Original Paper

Is Overexpression of Ki-67 a Prognostic Biomarker of Upper Tract Urinary Carcinoma? A Retrospective Cohort Study and Meta-Analysis

Bo Fan^a Hongshuo Zhang^b Huidan Jin^a Yu Gai^c Honglong Wang^d
Huafeng Zong^d Mingyu Jin^a Hao Yang^a Shuping Wan^e Jiabin Zhu^a Siyao Xu^a
Jianbo Wang^f Deyong Yang^f Xishuang Song^f

^aGraduate School, Dalian Medical University, Dalian, Liaoning, ^bDepartment of Biochemistry, Institute of Glycobiology, Dalian Medical University, Dalian, ^cDepartment of Pathology, Dalian Central Hospital Affiliated to Dalian Medical University, Dalian, ^dDepartment of Pathology, Dalian Friendship Hospital, Dalian, ^eDepartment of Urology, Land Reclamation Headquarters General Hospital, Erbin Ha, Heilongjiang, ^fDepartment of Urology, First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning, P.R. China

Key Words

Prognostic biomarker • Ki-67 • Upper urinary tract • Urothelial carcinoma • Meta-analysis

Abstract

Background: Upper tract urinary carcinoma (UTUC) is a relatively uncommon but aggressive disease. The Ki-67 antigen is a classic marker of cellular proliferation, but there is still controversy regarding the significance and importance of Ki-67 in tumor progression. **Methods:** In this study, we first detected Ki-67 expression in UTUC patients by immunohistochemistry (IHC). Subsequently, we quantitatively combined the results with those from the published literature in a meta-analysis after searching several databases. **Results:** IHC results demonstrated that patients with muscle-invasive tumors (T2-T4) had higher Ki-67 expression than those with non-muscle-invasive tumors (Tis-T1), suggesting that high Ki-67 expression may be associated with the aggressive form of UTUC. Kaplan-Meier curves showed that patients with high Ki-67 expression had significantly poorer cancer-specific survival (CSS) and disease-free survival (DFS). Furthermore, multivariate analysis suggested that Ki-67 expression was an independent prognostic factor for CSS (hazard ratio, HR=3.196) and DFS (HR=3.517) in UTUC patients. Then, a meta-analysis of the published literature investigating Ki-67 expression and its effects on UTUC prognosis was conducted. After searching the PubMed, Medline, Embase, Cochrane Library and Scopus databases, 12 articles met the eligibility criteria for this analysis. The

B. Fan and H. Zhang contributed equally to this work.

Xishuang Song, Deyong Yang
and Jianbo Wang

Department of Urology, First Affiliated Hospital of Dalian Medical University, Dalian,
Liaoning, 116011 (P.R. China)
E-Mail song_xishuang@sina.com/yang_deyong@sina.cn/wang_jianbo431@yeah.net

eligible studies included a total of 1740 patients with a mean number of 82 patients per study (range, 38-475). The combined results showed that increased Ki-67 levels were associated with poor survival and disease progression, with a pooled HR estimate of 2.081 and 2.791, respectively. In subgroup analysis, the pooled HR was statistically significant for cancer-specific survival (HR=2.276), metastasis-free survival (HR=3.008) and disease-free survival (HR=6.336).

Conclusions: In conclusion, high Ki-67 expression was associated with poor survival in patients with UTUC, as well as a high risk of disease progression, although these findings need to be interpreted with caution. Large-scale, adequately designed, prospective trials are needed to further confirm the value of Ki-67 in prognosis of UTUC patients.

© 2016 The Author(s)
Published by S. Karger AG, Basel

Introduction

Upper tract urinary carcinoma (UTUC) is a relatively uncommon but aggressive disease that accounts for only 5-10% of all urothelial malignancies [1, 2] and has an estimated annual incidence in Western countries of approximately 2 cases per 100,000 inhabitants [3]. As previously reported, the ratio of urothelial carcinoma in the renal pelvis and ureter is reported to be 3:1 [4, 5]. Sixty percent of UTUCs are invasive at diagnosis compared with 15-25% of bladder tumors [6, 7]. UTUCs have a peak incidence in individuals aged 70-90 years and are three times more common in men [8, 9]. Although radical nephroureterectomy (RNU) with bladder cuff excision is the standard treatment for UTUC, the urothelial cancer often recurs in the bladder after surgery. However, 22-50% of patients undergoing RNU develop postoperative intravesical recurrence during the follow-up period [10-12]. When UTUC invades the muscle wall, the 5-year specific survival rate is less than 50% for pT2/pT3 cases and less than 10% for pT4 cases, indicating poor prognosis [13-16]. A more accurate knowledge of the exact molecular mechanisms of tumor invasion and recurrence would improve UTUC prognostic accuracy and would benefit patients.

Cell proliferation is an important prognostic indicator of recurrence, aggressiveness and progression of tumors [17]. The Ki-67 antigen is a classic marker of cellular proliferation because it is preferentially expressed during the late G1, S, G2 and M phases of the cell cycle, whereas it is absent from resting cells in G0 phase; thus, it is an excellent marker for determining the growth fraction of a cell population [18-20]. Indeed, previous studies reported that Ki-67 expression was associated with tumor stage, grade, and recurrence in patient with bladder cancer [21]. However, there is still a debate regarding the significance and importance of Ki-67 in tumor progression because several studies identified it as a poor prognostic marker in UTUCs [22, 23], while others disagreed with these reports [24, 25]. Since evidence for its current role in UTUC is relatively poor, and few studies on Ki-67 molecular mechanisms in UTUC performed in Chinese mainland patients are available, it is of utmost importance to combine these data to reach a reasonable conclusion.

Therefore, the present study is focused on the retrospective evaluation of Ki-67 expression and its association with conventional pathological parameters and prognosis of patients. For this purpose, a systematic review and meta-analysis of published literature investigating the role of Ki-67 expression and its effects on UTUC prognosis were performed to help define individualized and closer follow-ups for marker-positive patients.

Materials and Methods

Study population

The present research was sanctioned by the Institutional Research Ethics Committee of the First Affiliated Hospital of Dalian Medical University (No. LCKY2015-08), and informed consent was provided by all 94 patients who underwent RNU between January 2008 and December 2013; diagnosis with UTUC was affirmed by clinical resection and pathology. Patients who received neoadjuvant chemotherapy, who had distant metastatic lesions at surgery and with previous or concurrent urothelial carcinoma of the

bladder were excluded from the present study. Surgery was performed by several surgeons according to the standard criteria for RNU.

Histopathological evaluation

All surgical specimens were processed using standard pathological procedures, and hematoxylin and eosin (H&E)-stained slides from all cases were reviewed by genitourinary pathologists without any knowledge of the clinical outcomes. Tumors were pathologically staged according to the 2002 TNM classification of the American Joint Committee on Cancer, and tumors were graded according to the 1998 WHO/International Society of Urologic Pathology consensus classification [26].

Immunohistochemical staining

Formalin-fixed, paraffin-embedded tissues were cut into 4 μ m thick sections and were then deparaffinized with xylene and rehydrated through a graded alcohol series. For antigen retrieval, the slides were placed in 10 mM citrate buffer (pH 6.0) and boiled for 20 min. Then, 3% hydrogen peroxide in methanol was used to block the endogenous peroxidase activity. The slides were incubated with the first antibody, rabbit polyclonal anti-Ki-67 (1:100, ProteinTech Group, Chicago, IL, USA), at room temperature for an hour and then with the second antibody. HRP-linked anti-rabbit/anti-mouse IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:1000). For immunohistochemical staining, an EliVision plus kit (Maixin-bio, Fuzhou, China) was used, diaminobenzidine (DAB) staining was performed and the slides were counterstained with hematoxylin. Images were captured with an Olympus BX51 microscope (Japan).

Immunohistochemical analysis

Immunostaining was evaluated and scored by two of the authors (Y.G and H.L. W), who were blinded to the clinicopathological data. We obtained the immunohistochemistry results using 10 random hotspots in the tumor cells with each specimen at 200-fold magnification. Staining for Ki-67 was performed on the primary tumor as described previously [22]. The samples were classified into the following groups: low Ki-67 expression ($\leq 20\%$ of cell nuclei stained positive for Ki-67) and high Ki-67 expression ($> 20\%$).

Statistical analysis

SPSS 20.0 statistical software (SPSS Inc., Chicago, USA) was used to assess Ki-67 prognostic significance. Pearson's χ^2 test and Spearman's correlation analysis were applied to investigate the association between Ki-67 expression and patient characteristics. The Kaplan–Meier method was used to compare the disease-free survival (DFS) and cancer-specific survival (CSS) between the groups using the log-rank test. DFS was considered the interval between the date of UTUC surgery and the time of appearance of the first locoregional recurrence or distant metastasis. CSS was defined as the interval between the date of UTUC surgery and the time of UTUC-related death [27]. The potential prognostic factors were established by univariate analysis, and only the significant factors were entered into multivariate Cox proportional hazard regression models. Differences were considered statistically significant when the *p* value was less than 0.05.

Meta-analysis

Search Strategy. We searched the PubMed, Medline, Embase, Cochrane Library and Scopus databases to identify relevant studies evaluating the immunohistochemical expression of Ki-67 and its effects on UTUC prognosis from inception until April 2015. The following words and medical subject headings that included all spellings were used: “Ki-67”, “Immunohistochemistry”, “Upper urinary tract”, “Renal pelvis”, “Ureter”, “Urothelial cancer”, “Urothelial carcinoma”, and “Prognosis”. To obtain all-sided data of relevant articles, further eligible bibliographies were hand-searched, and company reports were sought. No restrictions for language or time of publication were applied.

Selection Criteria. Studies satisfying the following criteria were included in our analysis: (1) the histologic type of the tumors was urothelial carcinoma; (2) Ki-67 protein expression was evaluated in the upper urinary tract tissue; (3) Ki-67 expression was evaluated in tissues by IHC; (4) the association between Ki-67 expression levels and survival outcome was investigated. Accordingly, the following exclusion criteria were applied: (1) studies in form of reviews, letters to the editor, commentaries, or case reports that lacked original data; (2) laboratory studies, such as studies on cancer cell lines and animal models; (3) studies that

did not provide information on survival; (4) studies in which the hazard ratio (HR) and its standard error could not be calculated based on the given information.

Data extraction. To decrease the risk of losing a publication, a rigorous process was independently carried out by two investigators (Bo Fan and Hongshuo Zhang), who considered potentially relevant studies based on the predefined criteria. A third reviewer (Xishang Song) assessed the resulting extractions and resolved any discrepancies between the independent search results. Data retrieved from the reports included author, year of publication, region, study type, recruitment period, number of patients, demographics (age, sex), pathologic grade, cut-off value, HR, and 95% confidence interval (CI) of reported endpoints, including disease-free survival (DFS), cancer-specific survival (CSS), overall survival (OS), metastasis-free survival (MFS) and recurrence-free survival (RFS). The definition of DFS and CSS has been introduced previously; OS was defined as the interval between the date of UTUC surgery and the time of death. MFS and RFS were considered the interval between the date of UTUC surgery and the time of appearance of distant metastasis or the first locoregional recurrence, respectively.

Statistical analysis. We estimated the HR with 95% confidence interval (CI) for dichotomous outcomes. Heterogeneity of the included studies was evaluated using the χ^2 test, a qualitative analysis to evaluate statistical heterogeneity, and the I^2 test, a quantitative analysis measuring the percentage of inconsistency. If the heterogeneity p -value was > 0.1 in the χ^2 test, indicating no heterogeneity among studies, fixed-effects models were applied to estimate the pooled HR of each study. Otherwise, the random effects models were used. The I^2 -value in the I^2 test delineated the proportion of total variation that is due to heterogeneity rather than errors of sampling, and an $I^2 > 50\%$ indicates high heterogeneity. The potential for bias was assessed by inspection of a funnel plot and Egger's test if the number of studies included in each analysis was ≥ 10 . Otherwise, publication bias was not tested because of the limited number (below 10). The results were considered statistically significant at two-sided p -values < 0.05 . All statistical analyses were performed using Stata 12.0 (Stata Corporation, College Station, TX, USA).

Results

Association of Ki-67 expressions with the clinicopathological characteristics of UTUC

To investigate the clinical relevance of Ki-67 expression in high-grade UTUC, the levels of Ki-67 in the patients' tumor lesions were determined by immunohistochemistry (IHC) (Fig. 1A-D). Based on the staining intensity, we subdivided 94 UTUCs patients into two groups: the "low Ki-67" UTUC group included 62 tissue samples characterized by weak and low Ki-67 staining ($\leq 20\%$ of cell nuclei stained positive for Ki-67), while the "high Ki-67"

Fig. 1. Representative Ki-67 immunohistochemical staining in UTUC specimens at 200 magnification. (A) No expression of Ki-67 in UTUC tissue (0% staining) as a contrast. (B) Low expression of Ki-67 in UTUC tissue (15% staining). (C) High expression of Ki-67 in UTUC tissue (40% staining). (D) Positive control of Ki-67 in UTUC tissue (80% staining). High expression level of Ki-67 appears as brown yellow or brown particles.

UTUC group included 32 tissue samples (>20%). The associations between Ki-67 expression and the clinicopathological factors in UTUC, including patient gender, patient age, tumor side, tumor location, tumor focality, pathological stage, lymph node status, operation method and intravesical chemotherapy, are summarized in Table 1. Pathological stage was positively correlated with Ki-67 expression ($P=0.031$). Spearman's correlation analysis identified a correlation coefficient of 0.222 ($P=0.031$) between Ki-67 and pathological stage.

The impact of Ki-67 expression on CSS in UTUC

To determine whether Ki-67 expression level is a significant predictor for CSS after radical nephroureterectomy, Kaplan-Meier curves were analyzed between high or low Ki-67 and CSS. The CSS of patients with high Ki-67 expression was significantly lower than those with low Ki-67 ($p=0.017$; Fig. 2A). Univariate analysis showed that lymph node metastases and high Ki-67 expression were related to a poor overall survival for patients with UTUCs. Multivariate analysis showed that high Ki-67 expression ($HR=3.196$; 95% $CI=1.013-10.086$; $p=0.048$) and lymph node metastases ($HR=3.469$; 95% $CI=1.031-11.670$; $p=0.045$) were independent prognostic factors for CSS. The results are shown in Table 2.

The impact of Ki-67 expression on DFS in UTUC

Kaplan-Meier analysis demonstrated that high Ki-67 expression was significantly associated with poor DFS ($p=0.020$) (Fig. 2B). In addition, the DFS was significantly shorter in the Ki-67 high expression group compared with the Ki-67 low expression group ($p=0.037$; Fig. 2). Furthermore, these associations were confirmed by Cox univariate analysis ($HR=3.517$; 95% $CI=1.006-12.299$; $p=0.049$) and multivariate analysis, with adjustments for lymph node status ($HR=3.207$; 95% $CI=0.999-10.299$; $p=0.050$) (Table 3).

Identification and eligibility of relevant studies in the meta-analysis

A detailed flowchart of the search and selection results is shown in Fig. 3. The initial

Table 1. Distribution of characteristics of patients with UTUC by Ki-67 expression

Patient characteristics	Ki-67 expression		Total	P Value
	Negative	Positive		
Gender				0.767
Male	33	16	49	
Female	29	16	45	
Age, years				0.759
≥70	35	17	52	
<70	27	15	42	
Tumor side				0.565
Left	31	18	49	
Right	31	14	45	
Tumor location				0.897
Calix or pelvis	34	17	51	
Ureter	25	14	39	
More than 1	3	1	4	
Tumor focality				0.950
Unifocal	52	27	79	
Multifocal	10	5	15	
Pathologic stage				0.031
Tis-T1	23	5	28	
T2-T4	39	27	66	
Lymph node status				0.138
No	56	27	83	
N1	6	3	9	
Nx	0	2	2	
Operation				0.220
Open	48	21	69	
Laparoscopic	14	11	25	
Intravesical chemotherapy				0.788
No	9	4	13	
Yes	53	28	81

Fig. 2. Kaplan-Meier curves for cancer specific survival (A) and disease-free survival (B) according to Ki-67 expression in patients with upper tract urothelial carcinoma (UTUC). Patients with high Ki-67 expression exhibited a significantly worse cancer-specific survival (CSS) rate and disease-free survival (DFS) rate than patients with low Ki-67 expression ($p=0.017$, 0.037, respectively, log rank test).

Table 2. Univariate and multivariate analyses for cancer-specific survival (CSS) including pre-operatively known parameters as well as Ki-67 in 94 patients treated with nephroureterectomy (RNU) for high-grade UTUC

Overall Survival	Univariate analysis		Multivariate analysis	
	HR	p Value	HR	p Value
Gender	1.103 (0.370–3.288)	0.860	-	-
Age	1.141 (0.372–3.501)	0.817	-	-
Tumor laterality	1.027 (0.342–3.082)	0.962	-	-
Tumor location	0.908 (0.332–2.487)	0.852	-	-
Tumor focality	0.406 (0.530–3.133)	0.387	-	-
Ki-67	3.615 (1.176–11.114)	0.025	3.196 (1.013–10.086)	0.048
Pathologic stage	3.956 (0.872–17.962)	0.075	-	-
Lymph node status	4.597 (1.351–15.642)	0.015	3.469 (1.031–11.670)	0.045
Type of surgery	1.442 (0.440–4.719)	0.546	-	-
Adjuvant chemotherapy	0.413 (0.111–1.533)	0.186	-	-

Table 3. Univariate and multivariate analyses for disease-free survival (DFS) including pre-operatively known parameters as well as Ki-67 in 94 patients treated with RNU for high-grade UTUC

Disease-free Survival	Univariate analysis		Multivariate analysis	
	HR	p Value	HR	p Value
Gender	2.245 (0.657–7.678)	0.197	-	-
Age	1.946 (0.515–7.349)	0.326	-	-
Tumor laterality	1.984 (0.577–6.820)	1.984	-	-
Tumor location	1.629 (0.581–4.568)	0.354	-	-
Tumor focality	1.123 (0.242–5.214)	0.882	-	-
Ki-67	3.892 (1.135–13.347)	0.031	3.517 (1.006–12.299)	0.049
Pathologic stage	6.362 (0.810–49.975)	0.078	-	-
Lymph node status	4.056 (1.221–13.476)	0.022	3.207 (0.999–10.299)	0.050
Type of surgery	1.120 (0.297–4.225)	0.868	-	-
Adjuvant chemotherapy	0.385 (0.102–1.457)	0.160	-	-

Fig. 3. Methodologic Flow Chart of the Systematic Review.

search yielded 81 relevant publications, of which 69 were excluded for duplicate studies and other reasons (such as reviews or commentaries, unavailable HRs and 95% CIs, animal studies, or not relevant to our analysis) on the basis of the title/abstract and full text, leaving 12 evaluable publications [22–25, 28–35] plus the current study for the meta-analysis. Finally, thirteen cohort studies that met our inclusion criteria were included in the meta-analysis.

Table 4. Main characteristics of eligible studies used in meta-analysis

First author (year)	Region	Study type	Recruitment period	No. of subjects	Demographics (age, sex)	Pathologic grade	Cut-off	Reported endpoints
Hayashi A[24] (2014)	Japan	Cohort; Retrospective	1996-2012	171	70 M:F=119:52	All	10	CSS:2.33 (1.07-5.07) MFS:2.24 (1.14-4.39)
Krabbe LM[27] (2014)	Muti- regions	Cohort; Retrospective	NR	475	69.7 M:F=262:213	High	20	CSS: 1.90 (1.29-2.90) RFS:1.60 (1.07-2.30)
Feng C[25] (2014)	China	Cohort; Retrospective	NR	88	69 M:F=59:29	All	20	CSS:1.29 (0.22-7.43) IRFS:0.50 (0.11-2.28)
García-Tello A[28] (2014)	Spain	Cohort; Prospective	2000-2008	82	66.9 M:F=65:17	All	6	CSS:0.32 (0.14-0.73)
Krabbe LM[29] (2013)	USA	Cohort; Prospective	2006-2013	101	70 M:F=79:22	High	20	CSS: 2.50 (0.06-11.40) RFS: 4.30 (1.00-18.50)
Jeon HG[22] (2010)	Korea	Cohort; Retrospective	1998-2005	107	64.1 M:F=80:27	All	20	CSS: 4.38 (1.48-12.94) MFS: 4.77 (1.81-12.54)
Chen M[34] (2008)	China	Cohort; Retrospective	2004-2007	71	63.8 M:F=41:30	All	30	OS:3.87 (1.14-13.11)
Joung JY[30] (2008)	Korea	Cohort; Retrospective	2001-2005	38	63.3 M:F=26:12	All	20	IRFS:6.44 (1.12-37.01)
Kamijima S[23] (2005)	Japan	Cohort; Retrospective	1975-1997	69	65 M:F=50:19	All	10	CSS: 1.35 (0.37-4.90)
Kamai T[31] (2000)	Japan	Cohort; Retrospective	1988-1995	37	74.7 M:F=19:18	All	30	OS: 19.04 (1.27-275.70) DFS:15.15 (2.566-89.49)
Chowdhury GM[32] (1996)	Japan	Retrospective	NR	67	67.9 M:F=48:19	All	24	CSS: 7.63 (1.16-50.81)
Masuda M[33] (1996)	Japan	Retrospective	1983-1993	70	64.7 M:F=52:18	All	13.3	OS: 4.49 (1.59-12.70)
Our study	China	Cohort; Retrospective	2008-2012	94	70 M:F=49:45	High	20	CSS:3.196 (1.013-10.086) DFS:3.517 (1.006-12.299)

The major characteristics of the thirteen included cohort studies are shown in Table 4. These studies were published between 1996 and 2014. In total, five were conducted in Japan [23, 24, 32], three in China [25, 35], two in Korea [22, 31], one in the USA [30], one in Spain [29], and one in a multi-institutional study [28]. The sample size of the studies ranged from 37 to 475 (a total of 546). Three studies enrolled patients with high grade UTUCs [28, 30], and the remaining 12 studies included patients with all grades of UTUCs [22-25, 29, 31-35]. The cut-off value used to define Ki-67 overexpression was less than or equal to 20% in ten studies [22-25, 28-31, 34], whereas in the remaining 3 studies [32, 33], the cut-off value was 24% or 30%.

Meta-analysis of Ki-67 expression and UTUC patient survival

Among twelve eligible studies, which included a total of 767 patients with survival data, nine studies reported the relation between Ki-67 expression and cancer-specific survival (CSS) in UTUC, and the remaining analyses investigated overall survival (OS). Fig. 4A shows the forest plot for the survival data. The combined HR was 2.081 (95% CI=1.550-2.793, $p<0.001$), indicating that increased Ki-67 levels were significantly associated with adverse survival in the pooled patient group. This result was not affected by study heterogeneity ($I^2=18.3\%$, $p=0.264$) and exhibited a low probability of publication bias, as determined by Egger's test ($p=0.046$, Fig. 4B). In subgroup analysis, increased Ki-67 levels were associated with CSS with a pooled HR estimate of 2.276 (95% CI= 1.702-3.045, $p<0.001$). However, a non-significant decreased risk of OS was observed in UTUC with high expression of Ki-67 ($p=0.200$).

Meta-analysis of Ki-67 expression and UTUC disease progression

According to the definition of related endpoints in several studies, including ours, recurrence-free survival (RFS), metastasis-free survival (MFS) and disease-free survival (DFS) are indicators of disease progression, such as locoregional recurrence or distant metastasis. Thus, we combined these overlapping endpoints for a higher power of analyses, as the included studies examining RFS, MFS and DFS were few. Forest plots of the meta-analyses for Ki-67 expression are depicted in Fig. 5A. Combined data from eight studies showed that increased Ki-67 levels were related to disease progression, with a pooled HR estimate of 2.791 (95% CI=1.611-4.835, $p<0.001$) and a moderate degree of heterogeneity ($I^2=56.5\%$, $p=0.024$). In subgroup analysis, increased Ki-67 levels were correlated with

Fig. 4. Forest plot (A) and funnel plot (B) for the association between Ki-67 expression and UTUC prognosis of patient survival. Hazard ratios for each trial are represented by squares; the horizontal line crossing the square represents the 95% confidence interval. The diamonds represent the estimated pooled effect using the Mantel-Haenszel random-effect model. Visual inspection of the Begg funnel plot did not identify substantial asymmetry.

Fig. 5. Forest plot (A) and funnel plot (B) for the association between Ki-67 expression and UTUC prognosis of disease progression. Hazard ratios for each trial are represented by squares; the horizontal line crossing the square represents the 95% confidence interval. The diamonds represent the estimated pooled effect using the Mantel-Haenszel random-effect model. Visual inspection of the Begg funnel plot did not identify substantial asymmetry.

MFS and DFS, with a pooled HR estimate of 3.008 (95% CI=1.460-6.197, $p=0.003$) and 6.336 (95% CI=1.558-25.772, $p=0.010$), respectively. However, the combined HR was 1.954 (95% CI=0.826-4.621, $p=0.127$), indicating no significant overall association between Ki-67 expression and RFS. Begg's funnel plot did not show the asymmetry typical of publication bias, and the p value for Egger's regression asymmetry test was 0.125, indicating no publication bias (Fig. 5B).

Discussion

The Ki-67 antigen, first described in 1983, is a labile, non-histone nuclear protein that is expressed in all phases of proliferating cells, except G0 and early G1 [36, 37]. This protein is encoded by the 15 exon-spanning MKI67 gene mapping to chromosome 10q26.2 [38, 39]. Moreover, Ki-67 expression is strongly associated with the percentage of growth, and this index is used for measuring tumor cell proliferation [40, 41]. Previous studies revealed that Ki-67 overexpression was an important event in patients with certain types

of cancer. Warth A analyzed the Ki-67 proliferation index in 1065 patients with non-small cell lung cancer (NSCLC) by IHC and found that the proliferation index is not only a clinically meaningful biomarker in NSCLC prognosis but may also potentially stratify patients with lung adenocarcinomas based on the need for adjuvant therapy [40]. In a meta-analysis evaluating the impact of Ki-67 on prognosis in patients with early breast cancer, Azambuja E showed that Ki-67 positivity was associated with a higher risk of relapse in both node-negative patients (HR=2.31, 95% CI: 1.83-2.92) and node-positive patients (HR=1.59, 95% CI: 1.35-1.87) [42]. Furthermore, Klatte et al. reported that Ki-67 could serve as an independent predictor of DFS in localized chromophobe renal cell carcinoma [43].

Our first results showed that 38% of the tumors from the patients enrolled in the current study expressed Ki-67 protein, although other researchers found frequencies ranging from 53–88%. However, these studies used different Ki-67 antibodies, sample sizes, cut-off staining values and elements that could have a role in the different results observed [22, 31, 35]. Moreover, we evaluated the association between Ki-67 expression and the clinicopathological features of patients with UTUC. Our results showed higher Ki-67 expression in patients with a muscle-invasive form of the tumor (T2-T4) compared with patients with the non-invasive form (Tis-T1), suggesting that the expression of this protein may be related to tumor aggressiveness. Krabbe et al. [28] reported Ki-67 expression in 14.9% of pT1 tumors and 60.8% of muscle-invasive tumors when the whole section was stained and taken into consideration, as well as a 20% expression threshold, results that are similar to ours. Thus, the detection and percentage of Ki-67 expression may be helpful for defining UTUC biological properties and may indirectly suggest the prognosis of patients. Above all, Ki-67 expression in UTUC patients' tissues detected by IHC was significantly correlated with CSS and DFS as determined by multivariate analysis, suggesting a role as an independent prognostic factor, which was also reported by another study [32].

However, among the studies related to Ki-67 expression and its prognostic value in UTUC, different results were found. Feng C and their colleagues found a negative association between high Ki-67 expression and tumor prognosis and intravesical recurrence [25]. In another study, Hayashi et al. [24] reported that high Ki-67 expression was not an independent predictor of CSS or MFS in 171 UTUC patients who underwent RNU. Why are the results on prognostic significance of Ki-67 in UTUC patients different or conflicting? First, different experimental conditions may lead to these differences, such as region-specific, race-specific, sex-specific or age-specific factors. Second, the varying threshold values of Ki-67, different technical platforms and varying concentrations of the antibody used may influence IHC results and produce statistical heterogeneity [44-46]. Finally, in breast cancer, the Ki-67 effect is in opposite directions for assessment of prognosis and for assessment of neoadjuvant response. Denkert et al. suggested that the biological explanation for these results is that there are different groups of tumors in breast cancer. When highly proliferating tumors are sensitive to chemotherapy, high Ki-67 may be related to an increased chance of pathological complete response and improved survival, suggesting that high Ki-67 is linked to good outcomes; however, when highly proliferating tumors are chemotherapy resistant, increased Ki-67 indicated poor outcome or reduced survival in breast cancer patients [47, 48]. Thus, Ki-67 may be both a prognostic and a predictive marker.

Since meta-analysis can combine studies on specific topics and RNU is less influenced by individual findings from a single study, we performed a systematic review of published articles related to the changes in Ki-67 and their effects on UTUC prognosis. Hence, our present report combined 12 independent studies, selected according to the enrollment criteria, which compared UTUC survival with Ki-67 expression in the primary tumor. The data were organized according to the assessment of patient survival (cancer-specific and overall survival) and disease progression (metastasis-free, recurrence-free and disease-free survival). Our results further clarify and elucidate the strong association between Ki-67 overexpression and progression, metastasis and mortality in UTUC.

Our cohort study has several limitations. First of all, it is a retrospective study of samples from patients with a relatively uncommon disease enrolled from only one university hospital.

In addition, we could not standardize the surgical procedure because different surgeons were involved and different surgical approaches were used. Furthermore, since the sample size was not large compared with previously reported multicenter studies, the analysis of several potential risk factors, such as pathological stage, lymph node status, and tumor focality, showed limited statistical power. Moreover, since UTUC is characterized by unique biological features as well as heterogeneous genetic backgrounds, it may be difficult to apply specific conventional parameters alone to predict postoperative outcomes in patients with UTUC [49-51]. Finally, some basic issues related to bias due to the exclusion of patients with distant metastatic lesions at surgery, those receiving neoadjuvant chemotherapy, and those undergoing systematic chemotherapy could affect our results. However, the clinical effects of such advanced cases are not based on bladder recurrence but rather on prognosis.

The meta-analysis results should be considered in the context of their strengths and limitations. The advantage of a meta-analysis is to provide a more precise estimation through the combination of the power of multiple studies with a large number of patients. Although we did not limit our literature search to only English language papers and extended the search to unpublished trials and abstracts that would likely include increased proportions of null results, potential bias cannot be completely avoided. Indeed, publication bias could arise as a result of selective 'positive' studies. In addition, the resulting HRs from multivariate survival analysis reported in the selected articles were included. When these results were not available, we extracted the univariate data [44]. When the individual HR together with its variance were not reported in the selected articles, we performed, when possible, a calculation from the survival comparison statistics and its variance. The estimated HR may be less reliable than the one obtained directly from published statistics [52]. Finally, when defining Ki-67 overexpression, the cut-off values for IHC varied from 6% to 30% among these studies [23, 29, 31, 35]. Indeed, there is no common threshold value to define Ki-67 positive expression in patients with UTUC. Therefore, standardized protocols will improve the quality of this study in the future.

Conclusion

Our retrospective cohort study and meta-analysis showed that Ki-67 overexpression may be a prognostic factor for patients with UTUC, although these findings need to be interpreted with caution. Ki-67 determination may help identify patients with UTUC at high risk of disease progression and poor prognosis, who might benefit from closer follow-up or more aggressive therapy. The value of Ki-67 for molecular staging of UTUC also needs to be further confirmed in adequately designed prospective trials involving larger number of patients before any definitive conclusions can be made.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 81271603, No. 31570953 and No. 81372761).

Disclosure Statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the reported research.

References

- 1 Roupřet M, Babjuk M, Compérat E, Zigeuner R, Sylvester R, Burger M, Cowan N, Böhle A, Van Rhijn BW, Kaasinen E, Palou J, Shariat SF: European guidelines on upper tract urothelial carcinomas: 2013 update. *Eur Urol* 2013;63:1059-1071.
- 2 Yeh CM, Chen PC, Hsieh HY, Jou YC, Lin CT, Tsai MH, Huang WY, Wang YT, Lin RI, Chen SS, Tung CL, Wu SF, Chang DC, Shen CH, Hsu CD, Chan MW: Methyloomics analysis identifies ZNF671 as an epigenetically repressed novel tumor suppressor and a potential non-invasive biomarker for the detection of urothelial carcinoma. *Oncotarget* 2015;6:29555-29572.
- 3 Roupřet M, Babjuk M, Compérat E, Zigeuner R, Sylvester RJ, Burger M, Cowan NC, Böhle A, Van Rhijn BW, Kaasinen E, Palou J, Shariat SF: European Association of Urology Guidelines on Upper Urinary Tract Urothelial Cell Carcinoma: 2015 Update. *Eur Urol* 2015;68:868-879.
- 4 Audenet F, Traxer O, Bensalah K, Roupřet M: Upper urinary tract instillations in the treatment of urothelial carcinomas: a review of technical constraints and outcomes. *World J Urol* 2013;31:45-52.
- 5 Lee YC, Wu WJ, Li WM, Lin HH, Huang CN, Chai CY, Chang LL, Lin HL, Ke HL: Prognostic value of p53 protein overexpression in upper tract urothelial carcinomas in Taiwan. *Anticancer Res* 2013;33:1091-1098.
- 6 Babjuk M, Oosterlinck W, Sylvester R, Kaasinen E, Böhle A, Palou-Redorta J, Roupřet M: EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder, the 2011 update. *Eur Urol* 2011;59:997-1008.
- 7 Margulis V, Shariat SF, Matin SF, Kamat AM, Zigeuner R, Kikuchi E, Lotan Y, Weizer A, Raman JD, Wood CG: Outcomes of radical nephroureterectomy: a series from the Upper Tract Urothelial Carcinoma Collaboration. *Cancer* 2009;115:1224-1233.
- 8 Shariat SF, Favaretto RL, Gupta A, Fritzsche HM, Matsumoto K, Kassouf W, Walton TJ, Tritschler S, Baba S, Matsushita K, Bastian PJ, Martínez-Salamanca JL, Seitz C, Pycha A, Otto W, Karakiewicz PI, Ficarra V, Novara G: Gender differences in radical nephroureterectomy for upper tract urothelial carcinoma. *World J Urol* 2011;29:481-486.
- 9 Lughezzani G, Sun M, Perrotte P, Shariat SF, Jeldres C, Budäus L, Latour M, Widmer H, Duclos A, Bénard F, McCormack M, Montorsi F, Karakiewicz PI: Gender-related differences in patients with stage I to III upper tract urothelial carcinoma: results from the Surveillance, Epidemiology, and End Results database. *Urology* 2010;75:321-327.
- 10 Raman JD, Ng CK, Boorjian SA, Vaughan ED, Sosa RE, Scherr DS: Bladder cancer after managing upper urinary tract transitional cell carcinoma: predictive factors and pathology. *BJU Int* 2005;96:1031-1035.
- 11 Zigeuner RE, Hutterer G, Chromecki T, Rehak P, Langner C: Bladder tumour development after urothelial carcinoma of the upper urinary tract is related to primary tumour location. *BJU Int* 2006;98:1181-1186.
- 12 Novara G, De Marco V, Dalpiaz O, Gottardo F, Bouygués V, Galfano A, Martignoni G, Patard JJ, Artibani W, Ficarra V: Independent predictors of metachronous bladder transitional cell carcinoma (TCC) after nephroureterectomy for TCC of the upper urinary tract. *BJU Int* 2008;101:1368-1374.
- 13 Abouassaly R, Alibhai SM, Shah N, Timilshina N, Fleshner N, Finelli A: Troubling outcomes from population-level analysis of surgery for upper tract urothelial carcinoma. *Urology* 2010;76:895-901.
- 14 Jeldres C, Sun M, Isbarn H, Lughezzani G, Budäus L, Alasker A, Shariat SF, Lattouf JB, Widmer H, Pharand D, Arjane P, Graefen M, Montorsi F, Perrotte P, Karakiewicz PI: A population-based assessment of perioperative mortality after nephroureterectomy for upper-tract urothelial carcinoma. *Urology* 2010;75:315-320.
- 15 Lughezzani G, Burger M, Margulis V, Matin SF, Novara G, Roupřet M, Shariat SF, Wood CG, Zigeuner R: Prognostic factors in upper urinary tract urothelial carcinomas: a comprehensive review of the current literature. *Eur Urol* 2012;62:100-114.
- 16 Hayashi M, Bernert H, Kagohara LT, Maldonado L, Brait M, Schoenberg M, Bivalacqua T, Netto GJ, Koch W, Sidransky D, Hoque MO: Epigenetic inactivation of VGF associated with Urothelial Cell Carcinoma and its potential as a non-invasive biomarker using urine. *Oncotarget* 2014;5:3350-3361.
- 17 Gerdes J: Ki-67 and other proliferation markers useful for immunohistological diagnostic and prognostic evaluations in human malignancies. *Semin Cancer Biol* 1990;1:199-206.
- 18 Yurakh AO, Ramos D, Calabuig-Fariñas S, López-Guerrero JA, Rubio J, Solsona E, Romanenko AM, Vozianov AF, Pellin A, Llombart-Bosch A: Molecular and immunohistochemical analysis of the prognostic value of cell-cycle regulators in urothelial neoplasms of the bladder. *Eur Urol* 2006;50:506-515; discussion 515.

- 19 Cattoretto G, Becker MH, Key G, Duchrow M, Schlüter C, Galle J, Gerdes J: Monoclonal antibodies against recombinant parts of the Ki-67 antigen (MIB 1 and MIB 3) detect proliferating cells in microwave-processed formalin-fixed paraffin sections. *J Pathol* 1992;168:357-363.
- 20 Sysel AM, Valli VE, Bauer JA: Immunohistochemical quantification of the cobalamin transport protein, cell surface receptor and Ki-67 in naturally occurring canine and feline malignant tumors and in adjacent normal tissues. *Oncotarget* 2015;6:2331-2348.
- 21 Rodríguez-Alonso A, Pita-Fernández S, González-Carrero J, Nogueira-March JL: p53 and ki67 expression as prognostic factors for cancer-related survival in stage T1 transitional cell bladder carcinoma. *Eur Urol* 2002;41:182-188; discussion 188-189.
- 22 Jeon HG, Jeong IG, Bae J, Lee JW, Won JK, Paik JH, Kim HH, Lee SE, Lee E: Expression of Ki-67 and COX-2 in patients with upper urinary tract urothelial carcinoma. *Urology* 2010;76:513.e7-12.
- 23 Kamijima S, Tobe T, Suyama T, Ueda T, Igarashi T, Ichikawa T, Ito H: The prognostic value of p53, Ki-67 and matrix metalloproteinases MMP-2 and MMP-9 in transitional cell carcinoma of the renal pelvis and ureter. *Int J Urol* 2005;12:941-947.
- 24 Hayashi A, Morikawa T, Kawai T, Kume H, Ishikawa S, Homma Y, Fukayama M: Clinicopathological and prognostic significance of EZH2 expression in upper urinary tract carcinoma. *Virchows Arch* 2014;464:463-471.
- 25 Feng C, Wang L, Ding G, Ding Q, Zhou Z, Jiang H, Wu Z: Predictive value of clinicopathological markers for the metachronous bladder cancer and prognosis of upper tract urothelial carcinoma. *Sci Rep* 2014;4:4015.
- 26 Holmäng S, Johansson SL: Urothelial carcinoma of the upper urinary tract: comparison between the WHO/ISUP 1998 consensus classification and WHO 1999 classification system. *Urology* 2005;66:274-278.
- 27 Cheng DD, Hu T, Zhang HZ, Huang J, Yang QC: Factors Affecting the Recurrence of Giant Cell Tumor of Bone After Surgery: A Clinicopathological Study of 80 Cases from a Single Center. *Cell Physiol Biochem* 2015;36:1961-1970.
- 28 Krabbe LM, Bagrodia A, Haddad AQ, Kapur P, Khalil D, Hynan LS, Wood CG, Karam JA, Weizer AZ, Raman JD, Remzi M, Rioux-Leclercq N, Haitel A, Roscigno M, Bolenz C, Bensalah K, Sagalowsky AI, Shariat SF, Lotan Y, Margulis V: Multi-institutional validation of the predictive value of Ki-67 in patients with high grade urothelial carcinoma of the upper urinary tract. *J Urol* 2015;193:1486-1493.
- 29 García-Tello A, Angulo JC, Andrés G, de Fata F R, Sánchez-Chapado M, López JI: Impact of p53, MIB-1 and PECAM-1 expression on the prognosis of urothelial carcinoma of the renal pelvis. *Actas Urol Esp* 2014;38:506-514.
- 30 Krabbe LM, Bagrodia A, Lotan Y, Gayed BA, Darwish OM, Youssef RF, John G, Harrow B, Jacobs C, Gaitonde M, Sagalowsky AI, Shariat SF, Kapur P, Margulis V: Prospective analysis of Ki-67 as an independent predictor of oncologic outcomes in patients with high grade upper tract urothelial carcinoma. *J Urol* 2014;191:28-34.
- 31 Joung JY, Yang SO, Jeong IG, Han KS, Seo HK, Chung J, Park WS, Lee GK, Lee KH: Identification of immunohistochemical factors that predict the synchronous or metachronous development of bladder tumors in patients with upper urinary tract tumors. *Urol Int* 2008;81:306-311.
- 32 Kamai T, Takagi K, Asami H, Ito Y, Arai K, Yoshida KI: Prognostic significance of p27Kip1 and Ki-67 expression in carcinoma of the renal pelvis and ureter. *BJU Int* 2000;86:14-19.
- 33 Chowdhury GM, Kojima K, Kanayama H, Tsuji M, Kurokawa Y, Kagawa S: The proliferation index of MIB-1 as a prognostic factor for patients with transitional cell carcinoma of the upper urinary tract. *Cancer* 1996;78:827-833.
- 34 Masuda M, Iki M, Takano Y, Asakura T, Noguchi S, Ikeda I, Kubota Y, Hosaka M: Prognostic significance of Ki-67 labeling index in urothelial tumors of the renal pelvis and ureter. *J Urol* 1996;155:1877-1880; discussion 1880-1881.
- 35 Pinho SS, Reis CA: Glycosylation in cancer: mechanisms and clinical implications. *Nat Rev Cancer* 2015;15:540-555.
- 36 Wang W, Liu X, Wang W, Li J, Li Y, Li L, Wang S, Zhang J, Zhang Y, Huang H: The Effects of Indoxyl Sulfate on Human Umbilical Cord-Derived Mesenchymal Stem Cells In Vitro. *Cell Physiol Biochem* 2016;38:401-414.
- 37 Lin W, Dai SH, Chen T, Kawai N, Miyake K, Okada M, Haba R, Yamamoto Y, Tamiya T, Fei Z: Expression of 58-kD Microspherule Protein (MSP58) is Highly Correlated with PET Imaging of Tumor Malignancy and Cell Proliferation in Glioma Patients. *Cell Physiol Biochem* 2016;38:635-645.

- 38 Pelosi G, Rindi G, Travis WD, Papotti M: Ki-67 antigen in lung neuroendocrine tumors: unraveling a role in clinical practice. *J Thorac Oncol* 2014;9:273-284.
- 39 Huang W, Wang J, Zhang D, Chen W, Hou L, Wu X, Lu Y: Inhibition of KIF14 Suppresses Tumor Cell Growth and Promotes Apoptosis in Human Glioblastoma. *Cell Physiol Biochem* 2015;37:1659-1670.
- 40 Warth A, Cortis J, Soltermann A, Meister M, Budczies J, Stenzinger A, Goeppert B, Thomas M, Herth FJ, Schirmacher P, Schnabel PA, Hoffmann H, Dienemann H, Muley T, Weichert W: Tumour cell proliferation (Ki-67) in non-small cell lung cancer: a critical reappraisal of its prognostic role. *Br J Cancer* 2014;111:1222-1229.
- 41 Cai J, Yang P, Zhang C, Zhang W, Liu Y, Bao Z, Liu X, Du W, Wang H, Jiang T, Jiang C: ATRX mRNA expression combined with IDH1/2 mutational status and Ki-67 expression refines the molecular classification of astrocytic tumors: evidence from the whole transcriptome sequencing of 169 samples. *Oncotarget* 2014;5:2551-2561.
- 42 de Azambuja E, Cardoso F, de Castro G, Colozza M, Mano MS, Durbecq V, Sotiriou C, Larsimont D, Piccart-Gebhart MJ, Paesmans M: Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. *Br J Cancer* 2007;96:1504-1513.
- 43 Klatte T, Seligson DB, LaRochelle J, Shuch B, Said JW, Riggs SB, Zomorodian N, Kabbavar FF, Pantuck AJ, Belldegrun AS: Molecular signatures of localized clear cell renal cell carcinoma to predict disease-free survival after nephrectomy. *Cancer Epidemiol Biomarkers Prev* 2009;18:894-900.
- 44 Ku JH, Byun SS, Jeong H, Kwak C, Kim HH, Lee SE: The role of p53 on survival of upper urinary tract urothelial carcinoma: a systematic review and meta-analysis. *Clin Genitourin Cancer* 2013;11:221-228.
- 45 Altman DG, Lausen B, Sauerbrei W, Schumacher M: Dangers of using "optimal" cutpoints in the evaluation of prognostic factors. *J Natl Cancer Inst* 1994;86:829-835.
- 46 Jeon C, Kim M, Kwak C, Kim HH, Ku JH: Prognostic role of survivin in bladder cancer: a systematic review and meta-analysis. *PLoS One* 2013;8:e76719.
- 47 Denkert C, Budczies J, von MG, Wienert S, Loibl S, Klauschen F: Strategies for developing Ki67 as a useful biomarker in breast cancer. *Breast* 2015;24:S67-72.
- 48 Laurinavicius A, Green AR, Laurinaviciene A, Smalyte G, Ostapenko V, Meskauskas R, Ellis IO: Ki67/SATB1 ratio is an independent prognostic factor of overall survival in patients with early hormone receptor-positive invasive ductal breast carcinoma. *Oncotarget* 2015;6:41134-41145.
- 49 Audenet F, Colin P, Yates DR, Ouzzane A, Pignot G, Long JA, Soulie M, Phé V, Bensadoun H, Guy L, Ruffion A, Valeri A, Cormier L, Droupy S, de La Taille A, Saint F, Faïs PO, Houlgatte A, Cussenot O, Rouprêt M: A proportion of hereditary upper urinary tract urothelial carcinomas are misclassified as sporadic according to a multi-institutional database analysis: proposal of patient-specific risk identification tool. *BJU Int* 2012;110:E583-589.
- 50 Wu CF, Pang ST, Shee JJ, Chang PL, Chuang CK, Chen CS, Liao SK, Weng WH: Identification of genetic alterations in upper urinary tract urothelial carcinoma in end-stage renal disease patients. *Genes Chromosomes Cancer* 2010;49:928-934.
- 51 van Oers JM, Zwarthoff EC, Rehman I, Azzouzi AR, Cussenot O, Meuth M, Hamdy FC, Catto JW: FGFR3 mutations indicate better survival in invasive upper urinary tract and bladder tumours. *Eur Urol* 2009;55:650-657.
- 52 Czachorowski MJ, Amaral AF, Montes-Moreno S, Lloreta J, Carrato A, Tardón A, Morente MM, Kogevinas M, Real FX, Malats N: Cyclooxygenase-2 expression in bladder cancer and patient prognosis: results from a large clinical cohort and meta-analysis. *PLoS One* 2012;7:e45025.