DFS, concurrent BC ($p < 0.001$, $p = 0.001$), LVI ($p < 0.001$, $p < 0.001$), tumor grade ($p = 0.007$, $p = 0.023$), and pT4 stage ($p < 0.001$, $p < 0.001$) were significantly different in both univariate and multivariate survival analyses, respectively.

Bladder Recurrence (BRFS)

There was no statistically significant difference between the LND (−) and LND (+) groups in terms of BRFS in both univariate and multivariate analyses. The overall 5-year BRFS rate was 62% in LND (−) and 66% in LND (+) patients (**Tables 2**, **3**, and **Figures 1D**, **2D**). Additionally, multiplicity ($p = 0.001$ and 0.030, respectively), previous BC ($p < 0.001$ and $p < 0.001$, respectively), concurrent BC ($p = 0.001$ and $p = 0.040$, respectively), and pT4 stage ($p = 0.027$ and $p = 0.042$, respectively) significantly correlated with BRFS in both analyses.

DISCUSSION

Whether routine LND at the time of NU should be performed in non-metastatic UTUC patients has always been debated, especially for patients with cN0 status. Previous studies have demonstrated the benefits of LND for oncological outcomes in advanced UTUC. Furuse et al. found that removal of defined systematic regional nodal areas can improve survival in cTanyN0M0, but that there was no significant benefit at the pTis-1 stage (13). Similar results were also observed in Dong's research; LND was associated with a better survival benefit in cN0 patients, especially in the muscle invasive stage. Even after receiving adjuvant therapy, patients receiving LND still have better outcomes than those who do not receive LND (14). In contrast, Inokuchi et al. indicated that there was no therapeutic benefit of LND, even in clinically advanced T stage disease (15). According to the latest EAU 2020 guidelines, LND is unnecessary in cases of non-muscle invasive disease due to the low risk of LN metastasis; therefore, we only focused on patients with pT2–4 stage disease (3). In the present study, using a multiple institution patient cohort from a real-world database, we found that for cN0 patients, LND did not improve survival in patients with pT2–4 stage disease.

The assumption of better survival outcomes after performing LND is based on the finding that LN metastasis is a poor risk factor for cancer prognosis in UTUC, which has been well established before (16, 17). Nevertheless, some other studies have failed to find a significantly better prognosis in patients with pN0 and pNx stage UTUC who underwent LND (18, 19). The incidence of LN

TABLE 1 | Clinicopathological data of cN0 UTUC patients receiving nephroureterectomy.

Variables	LND (-) (N=463)		LND (+) (N=195)		*p* value[a]
	N	%	N	%	
Gender					0.166
Men	196	(42.3)	94	(48.2)	
Women	267	(57.7)	101	(51.8)	
Age[b] Mean ±SD	69.8±10.8		67.7±10.6		0.022*
ECOG scores					<0.001**
0	188	(40.6)	40	(20.5)	
1	214	(46.2)	130	(66.7)	
2	47	(10.2)	23	(11.8)	
3	9	(1.9)	2	(1.0)	
4	5	(1.1)	0	(0.0)	
Tumor location					0.466
Renal pelvis	217	(47.1)	94	(48.5)	
Ureter	163	(35.4)	60	(30.9)	
Synchronous	81	(17.6)	40	(20.6)	
Tumor size					0.266
non-visible	2	(0.4)	1	(0.5)	
<1cm	14	(3.0)	3	(1.5)	
≥1 & < 2 cm	81	(17.5)	33	(16.9)	
≥2 & < 3 cm	117	(25.3)	37	(19.0)	
≥ 3cm	249	(53.8)	121	(62.1)	
Histological variant					0.008**
No	409	(88.3)	157	(80.5)	
Yes	54	(11.7)	38	(19.5)	
Tumor grade					0.166
Low grade	35	(7.6)	9	(4.6)	
High grade	427	(92.4)	186	(95.4)	
Multiplicity					0.184
No	298	(64.3)	113	(58.5)	
Yes	164	(35.7)	80	(41.5)	
Lymphovascular invasion					0.474
No	315	(69.7)	129	(66.8)	
Yes	137	(30.3)	64	(33.2)	
Preoperative hydronephrosis					0.089
No	150	(32.8)	77	(39.7)	
Yes	308	(67.2)	117	(60.3)	
History of BC		–		–	0.379
No	382	(82.5)	158	(81.0)	
Previous BC	22	(4.8)	6	(3.1)	
Concurrent BC	59	(12.7)	31	(15.9)	
Pathological stage T					<0.001**
pT2	195	(42.3)	47	(24.2)	
pT3	248	(53.8)	99	(51.0)	
pT4	18	(3.9)	48	(24.7)	
Clavien-Dindo classification					0.683
No	275	(60.0)	118	(61.8)	
Grade I	61	(13.3)	29	(15.2)	
Grade II	85	(18.6)	34	(17.8)	
Grade III	18	(3.9)	4	(2.1)	
Grade IV	8	(1.7)	4	(2.1)	
Grade V	11	(2.4)	2	(1.0)	
Post-OP Complication					
ESRD	62	(13.9)	19	(9.9)	0.170
Ileus	13	(2.9)	4	(2.1)	0.556
Follow up (months)[c] median	33.5		24.2		0.049*

[a]*Chi-Squared test calculated for the difference Variables.*

[b]*Student's t-test calculated for the difference in means. * < 0.05, ** < 0.01.*

[c]*Wilcoxon rank-sum test calculated for the difference in medians. * < 0.05, ** < 0.01.*

UTUC, upper tract urothelial carcinoma; LND, lymph node dissection; BC, bladder cancer; ESRD, end-stage renal disease; NU, nephroureterectomy.

TABLE 2 | Comparative univariate survival analysis of UTUC patients receiving NU.

Univariate analysis	OS		CSS		DFS		BRFS	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Group		0.396		0.340		0.432		0.772
LND (-)	1		1		1		1	
LND (+)	1.165 (0.818, 1.659)		1.222 (0.810, 1.844)		1.131 (0.833, 1.535)		1.049 (0.758, 1.451)	
Sex		0.901		0.694		0.395		<0.001**
Male	1		1		1		1	
Female	0.980 (0.710, 1.352)		0.927 (0.634, 1.354)		0.885 (0.669, 1.172)		0.573 (0.426, 0.770)	
Age		0.003**		0.172		0.419		0.770
<70	1		1		1		1	
>=70	1.633 (1.178, 2.263)		1.304 (0.891, 1.907)		1.123 (0.848, 1.488)		1.045 (0.779, 1.400)	
Histological variant		0.043*		0.120		0.070		0.729
No	1		1		1		1	
Yes	1.540 (1.014, 2.337)		1.482 (0.902, 2.434)		1.420 (0.972, 2.075)		0.924 (0.592, 1.444)	
ECOG scores		<0.001**		<0.001**		0.014*		0.836
0~1	1		1		1		1	
2~4	2.905 (1.991, 4.238)		2.465 (1.549, 3.923)		1.634 (1.105, 2.415)		0.951 (0.591, 1.531)	
Tumor size								
<1cm	1		1		1		1	
≥1 & < 2 cm	2.742 (0.649, 11.576)	0.170	3.485 (0.463, 26.248)	0.225	1.082 (0.373, 3.142)	0.885	0.850 (0.377, 1.918)	0.696
≥2 & < 3 cm	2.199 (0.525, 9.209)	0.281	3.180 (0.428, 23.618)	0.258	1.481 (0.531, 4.131)	0.453	0.776 (0.349, 1.728)	0.535
≥ 3cm	2.935 (0.722, 11.932)	0.132	4.166 (0.577, 30.055)	0.157	1.987 (0.734, 5.380)	0.177	0.769 (0.357, 1.657)	0.502
Tumor location								
Renal pelvis	1		1		1		1	
Ureter	1.361 (0.947, 1.957)	0.096	1.307 (0.849, 2.012)	0.223	1.217 (0.885, 1.647)	0.227	1.275 (0.911, 1.785)	0.156
Synchronous	1.442 (0.940, 2.213)	0.094	1.489 (0.905, 2.449)	0.117	1.397 (0.964, 2.023)	0.077	1.829 (1.255, 2.664)	0.002**
Multiplicity		0.026*		0.004**		0.006**		0.001**
No	1		1		1		1	
Yes	1.447 (1.045, 2.003)		1.761 (1.202, 2.578)		1.494 (1.124, 1.987)		1.671 (1.241, 2.249)	
History of BC								
No	1		1		1		1	
Previous BC	2.332 (1.253, 4.338)	0.008**	2.422 (1.167, 5.024)	0.018*	1.640 (0.888, 3.028)	0.114	3.214 (1.881, 5.493)	<0.001**
Concurrent BC	1.749 (1.140, 2.682)	0.010*	2.117 (1.317, 3.405)	0.002**	1.999 (1.398, 2.860)	<0.001**	1.926 (1.314, 2.823)	0.001**
Preoperative hydronephrosis		<0.001**		0.005**		0.088		0.383
No	1		1		1		1	
Yes	2.109 (1.405, 3.167)		1.966 (1.229, 3.147)		1.311 (0.960, 1.790)		1.151 (0.839, 1.577)	
Lymphovascular invasion		0.019*		0.002**		<0.001**		0.112
No	1		1		1		1	
Yes	1.502 (1.071, 2.107)		1.839 (1.242, 2.722)		1.979 (1.485, 2.639)		0.758 (0.539, 1.067)	
Tumor grade		0.035*		0.044*		0.007**		0.013*
Low grade	1		1		1		1	
High grade	2.609 (1.069, 6.364)		22.925 (1.087, 483.560)		3.405 (1.401, 8.276)		0.566 (0.363, 0.885)	
Pathological stage T								
pT2	1		1		1		1	
pT3	1.645 (1.150, 2.352)	0.006**	2.098 (1.341, 3.281)	0.001**	1.899 (1.376, 2.623)	<0.001**	0.889 (0.661, 1.194)	0.433
pT4	3.429 (1.881, 6.251)	<0.001**	4.891 (2.471, 9.681)	<0.001**	3.906 (2.297, 6.641)	<0.001**	0.206 (0.051, 0.836)	0.027*

CI, confidence; HR, hazard ratio; OS, overall survival; CSS, cancer-specific survival; DFS, disease-free survival; BRFS, Bladder Recurrence-free survival.

** < 0.05, ** < 0.01.*

UTUC, upper tract urothelial carcinoma; LND, lymph node dissection; BC, bladder cancer; ESRD, end-stage renal disease; NU, nephroureterectomy.

metastasis increases with higher T stage, with a reported incidence of approximately 60% for ≥pT3 disease (20). It remains difficult to declare that LND is needed if the patient has suspected LN metastasis, not to mention cN0 stage, which reflects a lack of suspicious LN involvement before surgery. Simultaneous LND increases the operation time and increases the risk of perioperative complications, including bleeding and chylous lymphatic leakage, which may be a concern for surgeons (21). In the current study, there was no significant benefit to the survival of patients with UTUC after receiving LND without clinical LN involvement. To ensure cohort homogeneity, we only included patients with ≥pT2 disease, which is considered to have a higher incidence of LN metastasis. We demonstrated that pT stage is a strong prognostic factor for OS, CSS, and DFS outcomes in multivariate analysis, which has been well established in previous studies (22, 23).

The number and extent of LNDs may also be associated with the subsequent prognosis. A meta-analysis showed that the

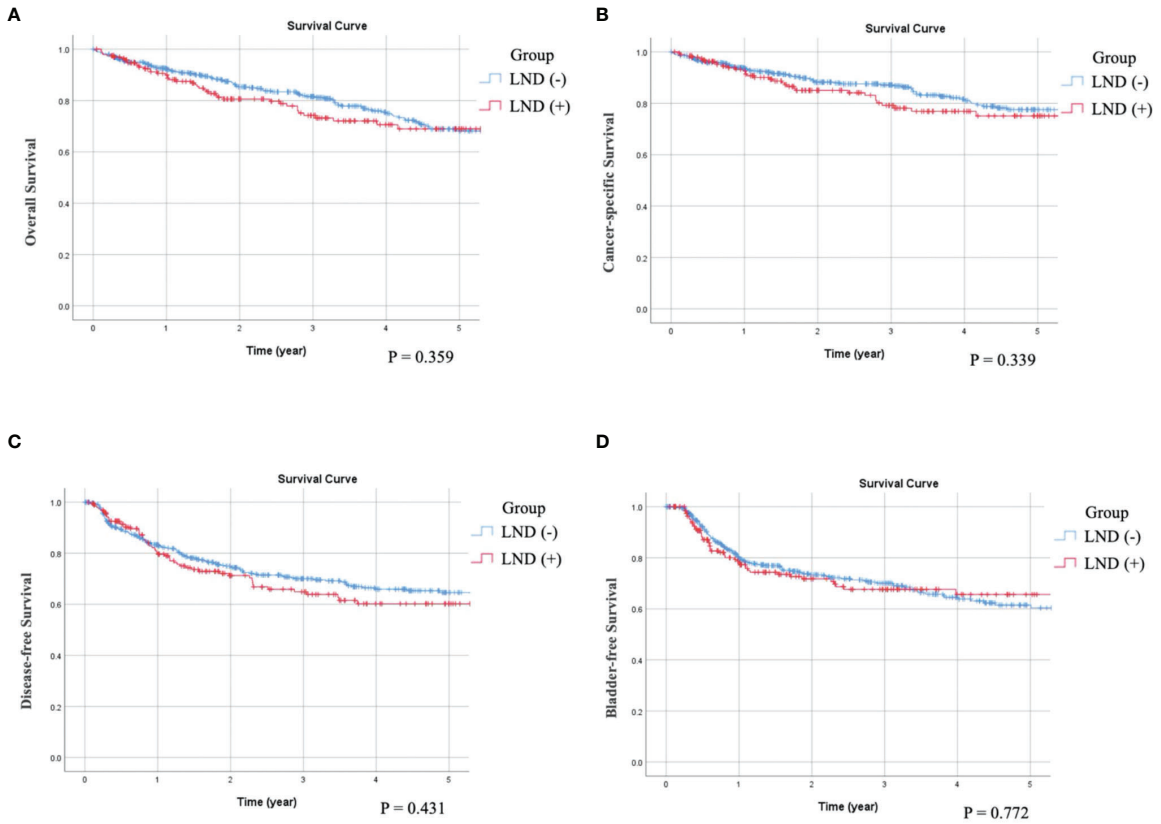

FIGURE 1 | Compare Kaplan-Meier curves between patients without receiving LND (LND (-)) or with receiving LND (LND (+)) by log-rank test. **(A)** Overall survival, p = 0.359. **(B)** Cancer-specific survival, p = 0.339. **(C)** Disease-free Survival, p = 0.431. **(D)** Bladder recurrence-free survival, p = 0.772.

removal of a higher number of LNs was associated with better survival outcomes in patients with UTUC (24). The minimal number of LNs requiring removal is variable, but a previous analysis showed that 8 LNs is the threshold for the improvement of survival in non-metastatic UTUC patients (25). Chappidi et al. demonstrated that removing over 5 LNs can improve CSS compared to that with 1–4 nodes removed (6). In our study, we did not find an association between number of nodes removed and survival, although the mean number of LNs removed (7.46 LNs) was comparable to that in previous studies (OS, p = 0.909; CSS, p = 0.893; and DFS, p = 0.196). Xylinas et al. (20) reported that there will be more missing positive lymph nodes if fewer LNs are removed; therefore, LND is necessary for accurate nodal metastasis status assessment and better postoperative clinical decision-making regarding the follow-up schedule. The distribution of LN metastasis differs between tumor locations; therefore, regional LN template removal according to tumor location is likely more important than the number of LNs that were removed. Kondo et al. also could not find whether the number of LNs removed affected survival in patients with pT2 or higher UTUC; instead, they discovered that the most critical factor regarding whether to remove regional LNs is completely based on the template according to tumor location (26). Matsumoto et al. demonstrated that template-based LND in patients with cN0 UTUC according to tumor anatomical location has better long-term oncological outcomes (27).

The location of UTUC tumors is a prognostic factor that was found to affect survival in previous studies and that ureter tumors have a worse survival rate than tumors in the renal pelvis. One possible hypothesis is that the thicker anatomic barrier of the renal pelvis than that of the ureter leads to more lymphovascular space with a lower chance of spreading out (6, 28). In the present study, we further compared the survival differences between the ureter and renal pelvis tumors in the LND (+) group. No significant differences in OS, CSS, DFS, and BRFS were observed between the renal pelvis and ureter tumors. This demonstrated that performing LND may provide a greater survival benefit in ureter cancer, which is considered to have a higher risk of lymphatic and hematogenous spread than renal pelvis cancer in cN0 stage patients. In the LND (+) group, 18.5% of the patients had pathological LN metastasis. Patients with pathological LN-positive disease (pN1+pN2) had significantly shorter CSS (p = 0.010) and DFS (p < 0.001) than those with no pathological LN metastasis (pN0) (**Figures 3A, B**). The results suggest that meticulous LND during NU may have a

TABLE 3 | Comparative multivariate survival analysis of UTUC patients receiving NU.

Multivariable analysis	OS		CSS		DFS		BRFS	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Group		0.672		0.770		0.489		0.170
LND (-)	1		1		1		1	
LND (+)	1.086 (0.742, 1.588)		1.069 (0.684, 1.671)		0.889 (0.637, 1.241)		1.272 (0.902, 1.793)	
Age		0.016*		0.309		0.483		0.446
<70	1		1		1		1	
>=70	1.552 (1.084, 2.221)		1.244 (0.817, 1.894)		1.116 (0.822, 1.515)		1.129 (0.827, 1.541)	
Histological variant		0.434		0.792		0.307		0.855
No	1		1		1		1	
Yes	1.201 (0.759, 1.899)		1.077 (0.621, 1.866)		1.238 (0.822, 1.863)		0.955 (0.582, 1.568)	
ECOG scores		0.001**		0.047*		0.237		0.642
0~1	1		1		1		1	
2~4	2.061 (1.363, 3.117)		1.676 (1.008, 2.788)		1.287 (0.847, 1.957)		0.886 (0.532, 1.476)	
Multiplicity		0.256		0.202		0.239		0.030*
No	1		1		1		1	
Yes	1.232 (0.859, 1.767)		1.318 (0.863, 2.014)		1.208 (0.882, 1.655)		1.446 (1.035, 2.020)	
History of BC								
No	1		1		1		1	
Previous BC	2.502 (1.294, 4.838)	0.006*	2.887 (1.323, 6.301)	0.008*	1.650 (0.869, 3.133)	0.126	2.829 (1.602, 4.997)	<0.001**
Concurrent BC	1.523 (0.953, 2.434)	0.079	1.865 (1.105, 3.147)	0.020*	1.952 (1.317, 2.892)	0.001**	1.581 (1.022, 2.446)	0.040*
Preoperative hydronephrosis		< 0.001**		< 0.001**		0.011*		0.457
No	1		1		1		1	
Yes	2.617 (1.697, 4.035)		2.522 (1.518, 4.191)		1.526 (1.100, 2.116)		1.132 (0.816, 1.571)	
Lymphovascular invasion		0.136		0.035		< 0.001**		0.449
No	1		1		1		1	
Yes	1.317 (0.917, 1.891)		1.565 (1.032, 2.374)		1.744 (1.289, 2.361)		0.872 (0.611, 1.244)	
Tumor grade		0.161				0.023*		0.026*
Low grade	1				1		1	
High grade	1.921 (0.771, 4.784)				2.846 (1.158, 6.995)		0.589 (0.369, 0.938)	
pathological stage T								
pT2	1		1		1		1	
pT3	1.530 (1.043, 2.244)	0.030*	1.859 (1.151, 3.002)	0.011*	1.737 (1.236, 2.440)	0.001**	0.875 (0.636, 1.203)	0.410
pT4	3.939 (1.985, 7.817)	<0.001**	5.038 (2.307, 11.000)	<0.001**	3.252 (1.770, 5.975)	<0.001**	0.127 (0.017, 0.928)	0.042*

CI, confidence; HR, hazard ratio; OS, overall survival; CSS, cancer-specific survival; DFS, disease-free survival; BRFS, Bladder Recurrence-free survival.
** < 0.05, ** < 0.01.*

therapeutic effect in LN-positive patients, provide accurate staging, and enable postoperative risk stratification for patient counseling. However, we discovered more patients with advanced T stage in the pN (+) subgroup of the LND (+) cohort (T2: 4/47 = 8.5%, T3+T4: 32/147 = 21.8%); the worse survival rate may be due to the more advanced T stage. Pelcovits et al. also observed that some patients with good OS in the pN (+) populations are more likely to have lower stage and lower grade disease (11). The positive predictive value of LND was low at the T2 stage; therefore, for more favorable disease biology (lower stage), NU without LND may be acceptable. The most crucial point is not the number of LNs removed, but the removal of positive LN metastasis. Until now, accurate preoperative clinical staging and assessment of LN invasion in UTUC is difficult (29). Misdiagnosis is a concern for surgeons, and LND may be considered a good method for LN staging, which is a key prognostic factor (24).

Although this study provides important insights into the impact of LND in cN0 stage UTUC, it has some limitations. First, it was performed with a retrospective design including multiple institutions in Taiwan, so a heterogeneous background may exist. Second, this database does not have information regarding the anatomical sites or extent of LND, which may be associated with survival outcomes in UTUC patients. The extent of LND is decided by individual surgeons, so we cannot analyze the effect of the extent of LND on survival outcomes. Furthermore, the lack of centralized image review before surgery may lead to misdiagnosis of clinical N stage. Moreover, because it includes a large cohort from many institutions, even if we have included many covariants, it may have some unavoidable selection bias. Therefore, further prospective studies are warranted to determine the benefits of LND.

CONCLUSIONS

We demonstrated that although pN (+) status has worse survival, performing LND in patients with muscle-invasive cN0 stage UTUC did not show a significant benefit regardless of the number of LNs removed. However, based on the results of the pN (+) subgroup of the LND (+) cohort, it may be reasonable to not perform LND in patients with cT2N0 stage disease due to the low positive predictive value of pN (+). In addition, it may

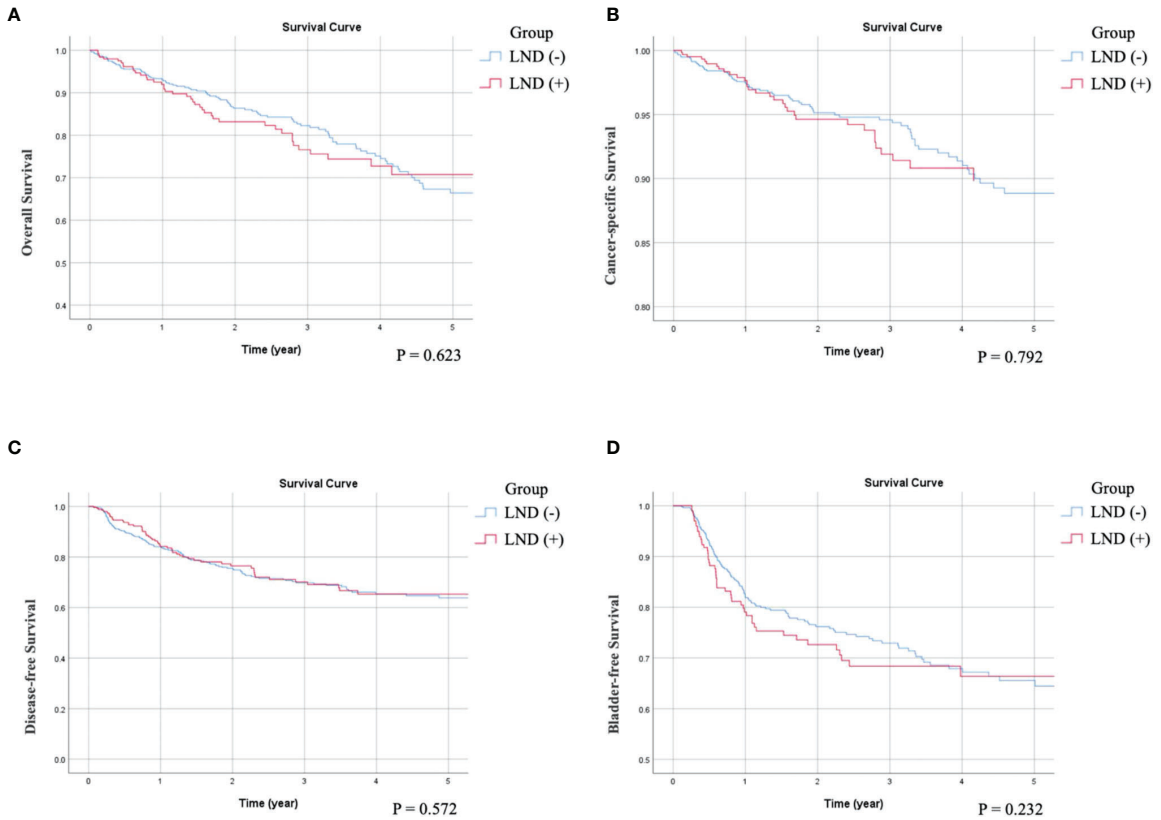

FIGURE 2 | Compare Kaplan-Meier curves between patients without receiving LND (LND (-)) or with receiving LND (LND (+)) after adjusting variables. **(A)** Overall survival, p = 0.623. **(B)** Cancer-specific survival, p = 0.792. **(C)** Disease-free Survival, p = 0.572. **(D)** Bladder recurrence-free survival, p = 0.232.

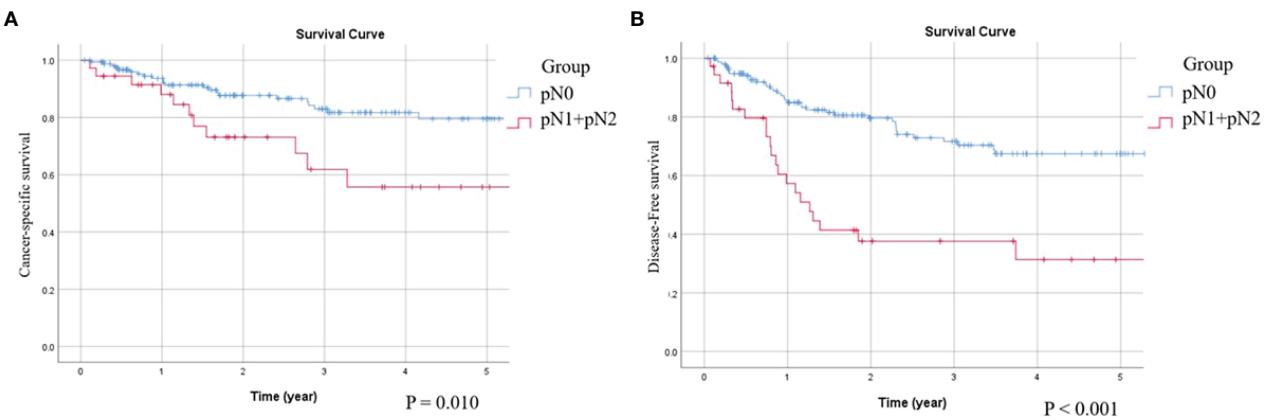

FIGURE 3 | Compare Kaplan-Meier curves in LND (+) group between patients with pN0 and pN1+pN2 by log-rank test. **(A)** Cancer-specific survival, p = 0.010. **(B)** Disease-free Survival, p < 0.001.

be suggested to perform LND for ureter cancer, which tends to result in lymphatic and hematogenous tumor spreading. Due to the potential increased risk of perioperative complications and considering the accurate staging benefit, a meticulous preoperative plan is needed to decide whether to perform LND.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Kaohsiung Medical University Hospital. KMUHIRB-E(I)-20180214. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements

AUTHOR CONTRIBUTIONS

Y-CT and H-YL conceived the project. All authors collected the data. H-YL analyzed the results. H-YL and H-CY drafted the manuscript. H-CY and H-LK edited the manuscript. All authors contributed to the article and approved the submitted version.

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Could Metabolic Syndrome Be a Predictor of Survival Outcomes in Upper Tract Urothelial Carcinoma? A Propensity Score Matching Study in a Large Chinese Center

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Purpose: To evaluate the prognostic value of metabolic syndrome (MetS) in upper tract urothelial carcinoma (UTUC) patients based on propensity score matching (PSM) analysis.

Patients and Methods: A total of 573 patients with UTUC after radical nephroureterectomy were included at Peking University People's Hospital from January 2007 to April 2021. MetS was diagnosed according to the criteria of Chinese Diabetes Society and was defined as the presence of 3 or more of the following 4 conditions (obesity, hyperglycemia, hypertension, high triglycerides and/or low high-density lipoprotein-cholesterol). Patients were divided into two groups based on whether they had MetS, whose variables were adjusted using 1:1 PSM analysis with a caliber of 0.02 to minimize selection bias. Univariate and multivariate Cox regression analysis were used to evaluate the association of MetS and its components with pathological outcomes after adjusting preoperative confounders by propensity score matching. The Kaplan-Meier method was used to estimate overall survival (OS), cancer-specific survival (CSS), and intravesical recurrence-free survival (IVRFS) after surgery.

Results: MetS was significantly correlated with older age, a history of coronary heart disease, high Charlson Comorbidity Index, low estimated Glomerular filtration rate, and low aspartate/alanine aminotransferase ratio (all $P<0.05$). Multivariate Cox regression analysis and Kaplan-Meier curves demonstrated that MetS showed no statistical correlation with lower OS or IVRFS and approaching significance with lower CSS ($P=0.063$) before PSM. After PSM, the 5-year OS, CSS, and IVRFS were 64.1%, 74.7%, and 77.2%, respectively, in the MetS group, compared with 67.4%, 78.8%, and 77.2%, respectively, in non-MetS group. Univariate Cox regression analyses showed that MetS and its components were not associated with decreased OS, CSS, or IVRFS (all $P>0.05$).

Conclusion: In our study, no statistical difference was found between MetS and survival outcomes in UTUC, except a marginal association with lower CSS. Further studies are needed to evaluate the role of MetS and its each single component on UTUC.

Keywords: metabolic syndrome, upper tract urothelial carcinoma, propensity score matching, prognosis, survival

INTRODUCTION

There is a high incidence of urothelial carcinoma and it ranks among the top ten malignant tumors worldwide. Bladder urothelial carcinoma accounts for its vast majority. Upper tract urothelial carcinoma (UTUC) accounts for only 5-10% of the total urothelial carcinoma but the proportion is higher in the Asian population, at about 9.3-29.9%, with an average of 17.9% (1). Therefore, UTUC may have different pathogenesis and clinical characteristics in the Asian population.

Metabolic Syndrome (MetS) is defined as a group of clinical manifestations including obesity, hyperglycemia, dyslipidemia (hypertriglyceridemia and/or hypo-high-density-lipoproteinemia), and hypertension. The components mentioned above seriously affected the health and showed aggregation at the onset. There is now increasing evidence of an association between MetS and tumor development and prognosis, such as colorectal cancer (2), breast cancer (3) and urinary cancer including prostate cancer (4) and bladder cancer (5). Few studies have investigated the link between MetS and UTUC, including one cohort study and two studies based on Surveillance, Epidemiology and End Results (SEER)-Medicare linked database (6–8). Although their studies showed that the patients with MetS had inferior survival outcomes, they may not be able to select the most suitable diagnostic criteria of MetS for the Chinese population. In 2004, the Chinese Diabetes Society (CDS) released MetS's diagnostic criteria for the Chinese population (9), and reaffirmed the criteria in the latest consensus in 2019. In the determination of obesity, CDS adopts the same index as the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) but with a lower cut-off (BMI≥25kg/m^2 vs BMI≥28kg/m^2). This is different from International Diabetes Federation (IDF), which selects waist circumference as the evaluation criteria of centripetal obesity. At the same time, the CDS combined hypertriglyceridemia and hypo-high-density-lipoproteinemia. In general, the CDS takes into account the baseline characteristics of the lower BMI in the Asian population and imposes stricter requirements on the diversity of metabolic abnormalities. The aim of our study was to evaluate the prognostic value of MetS in a large Chinese cohort based on the diagnostic criteria from CDS using propensity score matching (PSM) analysis.

MATERIAL AND METHODS

Study Population

This study received the approval from the Internal Ethics Review Board of the Peking University People's Hospital. We retrospectively collected the clinical and pathological records of 652 patients diagnosed with UTUC. We excluded 79 patients because they had non-urothelial carcinoma (n=48); were treated with ureteroscopic management (n=14); or did not show at the follow-up appointment (n=17). In total, 573 patients were included for further study (**Figure 1**). They were treated with radical nephroureterectomy (RNU) from January 2007 to April 2021. The type of bladder cuff removal included transvesical, extravesical, and endoscopic.

Lymph node dissection (LND) was performed when invasive UTUC was suspected or suspicious lymph node metastasis was found by preoperative imaging. The pathological staging was assessed according to the 2002 Union for International Cancer Control (UICC) Tumor-node-metastasis (TNM) classification system. Tumor grade was determined according to the World Health Organization/International Society of Urologic Pathology 2004 classification (WHO/ISUP) grading system. Other pathological features were simultaneously retrieved from the pathological reports.

MetS Definitions

MetS was diagnosed according to the criteria of CDS and was defined as the presence of 3 or more of the following 4 conditions: (1) obesity: body mass index (BMI)≥25kg/m^2; (2) hyperglycemia: fasting plasma glucose (FPG) ≥ 6.1mmol/L and/or 2-hour postprandial blood glucose (2hPG) ≥ 7.8mmol/L or drug treatment for any type of diabetes mellitus, (3) high blood pressure: systolic blood pressure≥140 mmHg or diastolic blood pressure≥90 mmHg or antihypertensive drug treatment, or (4) high triglycerides (defined as ≥1.7mmol/L) and/or low high-density lipoprotein-cholesterol (defined as <0.9mmol/L in males and <1.0mmol/L in females).

Follow-Up

Before the operation, blood and urinary samples were routinely obtained. For patients who were followed, cystoscopy was done every 3 months for the first 2 years after RNU and once a year thereafter. Computed tomography or magnetic resonance imaging, blood and urinary laboratory tests, and other evaluations were also performed. Overall survival (OS) was evaluated from the date of surgery to the date of death from all causes. Cancer-specific survival (CSS) was defined as the interval between surgery and cancer-specific death. Intravesical recurrence-free survival (IVRFS) was defined as the interval between surgery and identification of a subsequent bladder tumor during cystoscopy confirmed by pathological evaluation.

Statistical Analysis

Differences in clinical and pathological characteristics among MetS and non-MetS groups were compared using Chi-square test for categorical variables and Student's t-test or Mann-

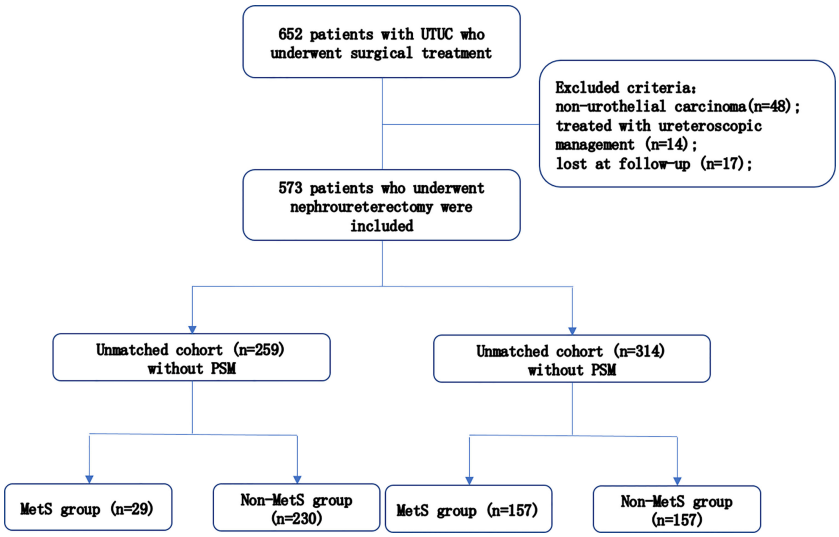

FIGURE 1 | Patient selection flowchart.

Whitney U test for continuous variables. Continuous variables with normal distribution were present as mean ± standard deviation (SD) and non-normal variables were reported as median (interquartile range). Univariate and multivariate Cox regression analysis were used to evaluate the association of MetS and its components with pathological outcomes after adjusting preoperative confounders by propensity score matching. All factors with p-value<0.1 in univariate Cox regression analysis were included in multivariate analysis. Patients were divided into two groups based on whether they had MetS, or whether variables were adjusted using 1:1 PSM analysis with a caliber of 0.02 to minimize selection bias. Standardized differences were used to compared the balance of baseline characteristics and covariates between MetS and non-MetS patients before and after matching, expressed as absolute standardized difference. The Kaplan-Meier method was used to estimate OS, CSS, and IVRFS after surgery. Statistical analyses were performed using IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA) and Stata version 15 (StataCorp LLC, Texas, USA). A two-sided P value <0.05 was considered statistically significant.

RESULTS

Clinicopathological Characteristics Before PSM

Of the 573 UTUC patients in the entire cohort, 186 (32.5%) were diagnosed with MetS. The baseline characteristics of patients before PSM are demonstrated in **Table 1**. Median age of patients at surgery was 67.27 ± 9.97 year, with a median follow-up duration of 32.8 months (range from 1 to 179 months). Low-grade and high-grade UTUC were seen in 106 patients (18.5%) and 456 patients (79.6%), respectively, and 11 patients (1.9%) were in unclear classification. Non-muscle-invasive UTUC (pTa-Tis-T1) was seen in 145 (25.3%) of patients, 125 (21.8%) showed pT2, 274 (47.8%) showed pT3 or pT4, and 29 (5.1%) showed unclear results. A total of 230 patients (40.1%) were diagnosed with obesity, 176 patients (30.7%) with hyperglycemia, 371 patients (64.7%) with hypertension, and 313 patients (54.6%) with hyperlipidemia. Patients with MetS were significantly older (P=0.04), more likely to have a history of coronary heart disease (CHD) (P=0.002); had a higher Charlson Comorbidity Index (CCI) (P<0.001), a lower estimated Glomerular filtration rate(eGFR) (P=0.014), and lower AST/ALT ratio (P<0.001). Each component of MetS was also significantly higher in the MetS group, compared with patients in the non-MetS group. Although differences of these baseline characteristics and laboratory tests were statistically significant, there was no statistical difference in pathological characteristics including tumor stage and grade.

Survival Outcomes Before PSM

Before PSM, with a median follow-up of 32.8 months, there were 169 (29.5%) overall deaths after a median (interquartile range [IQR]) of 20 (9.8-40.0) months and 121 (21.1%) cancer-specific deaths after a median (IQR) of 15.1 (8.4-27.5) months, postoperatively. Also, 79 patients (13.8%) experienced intravesical recurrence (IVR) after a median (IQR) of 10.5 (6.4-28.5) months. After controlling for clinicopathological characteristics, there was a borderline effect in which members of the MetS group demonstrated a somewhat lower CSS compared with patients without MetS (95% confidence interval [CI] 0.978-2.351, P=0.063) based on multivariate Cox regression analyses, as shown in **Table 2**. Moreover, patient gender, pathologic T stage, tumor grade, and tumor size were revealed

TABLE 1 | Clinicopathological characteristics of the entire cohort and subgroups according to MetS before propensity score matching.

Characteristics	Subgroup	Entire cohort	MetS	Non-MetS	P-value
Number		573	186	387	
Preoperative characteristics					
Age, years		67.27 ± 9.97	68.51 ± 9.41	66.68 ± 10.19	0.040
Gender	Male	292 (51.0%)	91 (48.9%)	201 (51.9%)	0.500
	Female	281 (49.0%)	95 (51.1%)	186 (48.1%)	
Tobacco	Yes	98 (17.1%)	34 (18.3%)	64 (16.5%)	0.605
	No	475 (82.9%)	152 (81.7%)	323 (83.5%)	
CHD	Yes	77 (13.4%)	37 (19.9%)	40 (10.3%)	0.002
	No	496 (86.6%)	149 (80.1%)	347 (89.7%)	
CCI		3.15 ± 1.55	3.54 ± 1.67	2.96 ± 1.46	<0.001
ASA	1	42 (7.3%)	34 (8.8%)	8 (4.3%)	0.069
	2	436 (76.1%)	293 (75.7%)	143 (76.9%)	
	3	95 (16.6%)	60 (15.5%)	35 (18.8%)	
PLR		153.13 ± 74.16	144.99 ± 65.16	157.04 ± 77.89	0.068
Hb, g/dl		125.4 ± 18.09	124.12 ± 19.14	126.01 ± 17.56	0.242
eGFR, ml/min/1.73m^2		67.28 ± 23.51	63.80 ± 25.12	68.95 ± 22.55	0.014
FAR		8.64 ± 2.57	8.74 ± 2.70	8.59 ± 2.50	0.497
AAR		1.36 ± 0.59	1.21 ± 0.43	1.43 ± 0.63	<0.001
TG, mmol/L		1.58 ± 1.14	2.00 ± 1.66	1.38 ± 0.70	<0.001
HDL-C, mmol/L		1.11 ± 0.26	1.00 ± 0.24	1.17 ± 0.25	<0.001
GLU, mmol/L		5.89 ± 1.83	6.96 ± 2.44	5.37 ± 1.13	<0.001
Components of MetS					
BMI	≥25	230 (40.1%)	152 (81.7%)	78 (20.1%)	<0.001
	<25	343 (59.9%)	34 (18.3%)	309 (79.8%)	
Diabetes	Yes	176 (30.7%)	122 (65.6%)	54 (14.0%)	<0.001
	No	397 (69.3%)	64 (34.4%)	333 (86.0%)	
Hypertension	Yes	371 (64.8%)	172 (92.5%)	199 (51.4%)	<0.001
	No	202 (35.3%)	14 (7.5%)	188 (48.6%)	
Hyperlipidemia	Yes	313 (54.6%)	167 (89.8%)	146 (37.7%)	<0.001
	No	260 (45.4%)	19 (10.2%)	241 (62.3%)	
Pathological characteristics					
T stage	T_a-T_1	145 (25.3%)	49 (26.3%)	96 (24.8%)	0.252
	T_2	125 (21.8%)	45 (24.2%)	80 (20.7%)	
	T_{3-4}	274 (47.8%)	80 (43.0%)	194 (50.1%)	
	Undefined	29 (5.1%)	13 (7.0%)	16 (4.1%)	
N stage	N_0	71 (12.4%)	20 (10.8%)	51 (13.2%)	0.360
	N_1	18 (3.1%)	3 (1.6%)	15 (3.9%)	
	N_x	484 (84.5%)	163 (87.6%)	321 (82.9%)	
WHO/ISUP grade	Low Grade	106 (18.5%)	37 (19.9%)	69 (17.8%)	0.599
	High Grade	456 (79.6%)	147 (79.0%)	309 (79.8%)	
	Undefined	11 (1.9%)	3 (1.6%)	8 (2.1%)	
Tumor diameter, cm		3.21 ± 1.98	3.09 ± 1.84	3.27 ± 2.04	0.306
Multifocality	Single	447 (78.0%)	151 (81.2%)	296 (76.5%)	0.204
	Multi	126 (22.0%)	35 (18.8%)	91 (23.5%)	
CIS	Yes	28 (4.9%)	5 (2.7%)	23 (6.0%)	0.091
	No	545 (95.1%)	181 (97.3%)	364 (94.1%)	

CHD, coronary heart disease; CCI,Charlson Comorbidity Index; ASA, American Society of Anesthesiologists classification; PLR, platelet-lymphocyte ratio; Hb, hemoglobin; eGFR, estimated Glomerular filtration rate; FAR, fibrinogen-albumin ratio; AAR, aspartate/alanine aminotransferase ratio; TG, triglyceride; HDL-C, high density liptein cholesterol; Glu, glucose; BMI, body mass index; CIS, carcinoma in situ.

as significant co-predictors of CSS. However, MetS was not found to be an independent predictor for OS (95%CI 0.683-1.300, p=0.716) and IVRFS (95%CI 0.590-1.122, p=0.361). For OS, patient gender (P=0.029), pathologic T stage (P=0.010), tumor grade (P=0.002), and tumor size (P=0.001) were also significant co-predictors. Patient gender (P=0.009), PLR (P=0.001) and tumor multifocality (P=0.002) were revealed as independent predictors for IVRFS. Kaplan-Meier curves demonstrated that MetS showed no statistical correlation with lower OS and IVRFS and a marginal association with lower CSS (P=0.06) than those without MetS (**Figures 2A–C**).

Clinicopathological Characteristics After PSM

After PSM, the distributions of baseline and clinicopathological characteristics between MetS and non-MetS groups are summarized **Table 3**. Absolute standardized differences for all observed covariates were below 15%, suggesting an acceptable improvement in covariate balance. Only the standard difference of CHD, which is closely associated with MetS, declined less than other covariates. Age, gender, CCI, ASA classification, PLR, eGFR, AST/ALT ratio, and T stage were controlled in matching with a caliber of 0.02 (**Figures 3–5**).

TABLE 2 | Univariate and multivariate cox regression analyses for OS, CSS and IVRFS before propensity score matching.

Characteristics	OS				CSS				IVRFS			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	P	95%CI	P	95%CI	P	95%CI	P	95%CI	P	95%CI	P	95%CI
Age, years	**0.001**	**1.012-1.045**	0.154	0.994-1.039	0.076	0.998-1.036	0.483	0.984-1.035	**0.026**	**1.003-1.051**	0.264	0.987-1.050
Gender	**0.031**	**0.528-0.970**	**0.029**	**0.462-0.959**	**0.037**	**0.475-0.977**	**0.029**	**0.408-0.953**	**0.018**	**0.368-0.911**	**0.009**	**0.339-0.856**
Tobacco	0.611	0.611-1.335			0.224	0.444-1.210			0.365	0.406-1.392		
coronary	0.117	0.919-2.129			0.924	0.597-1.766			0.425	0.694-2.380		
CCI	**0.001**	**1.110-1.348**	0.488	0.906-1.231	**0.032**	**1.011-1.272**	0.467	0.893-1.280	0.053	0.998-1.327	0.422	0.886-1.336
ASA												
1	–	Referent			–	Referent			–	Referent		
2	**0.006**	**0.141-0.726**	0.203	0.238-1.356	0.169	0.207-1.318			0.734	0.316-2.252		
3	**0.023**	**0.431-0.941**	0.868	0.602-1.535	0.712	0.549-1.507			0.760	0.487-1.691		
PLR	**0.002**	**1.001-1.004**	0.381	0.999-1.003	**0.002**	**1.001-1.005**	0.669	0.998-1.003	**0.001**	**1.002-1.006**	**0.001**	**1.002-1.006**
Hb, g/dl	**0.001**	**0.979-0.995**	0.278	0.983-1.005	**0.004**	**0.977-0.995**	0.103	0.977-1.002	0.778	0.989-1.014		
eGFR, ml/min/1.73m^2	**0.001**	**0.983-0.995**	0.612	0.990-1.006	0.061	0.986-1.000	0.657	0.993-1.011	0.248	0.986-1.004		
AAR	0.458	0.876-1.342			0.149	0.943-1.473			0.879	0.677-1.397		
FAR	**0.001**	**1.058-1.152**	0.443	0.967-1.081	**0.001**	**1.059-1.171**	0.306	0.968-1.109	0.139	0.454-1.116		
MetS	0.716	0.683-1.300			0.094	0.943-2.117	0.063	0.978-2.351	0.088	0.990-1.153	0.361	0.59-1.122
BMI (>25kg/m^2)	0.421	0.646-1.200			0.716	0.491-1.043			0.596	0.723-1.758		
Diabetes	0.143	0.924-1.731			0.998	0.680-1.473			0.779	0.666-1.720		
Hypertension	0.673	0.779-1.473			0.654	0.636-1.329			0.726	0.680-1.739		
Hyperlipidemia	0.481	0.663-1.214			0.294	0.578-1.181			0.901	0.624-1.514		
T (≥2) stage	**0.001**	**2.498-7.025**	**0.010**	**0.257-0.828**	**0.001**	**3.600-18.630**	**0.006**	**0.127-0.705**	0.123	0.894-2.565		
WHO grade	**0.001**	**2.374-7.403**	**0.002**	**0.202-0.694**	**0.001**	**2.687-13.931**	**0.003**	**0.100-0.634**	0.437	0.710-2.210		
Tumor diameter(>=3cm)	**0.001**	**1.265-2.389**	**0.001**	**1.113-1.313**	**0.011**	**1.115-2.357**	**0.001**	**1.095-1.315**	0.291	0.814-1.986		
Multifocality	0.704	0.635-1.359			0.662	0.717-1.688			**0.005**	**1.231-3.149**	**0.002**	**0.294-0.761**
CIS	0.426	0.318-1.623			0.995	0.439-2.267			0.584	0.228-2.298		

CCI, Charlson Comorbidity Index; ASA. American Society of Anesthesiologists classification; PLR, platelet-lymphocyte ratio; Hb, hemoglobin; eGFR, estimated Glomerular filtration rate; FAR, fibrinogen-albumin ratio; BMI, body mass index; CIS, carcinoma in situ.

Bold values represent statistical differences.

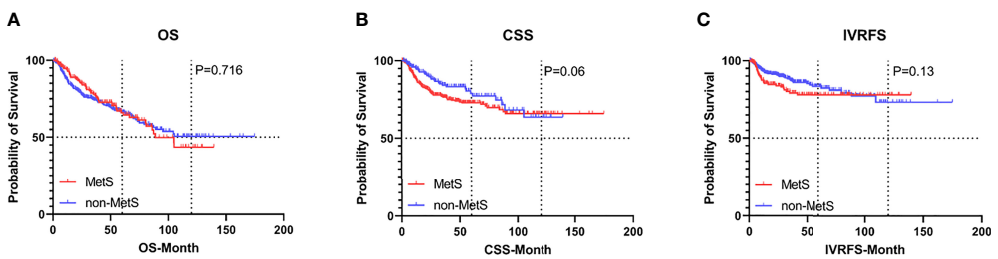

FIGURE 2 | Kaplan-Meier curves for survival outcomes in UTUC patients according to the presence of MetS before propensity score matching. **(A)** OS, **(B)** CSS, and **(C)** IVRFS. OS, overall survival; CSS, cancer-specific survival; IVRFS, intravesical recurrence-free survival.

As shown in **Table 3**, nearly all clinicopathological characteristics did not have any statistical difference between MetS and non-MetS group, except each component of MetS diagnosis (all P<0.05).

Survival Outcomes After PSM

With a median (IQR) follow-up duration of 33.1 (14.0-58.6) months postoperatively, there were 90 (28.7%) overall deaths after a median (IQR) of 32.6 (13.9-68.1) months and 58 (18.5%) cancer-specific deaths after a median (IQR) of 17.7 (9.7-39.1) months. Also, 50 patients (15.9%) experienced IVR after a median (IQR) of 10.1 (6.4-23.5) months. The 5-year OS, CSS, and IVRFS were 64.1%, 74.7%, and 77.2%, respectively, in the MetS group, as compared with 67.4%, 78.8%, and 77.2%, respectively, in non-MetS group. Kaplan-Meier curves demonstrated that MetS patients had almost the same CSS, RFS, and OS as those without MetS (all P>0.05; **Figure 6**). Univariate Cox proportional hazards regression analyses showed that MetS and its components were not associated with decreased OS, CSS, and IVRFS (all P>0.05; **Table 4**). After adjusting clinical confounders, multivariate Cox regression analysis showed that age, pathological T stage, tumor grade, and tumor size were significant co-predictors of OS (all P<0.05). T stage and tumor grade were also significant co-predictors of CSS (both P<0.05). Age and patient gender were significant co-predictors of IVRFS (both P<0.05) (**Table 4**).

TABLE 3 | Clinicopathological characteristics of the entire cohort and subgroups according to MetS after propensity score matching.

Characteristics	Subgroup	Entire cohort	MetS	Non-MetS	P-value
N		314	157	157	
Preoperative characteristics					
Age, years		67.92 ± 9.60	67.92 ± 9.43	67.92 ± 9.80	1.000
Gender	Male	156 (49.7%)	80 (51.0%)	76 (48.4%)	0.653
	Female	158 (50.3%)	77 (49.0%)	81 (51.6%)	
Tobacco	Yes	54 (17.2%)	29 (18.5%)	25 (15.9%)	0.551
	No	260 (82.8%)	128 (81.5%)	132 (84.1%)	
CHD	Yes	45 (14.3%)	26 (16.6%)	19 (12.1%)	0.260
	No	269 (85.7%)	131 (83.4%)	138 (87.9%)	
CCI		3.32 ± 1.53	3.37 ± 1.62	3.26 ± 1.43	0.531
ASA	1	15 (4.8%)	7 (4.5%)	8 (5.1%)	0.800
	2	248 (79.0%)	124 (79.0%)	124 (79.0%)	
	3	51 (16.2%)	26 (16.6%)	25 (15.9%)	
PLR		146.35 ± 60.43	149.28 ± 67.4	143.42 ± 52.61	0.391
Hb, g/dl		124.63 ± 18.17	124.44 ± 19.04	124.81 ± 17.32	0.859
eGFR, ml/min/1.73m^2		65.76 ± 23.37	66.03 ± 24.21	65.48 ± 22.57	0.837
FAR		8.64 ± 2.40	8.61 ± 2.38	8.67 ± 2.43	0.849
AAR		1.24 ± 0.42	1.24 ± 0.44	1.24 ± 0.41	0.980
TG, mmol/L		1.69 ± 1.38	1.97 ± 1.77	1.41 ± 0.68	<0.001
HDL-C, mmol/L		1.07 ± 0.25	1.01 ± 0.25	1.13 ± 0.24	<0.001
GLU, mmol/L		6.12 ± 2.04	6.85 ± 2.48	5.40 ± 1.08	<0.001
Components of MetS					
BMI		24.91 ± 3.46	26.62 ± 2.97	23.21 ± 3.05	<0.001
Diabetes	Yes	125 (39.8%)	100 (63.7%)	25 (15.9%)	<0.001
	No	189 (60.2%)	57 (36.3%)	132 (84.1%)	
Hypertension	Yes	232 (73.9%)	146 (93.0%)	86 (54.8%)	<0.001
	No	82 (26.1%)	11 (7.0%)	71 (45.2%)	
Hyperlipidemia	Yes	202 (64.3%)	141 (89.8%)	61 (38.9%)	<0.001
	No	112 (35.7%)	16 (10.2%)	96 (61.1%)	
T stage	T_a-T_1	90 (28.7%)	43 (27.4%)	47 (29.9%)	0.948
	T_2	71 (22.6%)	40 (15.5%)	31 (19.7%)	
	T_{3-4}	153 (48.7%)	74 (47.1%)	79 (50.3%)	
N stage	N_0	40 (12.7%)	20 (12.7%)	20 (12.7%)	0.377
	N_1	7 (2.2%)	2 (1.3%)	5 (3.2%)	
	N_x	267 (85.0%)	135 (86.0%)	132 (84.1%)	
WHO/ISUP	LG	58 (18.5%)	29 (18.5%)	29 (18.5%)	0.957
	HG	252 (80.3%)	127 (80.9%)	125 (79.6%)	
	Undefined	4 (1.3%)	1 (0.6%)	3 (1.9%)	
Tumor diameter		3.06 ± 1.75	3.19 ± 1.90	2.93 ± 1.57	0.474
Multifocality	Single	249 (79.3%)	127 (80.9%)	122 (77.7%)	0.488
	Multi	65 (20.7%)	30 (19.1%)	35 (22.3%)	
CIS	Yes	17 (5.4%)	5 (3.2%)	12 (7.6%)	0.081
	No	297 (94.6%)	152 (96.8%)	145 (92.4%)	

CHD, coronary heart disease; CCI, Charlson Comorbidity Index; ASA, American Society of Anesthesiologists classification; PLR, platelet-lymphocyte ratio; Hb, hemoglobin; eGFR, estimated Glomerular filtration rate; FAR, fibrinogen-albumin ratio; AAR, aspartate/alanine aminotransferase ratio; TG, triglyceride; HDL-C, high density liptein cholesterol; Glu, glucose; BMI, body mass index; CIS, carcinoma in situ.

DISCUSSION

In our present single-center study, 573 patients with UTUC treated with RNU were included and we observed whether MetS had negative impact on survival outcomes. Moreover, we re-evaluated the association between MetS and outcomes of UTUC after adjusting preoperative confounders by propensity score matching with a sample size of 314 patients. The results showed that the existence of MetS was not an independent factor for worse pathological outcomes and survival outcomes including death or IVR. However, it is worth noting that there presented a correlation which was of marginal significance between MetS and lower CSS in both multivariate Cox regression analysis and Kaplan-Meier survival analysis, indicating that MetS may be associated with increased risk of UTUC.

Evidence based on several current studies showed that patients diagnosed with MetS were more likely to have worse survival outcomes of several types of malignant tumors (10), such as breast cancer (3) and bladder cancer (5). But the inverse relationship between MetS and outcomes were presented in patients with ovarian (11) or renal cancer (12). Even within the same cancer, the results remain controversial. The results of evaluating prognostic value of MetS in localized clear cell renal cell carcinoma (ccRCC) (12, 13) were diametrically opposed. As for UTUC, a recent study based on the Chinese population demonstrated that MetS was a negative prognostic factor of CSS

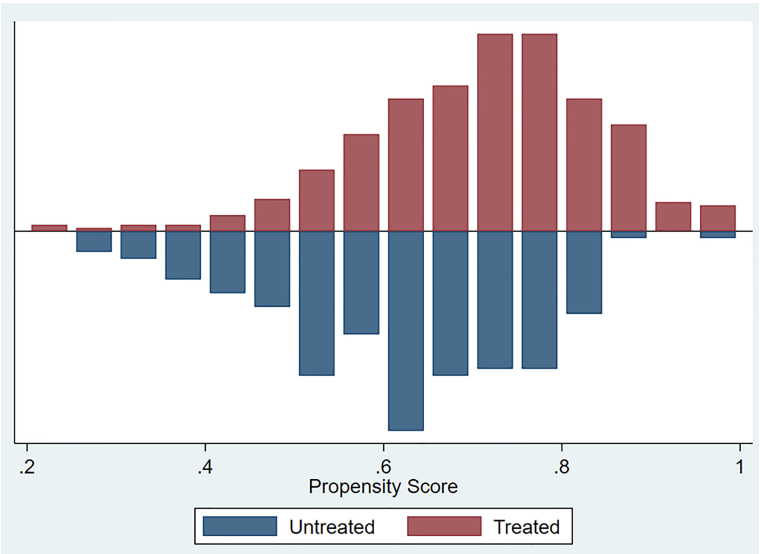

FIGURE 3 | Covariate balance test of matching.

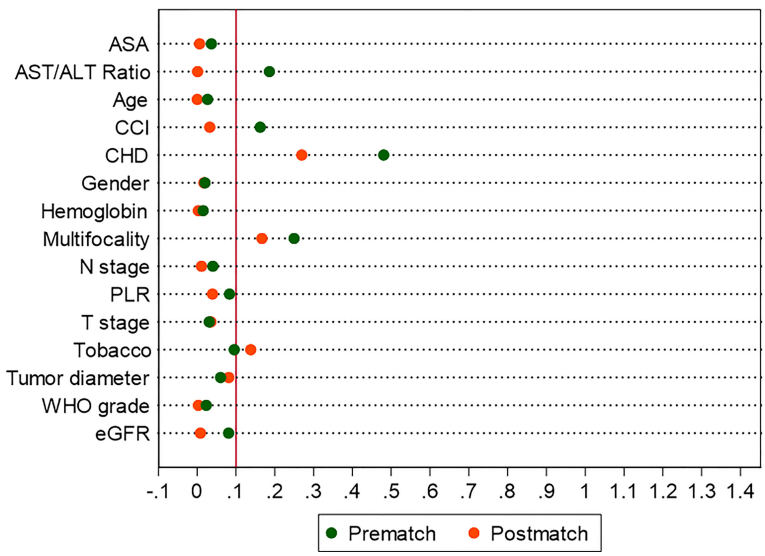

FIGURE 4 | Absolute standardized differences in all characteristics between MetS and non-MetS group, before and after propensity score matching.

in UTUC and the trend was particularly persisted in patients with non-muscle-invasive UTUC, high-grade disease, and large tumor size (8). As described by another study based on Surveillance, Epidemiology and End Results-Medicare-Linked Database (SEER), MetS and its components were significant risk factors for UTUC among people aged over 65 (7). Whether MetS can be a predictor of UTUC still remains a topic of concern.

MetS, as a cluster of metabolic abnormalities, has complex clinical manifestations and diagnostic criteria. Diagnostic criteria suitable for western populations, which were mostly from large international or European and American institutions, such as the American Heart Association (AHA), the National Heart, Lung, and Blood Institute (NHLBI), and the International Diabetes Federation (IDF). Although some researchers have modified the diagnostic criteria to accommodate local populations, it could also be one of important reasons for differences in results. We adopted the latest version of diagnostic criteria released by CDS, making our best efforts to ensure that the diagnostic criteria was

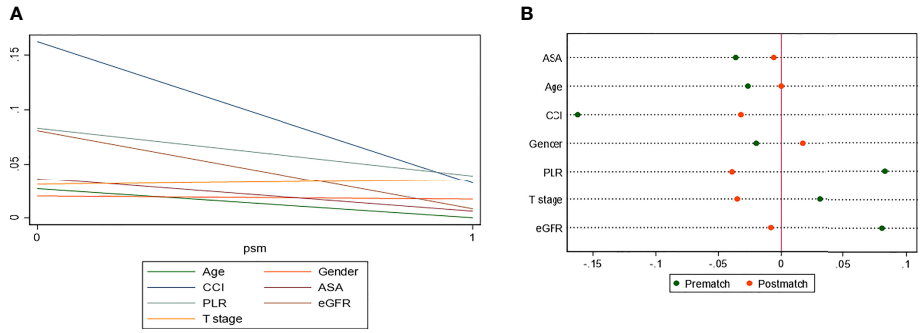

FIGURE 5 | Standardized differences in covariates between MetS and non-MetS group, prematch and postmatch: **(A)** Line-plot; **(B)** Dot-plot.

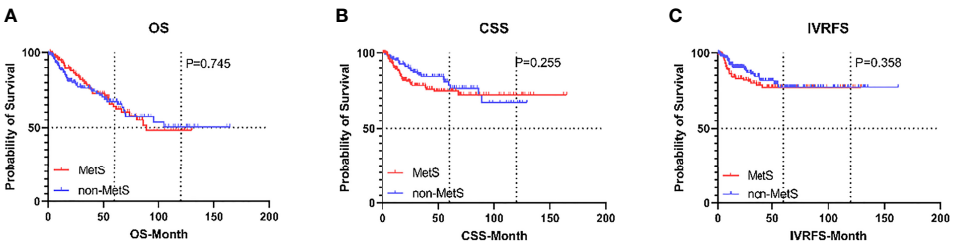

FIGURE 6 | Kaplan-Meier curves for survival outcomes in UTUC patients according to the presence of MetS after propensity score matching. **(A)** OS, **(B)** CSS, and **(C)** IVRFS. OS, overall survival; CSS, cancer-specific survival; IVRFS, intravesical recurrence-free survival.

appropriate for the Chinese population. We also collected the use of therapeutic drug for MetS during follow-up according to diagnostic criteria to ensure that MetS could be diagnosed accurately when patients were admitted with normal blood pressure or blood glucose level. MetS may be more easily diagnosed with CDS criteria than others, due to its lower cut-off of BMI. In our study, patients diagnosed with MetS accounted for 32.5% of the total patients, which was higher than 24.4% based on IDF criteria in Xu's research (8) and 17.1% based on National Cholesterol Education Program-Adult Treatment Panel III (ATPIII) criteria in Lu's research (7). Difference of diagnostic criteria is expected to affect the level of baseline and clinicopathological characteristics but whether it will result in inconsistency in the prognostic value of MetS on UTUC prognosis still needs further research.

Obesity, as a major component of MetS, has been determined in many studies to be associated with poor prognosis of renal cancer (14) or other cancers. In IDF and ATPIII diagnostic criteria, obesity was defined using waist circumference as the indicator and recent studies have also shown that waist circumference could reflect centripetal obesity more directly than BMI. But the cut-off value of waist circumference was determined from data obtained from the American population. Studies showed that there was significant heterogeneity in waist circumference and BMI among different population and the baseline BMI of the Chinese population was significantly lower than that of western population. A study including 971 Chinese patients showed that BMI was a better predictor of cardiovascular events than waist circumference (9). Unfortunately, the cut-off values of waist circumference or waist-to-hip ratio in Chinese population have not been determined yet, which requires further collaborative research.

A meta-analysis concerning the impact of BMI on urothelial carcinoma provided conclusions that being obese and underweight were predictors for predicted worse survival outcomes, while being overweight was a protective factor (15). But in UTUC patients, previous studies revealed contradictory results. Ehdaie et al. (16) and Dabi et al. (17) showed that increased BMI impacts oncological outcomes in western patients with UTUC, but Kang et al. (18) Liu et al. (19) and Inamoto et al. (20) demonstrated that a preoperative decreased BMI was an independent predictor for OS and CSS after analyzing data from Korean, Chinese, and Japanese populations. Given the large differences in baseline BMI between Asian, and Western populations, collaborative international studies are needed to explained the controversy after rigorous matching. A meta-analysis including 10 studies showed that diabetes increased the risk of IVR in UTUC patients (21), and an international retrospective study discovered that hypertension was a significant risk factor for IVR based on data set from 17 centers worldwide (22). However, the effect of diabetes and hypertension on overall or cancer-specific survival has not been proven. An earlier study showed a weak inverse association between HDL-cholesterol and progress of bladder urothelial carcinoma (23). Xu et al. (8) discovered that patients with hypertriglyceridemia or low HDL-cholesterol were more

TABLE 4 | Univariate and multivariate cox regression analyses for OS, CSS and IVRFS after propensity score matching.

Characteristics	OS				CSS				IVRFS			
	Univariate		Multivariate		Univariate		Multivariate		Univariate		Multivariate	
	P	95%CI	P	95%CI	P	95%CI	P	95%CI	P	95%CI	P	95%CI
Age, years	**0.005**	**1.010-1.058**	**0.026**	**1.003-1.057**	0.159	0.992-1.049			**0.014**	**1.008-1.073**	**0.010**	**1.010-1.075**
Gender	0.056	0.438-1.011	0.133	0.404-1.127	0.110	0.387-1.101			0.062	0.972-3.019	**0.047**	**0.315-0.980**
Tobacco	0.949	0.586-1.651			0.481	0.634-2.633			0.395	0.636-3.145		
coronary	0.440	0.689-2.354			0.664	0.557-2.501			0.487	0.613-2.792		
CCI	0.615	0.414-1.684			0.820	0.427-2.933			0.875	0.348-2.454		
ASA												
1	–	Referent			–	Referent			–	Referent		
2	0.302	0.102-2.030			0.568	0.062-4.584			0.605	0.420-4.437		
3	0.797	0.500-1.702			0.267	0.671-4.225			0.332	0.335-1.446		
PLR	0.735	0.996-1.003			0.993	0.996-1.004			0.736	0.997-1.005		
Hb, g/dl	0.069	0.979-1.001	0.394	0.979-1.009	**0.032**	**0.972-0.999**	0.119	0.974-1.003	0.406	0.991-1.023		
eGFR, ml/min/1.73m^2	0.062	0.984-1.000	0.915	0.991-1.011	0.358	0.985-1.006			0.310	0.983-1.006		
AAR	**0.004**	**1.035-1.196**	0.785	0.917-1.121	0.065	0.994-1.200			0.583	0.924-1.150		
FAR	0.172	0.878-2.081			0.069	0.964-2.637			0.562	0.650-2.211		
MetS	0.745	0.617-1.412			0.257	0.440-1.245			0.360	0.743-2.269		
BMI (>25kg/m^2)	0.953	0.670-1.531			0.628	0.678-1.904			0.452	0.463-1.409		
Diabetes	0.334	0.537-1.235			0.871	0.615-1.777			0.442	0.700-2.259		
Hypertension	0.392	0.771-1.942			0.155	0.860-2.580			0.356	0.726-2.441		
Hyperlipidemia	0.330	0.809-1.881			0.303	0.780-2.226			0.901	0.582-1.849		
T (≥2) stage	**0.001**	**0.132-0.491**	**0.037**	**0.209-0.952**	**0.001**	**0.036-0.369**	**0.033**	**0.080-0.902**	0.213	0.346-1.268		
WHO grade	**0.001**	**0.121-0.569**	**0.048**	**0.189-0.993**	**0.004**	**0.030-0.501**	**0.072**	**0.062-1.125**	0.186	0.261-1.298		
Tumor diameter(>=3cm)	**0.003**	**0.329-0.790**	**0.001**	**1.090-1.387**	**0.024**	**0.308-0.922**	**0.004**	**1.065-1.392**	0.117	0.366-1.118		
Multifocality	0.369	0.740-2.246			0.850	0.552-2.055			0.248	0.366-1.296		
CIS	0.488	0.475-4.760			0.990	0.310-3.175			0.613	0.350-5.928		

CCI, Charlson Comorbidity Index; ASA, American Society of Anesthesiologists classification; PLR, platelet-lymphocyte ratio; Hb, hemoglobin; eGFR, estimated Glomerular filtration rate; FAR, fibrinogen-albumin ratio; BMI, body mass index; CIS, carcinoma in situ.

Bold values represent statistical differences.

likely to have adverse pathological features and lower OS, CSS, and RFS of UTUC in univariable Cox regression analyses, but not confirmed in multivariate Cox regression analyses. According to our data and analysis, all components found no statistically significant association with survival outcomes. Further studies are needed to confirm the impact of MetS and its components on progress or prognosis of UTUC.

There is increasing evidence to indicate that there is a certain relationship between MetS and survival prognosis in certain types of cancers. However, numerous mechanisms of how MetS affected pathological features and survival outcomes have been proposed but fail to elaborate at the molecular level, involving insulin-like growth factor (IGF) axis, pro-inflammatory cytokines, circulating factors, angiogenesis, and other important aspects (10). Meanwhile, a network containing these factors and a variety of complex signaling pathways regulates the relation between MetS and tumor progress. The insulin-like growth factor (IGF) system, composed of different subtypes with their receptors and binding proteins, plays an important role on tumor formation, differentiation, and progression. Increased IGF strongly correlates with insulin resistance and obesity, promoting proliferation and migration of pathological cell and overexpression of IGF-1R (24) and IGFBP-5 (24) in UTUC which was proved by *in vitro* experiment. Central fat distribution and low HDL-cholesterol is also associated with production of many pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), and elevated level of reactive oxygen species (ROS). In addition, MetS patients tend to have high level of adipose tissue, which correlated with an elevated level of serum leptin and reduction of adiponectin. All these factors have been demonstrated to stimulate angiogenesis, which promotes epithelial cell proliferation (10).

Abundant evidence has described the closed association between each single component of MetS with tumor, and combining all the components of MetS as a single condition may not be appropriate. But at the same time, considering MetS and its components as independent factors for UTUC may reveal justification for pre-operative use of MetS-specific drugs including as statin and metformin.

In our study, some potential predicting factors were not included in the multiple Cox analysis, such as N stage, type of bladder cuff management and lymphovascular invasion (LVI). LND is not currently included in UTUC's standard procedure and is performed only when lymph node metastasis is suspected. Only 89 patients (15.5%) underwent LND as shown in **Tables 1**, **3**, which may result in potential sampling error. Common types of bladder cuff removal included transvesical, extravesical, and endoscopic approaches. The result of a study comparing different methods of bladder cuff management showed that there was no difference in terms of OS, CSS, and RFS among the three distal ureteral management, but patients who underwent the endoscopic approach had a higher risk of IVR (25). Meanwhile, other researchers have also reached different conclusions (26) and the impact of methods of bladder cuff

management on oncological outcome is still controversial. The diagnosis of some pathological features including LVI are extremely dependent on pathological reports and sometimes it is difficult to obtain accurate information on some pathological features due to non-standard reporting formats. Smoking, as a factor closely associated with dyslipidemia, is known to increase the risk of experiencing adverse events and IVR in urothelial carcinoma patients (27, 28) but the relation and biological basis between smoking and MetS remains unclear. A recent annual cross-sectional survey showed that life-course cigarette smoking is associated with increased odds of MetS and low high-density lipoprotein-cholesterol (29), which may portend an association between MetS and tumor recurrence.

Our study is not devoid of limitations. First, limited by retrospective data, we could not detail the duration of each component and use of a corresponding drug. Also, small sample size prevented further subgroup analysis based on medication status, such as statin (n=23) or metformin (n=24). Thus, although we adjusted preoperative confounders by propensity score matching with a small caliber of 0.02, some confounders which may affect survival outcomes, like tobacco consumption and some pathological features, were not included. Finally, the duration, dose, and regimen of adjuvant chemotherapy were incomplete, so we were not able to evaluate its potential mitigation effect on survival outcomes.

CONCLUSION

In our study, no statistical difference was found between MetS and survival outcomes in UTUC, except a marginal association with lower CSS. Further studies are needed to evaluate the role of MetS and its each single components on UTUC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics committee of Peking University People's Hospital. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

XD carried out the study, participated in the data analysis and drafted the manuscript. XD, YD, FW, SH, and ZH collected the data. XD, YD, CQ, SL, YS, and TX participated in designing the study. XD, YD, XZ, and TX revised the manuscript for important intellectual content. All authors gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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