의학석사 학위논문

상부요로상피암에서의 예후 예측용 표지자로서의 Ki-67: 체계적 문헌고찰과 메타분석

Ki-67 as a Prognostic Marker in Upper Urinary Tract Urothelial Carcinoma: a Systematic Review and Meta-analysis

2017 년 10 월

서울대학교 대학원

의학과 비뇨기과학 전공

안 치 현

A thesis of the Master's degree

Ki-67 as a Prognostic Marker in Upper Urinary Tract Urothelial Carcinoma: a Systematic Review and Meta-analysis

상부요로상피암에서의 예후 예측용 표지자로서의 Ki-67: 체계적 문헌고찰과 메타분석

October 2017

Chihyun Ahn, MD

College of Medicine, Urology

The Graduate School

Seoul National University

상부요로상피암에서의 예후 예측용 표지자로서의 Ki-67: 체계적 문헌고찰과 메타분석

지도교수 구 자 현

이 논문을 의학석사 학위논문으로 제출함

2017 년 10 월

서울대학교 대학원

의학과 비뇨기과학 전공

안 치 현

안치현의 의학석사 학위논문을 인준함

2017 년 10 월

위 원 장 오 승 준 (인)

부위원장 구 자 현 (인)

위 원 조 정 연 (인)

Abstract

Purpose: We tried to systematically evaluate the prognostic significance of Ki-67 on survival outcomes in patients with upper urinary tract urothelial carcinoma (UTUC) treated with radical nephroureterectomy (RNU).

Materials and Methods: We searched Embase, Scopus, and PubMed databases for all articles published up to February 2016 in line with the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines. Outcomes of interest included intravesical recurrence (IVR) free survival, disease free survival (DFS), cancer-specific survival (CSS), and overall survival (OS). The associations between Ki-67 and survival outcomes were expressed with pooled hazard ratio (HR) and 95% confidence interval (CI).

Results: A total of 12 articles, consisting of 1,351 patients, ranging from 37 to 475, met the eligibility criteria and were finally selected for this meta-analysis. The overexpression of Ki-67 was significantly associated with worse DFS (HR 2.74, 95% CI 1.58-4.74), CSS (HR 2.26, 95% CI 1.70-3.01), and OS (HR, 3.71; 95% CI, 1.78-7.75), but not IVR free survival (HR, 0.77; 95% CI, 0.10-5.82). Inter-study heterogeneity was observed in the analysis of DFS (I^2 = 54%; p = 0.05) and IVR free survival (I^2 = 81%, p = 0.005). There was no significant publication bias in the meta-analysis of survival outcome as a result of funnel plot test.

Conclusions: The results drawn in this meta-analysis suggest that the overexpression of Ki-67 may be a promising prognostic indicator predicting the survival outcome after RNU for UTUC. However, a large, well-designed,

prospective study is necessary to establish the prognostic value of Ki-67 in UTUC.

Keywords: upper urinary tract, ureter, renal pelvis, urothelial carcinoma, Ki-67, prognosis, meta-analysis

Student number: 2016-21946

Table of contents

Legends of Tables and Figures

Introduction

Upper urinary tract urothelial carcinoma (UTUC) is a rare urologic malignancy, accounting for only 5% to 10% of all urothelial carcinomas (UCs), but shows an aggressive nature with high recurrence and progression rates [1]. Although radical nephroureterectomy (RNU) with ipsilateral bladder cuff excision has been applied as a standard treatment for non-metastatic UTUC, the prognosis of UTUC is generally poorer than that of UC of the bladder. This can be explained by the difference of the natural history between two malignancies, given that 20% to 30% of bladder cancers show muscle invasive feature at the initial diagnosis, while 60% of UTUCs are initially diagnosed with muscle invasive status [2]. In particular, muscle invasive UTUC usually shows a very worse prognosis, presenting 5-year cancer specific survival rate of less than 50% for pT2/pT3 disease and less than 10% for pT4 [3, 4].

Therefore, risk stratification based on the factors to predict the prognosis may help in selecting the proper treatment modalities and clinical follow-up strategies in UTUC patients. Many potential prognostic factors have been identified, including clinical factors, surgical factors, tumor related factors, and molecular factors [5-8]. Tumor related factors, such as pathological tumor stage, grade, lymphovascular invasion (LVI), and lymph node invasion (LNI), are suggested as crucial primary prognosticators for postoperative tumor recurrence and survival [5-8]. Additionally, several demographic and clinical factors, including advanced age, worse performance status, ureteral tumor location, preoperative hydronephrosis, and the presence of previous or

synchronous bladder cancer, may adversely affect the recurrence and survival in patients with UTUC [6-8].

In recent years, many investigators have focused their research on molecular biomarkers related to the prognosis of UTUC patients [6-9]. Several studies have assessed the prognostic implication of various tissue-based markers, including E-cadherin, CD 24, p27, p53, Epidermal growth factor receptor (EGFR), hypoxia-inducible factor (HIF)-1a, MET, bcl-2, survivin, and Ki-67 in UTUC [6-9]. Among these tissue-based markers, Ki-67, which is a nuclear protein presenting cell proliferation that is especially active in malignant tumors, is a well-studied and potential biomarker in UTUC [10-21]. However, due to the conflicting results among previously reported studies, the prognostic value of Ki-67 in UTUC remains controversial, especially in terms of survival outcomes.

Our study aimed to elucidate the prognostic impact of Ki-67 on survival outcomes in UTUC patients treated with RNU through a systematic review of relevant published articles and meta-analysis of available data.

Materials and Methods

Search Strategy

A systematic review was performed in accordance with Cochrane Collaboration and Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines [22]. A literature search was performed using Embase, Scopus, and PubMed databases on all articles in English published up to February 26, 2016. The following search terms were used: “upper”, “urothelial”, “cancer”, and “Ki-67”. Citation lists of all retrieved studies were then used to identify other potentially relevant publications. Article selection was performed by two independent evaluators. Conflicts between reviewers were resolved by consensus.

Study Inclusion/Exclusion Criteria

According to the PRISMA guidelines, we used the Population, Intervention, Comparator, Outcome, and Study design (PICOS) approach to define study eligibility [22].

Population: patients with UTUC

Intervention: RNU

Comparator: expression of Ki-67

Outcome: intravesical recurrence (IVR), progression, cancer-specific mortality, and any-cause mortality

Study design: univariate and/or multivariate Cox regression analyses

Studies were considered eligible for further evaluation if they met the following inclusion criteria: (1) original article, (2) human research, (3) English language, (4) UTUC, (5) treatment exclusively with RNU, (6) expression of the Ki-67 was evaluated in by immunohistochemistry (IHC), (7) the association between Ki-67 expression levels and survival outcome was investigated, and (8) availability of Kaplan-Meier/Cox regression-derived results about the prognostic value of Ki-67 on UTUC outcomes according to the Reporting recommendations for tumor marker prognostic studies (REMARKS) guidelines for assessment of a prognostic marker [23].

The exclusion criteria were as follows: (1) letters, commentaries, case reports, reviews, and conference abstracts because of limited data; (2) studies conducted using animals or cell lines; and (3) studies using other analyses instead of survival analysis. Where part or all of the same patient series was included in more than one publication, only the most recent or most complete study was included in the analysis in order to avoid duplication of the same survival data. Two reviewers independently determined study eligibility. Agreements were reached for discrepant opinions through discussion.

In the present meta-analysis, IVR-free survival was defined as the interval between surgery and the occurrence of urothelial cancer in the bladder. Disease-free survival (DFS) was defined as the interval between surgery and the subsequent appearance of either local failure at the operative site or in the regional lymph nodes, or distant metastasis, while recurrence in the bladder was not considered for DFS analysis. If DFS was defined as recurrence both in bladder and non-bladder lesions, these studies were excluded from the analysis for DFS. Cancer-specific survival (CSS) and overall survival (OS)

was defined as the interval between surgery and death from UTUC and any cause, respectively.

Data Extraction

We performed data extraction including study characteristics and outcome data, and data were subsequently crosschecked to ensure their accuracy. Any discrepancies in extracting data were resolved. We did not contact the authors of eligible studies for additional data. Information was retrieved according to the REMARK guidelines for reporting prognostic markers including (i) publication data: name of first author, country, year of publication, geographic location, study design, period of recruitment, (ii) characteristics of the studied population: sample size, mean or median age, gender, inclusion and exclusion criteria, treatment, endpoint definition, and follow-up period, (iii) tumor characteristics; tumor stage and grade, (iv) IHC data: cut-off value of positive expression, the antibodies used, and adoption of the blind method, and (v) statistical data: survival curves, the exact data of total and exposed number in case and control groups, as well as hazard ratios (HRs) and their confidence intervals (Cis). Discrepancies were discussed to reach consensus.

Statistical Analysis

A meta-analysis was performed using the DerSimonian and Laird random effects model, applying the inverse of variance as a weighing factor. Survival outcome data were synthesized using the time-to-event HR as the operational measure. For each trial, this HR was estimated by a method depending on the data provided in the publications. If HRs and the corresponding standard errors were not directly reported, previously reported indirect methods were

utilized for extracting the logHR and variance, due to the paucity of prognostic literature directly reporting these values [24].

A test of heterogeneity of the combined HRs was carried out using the Chi-square test and Higgins I-squared statistic. For the Chi-square test, we judged that heterogeneity was significant when the p value was <0.05. I^2 describes the proportion of total variation in meta-analysis estimates, which is due to inter-study heterogeneity, rather than sampling error, and is measured from 0% to 100%, with increasing I^2 values indicating a larger effect of between-study heterogeneity in the meta-analysis [25]. Publication bias was evaluated using the funnel plot. The graph should resemble a symmetrical inverted funnel in the absence of bias; in the presence of bias, the plot should appear skewed and asymmetrical.

The nominal level of significance was set at 5%. All 95% CIs were two-sided. The Version 5.0 RevMan statistical software (the Cochrane Collaboration, Copenhagen) was employed in this study.

Results

Study Selection

The database searches identified 120 articles for initial evaluation. Of these, 55 articles were excluded because of duplicate publications. After reviewing the corresponding abstracts, we excluded 35. A total of 30 articles remained for full test review. By further review, 14 were excluded because these studies did not apply survival analysis, three were excluded because the estimation of HRs in these studies was not allowed because of the insufficient data provided by the authors, and one was excluded because it had overlapped data with another study. Finally, 12 articles including 1,351 patients, ranging from 37 to 475 per study were included in this study [10-21]. Figure 1 shows a flow diagram of the selection process for relevant studies.

Study Characteristics

The characteristics of the 12 eligible studies are extracted and summarized in Tables 1-3. Of the 12 studies, one was a global study [20], two were conducted in Europe [16, 18], and the remaining nine studies were performed in Asian countries [10-15, 17, 19, 21]. Among included studies, data were collected prospectively in one study [18]. Three of these studies included <50 patients, and four studies enrolled >100 patients. The follow-up ranged from 2 months to 252 months, while one study did not provide clear follow-up time. These eligible studies were published from 1996 and 2015. The threshold for dichotomizing the level of Ki-67 also varied widely among studies (Table 4). The cut-off value used to define Ki-67 overexpression was 20% in five studies

[14, 15, 17, 20, 21] and 10% in three studies [13, 16, 19], respectively. Whereas, in the remaining studies, the cut-off values were defined as 6% [18], 13.3% and 21.7% [11], 24% [10] and 30% [12]. Immunopositive cells were defined according to the percentage of nuclear in nine studies. Only two studies used the blind method to assess the Ki-67 expression (Table 4). Some studies did not perform multivariate analysis (Tables 5-8).

Meta-analysis

Owing to prior assumptions about the likelihood for heterogeneity between primary studies, the pooled HR estimate of each study was calculated by the random effects model. Figure 2 demonstrates a forest plot of the individual HRs and results from the meta-analysis. When 6 eligible studies were pooled into the meta-analysis for DFS, we found that Ki-67 was significantly associated with worse DFS (pooled HR, 2.74; 95% CI, 1.58-4.74; Z = 3.60). Cochrane Q test (Chi^2 = 10.88; p = 0.05) and test of inconsistency (I^2 = 54%) could not exclude a significant heterogeneity (Fig. 2a). The pooled analysis of CSS was based on eight publications. The pooled HR was 2.26 (95% CI, 1.70-3.01; Z = 5.57) without heterogeneity (Chi^2 = 5.93, p = 0.55; I^2 = 0%) (Fig. 2b). Three studies provided data on OS. Results suggested that Ki-67 correlated with poor OS (pooled HR, 3.71; 95% CI, 1.78-7.75; Z = 3.49). No significant heterogeneity existed between the studies (Chi^2 = 2.22; p = 0.33, I^2 = 10%) (Fig. 2c). Three studies investigated IVR free survival and the pooled HR (95% CI) was 0.77 (0.10-5.82) with large heterogeneity in the data (Chi^2 = 10.49; p = 0.005, I^2 = 81%) (Fig. 2d).

Publication Bias

No significant publication bias was found in the meta-analysis of survival outcome. Funnel plots for publication bias of the association between Ki-67 and outcome demonstrated a certain degree of asymmetry (Fig. 3).

Discussion

Although RNU can provide effective local control as a radical extirpative therapeutic modality for non-metastatic, high-grade UTUC, the overall prognosis for UTUC after surgery is not quite promising [7]. It has been reported that about a quarter of patients die from the UTUC in less than five years [1]. Therefore, it is important to evaluate the status of the disease and predict the prognosis of individual UTUC patients. Based on the predictors related to the prognosis of UTUC, we can further determine proper candidates for additional treatment strategies including neoadjuvant and/or adjuvant chemotherapy [5-8, 26]. There have been several tumor-related factors considered to have prognostic values in UTUC. Although the prognostic implication of tumor related factors somehow varied among the studies, several parameters, including tumor stage, tumor grade, LVI, LNI, extensive tumor necrosis and sessile tumor architecture, are generally widely accepted as independent prognostic factors for UTUC [5-8, 27].

Recently, the approach to improve prognostic predictability in UTUC has focused on evaluating the prognostic role of molecular factors other than tumor related factors [6-9]. In particular, as for Ki-67, which is an established molecular marker representing cell proliferation, there have been several studies to investigate the prognostic value of Ki-67 in various types of human cancers. It was demonstrated that high expression of Ki-67 and p53 are associated with a worse OS in patients with colorectal cancer [28]. Similarly, increased Ki-67 expression and tumor size were related to increased risk of disease recurrence and decreased OS in pancreatic neuroendocrine neoplasms

[29]. As for prostate cancer, Ki-67 positivity is an independent predictor of biochemical recurrence after radical prostatectomy, especially in patients expected to have favorable prognostic characteristics, such as no LNI and negative surgical margins [30]. In a study to investigate the relationship between Ki-67 and survival outcomes in clinically localized clear-cell carcinoma, Ki-67 overexpression was identified to be a significant predictor of worse DFS [31]. The prognostic value of Ki-67 was also assessed in non-muscle invasive bladder cancer (NMIBC) [32, 33]. Ki-67 positivity was an independent predictor of tumor recurrence and progression in NMIBC patients so that combination of Ki-67 expression with European Organization for Research and Treatment of Cancer risk scores could improve the risk prediction for both recurrence and progression in NMIBC [32]. Likewise, it was reported that the novel molecular grading model combining Ki-67 labeling index and VEGF is helpful for predicting tumor recurrence and progression in NMIBC [33].

This prognostic implication of Ki-67 has been also investigated in UTUC, but there have shown some conflicting results among studies. In several studies, it was identified that high Ki-67 labeling index is closely related to poor oncologic (high tumor stage and grade) and survival outcome [10-13, 15, 16]. This unfavorable correlation between high Ki-67 expression and prognosis was recently further validated in a large, multi-institutional UTUC cohort [20]. Likewise, a meta-analysis pooling a total of 13 studies demonstrated that Ki-67 overexpression significantly correlated with higher disease specific mortality rate, lower 5-year DFS and OS rates [34]. Therefore, these results suggest that we can obtain additional information regarding the patients' prognosis by measuring a degree of Ki-67 expression in UTUC

specimens after RNU. Whereas, in a recent study performed by Wu et al., low-level Ki-67 expression was an independent predictor of IVR [21].

In this study, we tried to elucidate the prognostic value of Ki-67 in terms of survival outcomes in UTUC patients treated with RNU by applying meta-analysis method. As mentioned above, Ki-67 has been recognized as an established molecular biomarker showing significant correlation with the prognosis of urothelial carcinomas in many studies [32-35]. In the present study, we focused on the patients who underwent RNU for UTUC. It was revealed that Ki-67 overexpression portends the increased risk of worse DFS, CSS and OS, except for IVR free survival. Our meta-analysis results may provide some insights which can aid in the specific and real clinical scenarios we face in clinical practice, such as patients' counseling based on the prognosis of each patient after surgery and clinical decision-making for additional treatment plan (i.e. chemotherapy). Furthermore, the results of the study can give a clue to make up a follow up protocol or reinforce the current protocol for UTUC patients in terms of evaluating the significance on the relationship of Ki-67 with IVR free survival. Even if its relationship was not significant in this study, the results should be further identified through additional investigation to justify the inclusion of periodic cystoscopy in the follow up protocol to monitor intravesical disease recurrence following RNU for UTUC.

The current study has several limitations. First, owing to the characteristics of meta-analysis based on previously published studies, the association of Ki-67 and survival outcomes could not be adjusted in a multivariate analysis incorporating other prognostic factors, such as tumor stage, grade and LVI. Consequently, the prognostic values of Ki-67 were reported as the univariate

analysis results. A further prospective investigation is needed to make such a determination. Second, the cut-off points to define Ki-67 overexpression differed among the enrolled studies (Table 4). These differences may be attributable to the difficulty in defining a unified standard threshold in real clinical practice. However, it was suggested that the immunohistochemical cut-off points should be determined in consideration of various clinical scenarios [36]. For instance, a lower cut-off of 10% would avoid overtreatment if Ki-67 is used to exclude patients with slow-growing tumors from chemotherapy. However, if Ki-67 is used to discriminate chemotherapy-sensitive patients, a higher cut-off of 30% would be reasonable. Third, significant inter-study heterogeneity was found in the analysis of DFS (I^2 = 54%) and IVR free survival (I^2 = 81). These results may result from the use of diverse Ki-67 cut-off points among the included studies and the variations in healthcare delivery, environmental and ethnogenetic factors among the various geographic regions included in this study. Lastly, we cannot completely exclude the probability of language bias by including only the articles published in English [37], in spite of no definite evidence of significant publication bias.

Conclusions

Our analysis results suggest that overexpression of Ki-67 can be used as a potential prognostic biomarker predicting survival outcomes in UTUC patients who underwent RNU for UTUC. Also, it may be helpful to justify and improve current follow up protocol. Further well-designed studies with larger cohort population are needed to confirm and establish the use of this molecular biomarker in clinical practice.

References

1. Pierre Colin, Wassim Kassouf, Badrinath R. Konety, Yair Lotan, Morgan Rouprêt. Diagnosis and Evaluation of Upper Tract Urothelial Carcinoma (UTUC). In: S.F. Shariat, E. Xylinas. Upper Tract Urothelial Carcinoma. Springer; 2015;31-43.
2. Rouprêt M, Babjuk M, Compérat E, Zigeuner R, Sylvester R, Burger M, et al. European guidelines on upper tract urothelial carcinomas: 2013 Update. European Urology 2013;63(6):1059-71.
3. Abouassaly R, Alibhai SMH, Shah N, Timilshina N, Fleshner N, Finelli A. Troubling Outcomes From Population-level Analysis of Surgery for Upper Tract Urothelial Carcinoma. Urology 2010;76(4):895-901.
4. Jeldres C, Sun M, Isbarn H, Lughezzani G, Budäus L, Alasker A, et al. A Population-based Assessment of Perioperative Mortality After Nephroureterectomy for Upper-tract Urothelial Carcinoma. Urology 2010;75(2):315-20.
5. Cho DS, Hong SY, Kim YK, Kim SI, Kim SJ. Prognostic Factors in Transitional Cell Carcinoma of the Upper Urinary Tract after Radical Nephroureterectomy. Kor J Urol 2011;52(5):310-6.
6. Chromecki TF, Bensalah K, Remzi M, Verhoest G, Cha EK, Scherr DS, et al. Prognostic factors for upper urinary tract urothelial carcinoma. Nature Reviews Urology 2011;8(8):440-7.
7. Verhoest G, Shariat S, Chromecki T, Raman J, Margulis V, Novara G, et al. Predictive factors of recurrence and survival of upper tract urothelial carcinomas. World J Urol 2011;29(4):495-501.

8. Lughezzani G, Burger M, Margulis V, Matin SF, Novara G, Roupret M, et al. Prognostic factors in upper urinary tract urothelial carcinomas: a comprehensive review of the current literature. Eur Urol 2012;62(1):100-14.
9. Eltz S, Comperat E, Cussenot O, Roupret M. Molecular and histological markers in urothelial carcinomas of the upper urinary tract. BJU Int 2008;102(5):532-5.
10. 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(4):827-33.
11. Masuda M, Iki M, Takano Y, Asakura T, Noguchi S, Ikeda I, et al. Prognostic significance of Ki-67 labeling index in urothelial tumors of the renal pelvis and ureter. J Urol 1996;155(6):1877-80.
12. 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(1):14-9.
13. Kamijima S, Tobe T, Suyama T, Ueda T, Igarashi T, Ichikawa T, et al. 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(11):941-7.
14. Joung JY, Yang SO, Jeong IG, Han KS, Seo HK, Chung J, et al. Identification of immunohistochemical factors that predict the synchronous or metachronous development of bladder tumors in patients with upper urinary tract tumors. Urologia Int 2008;81(3):306-11.

15. Jeon HG, Jeong IG, Bae J, Lee JW, Won J-K, Paik JH, et al. Expression of Ki-67 and COX-2 in Patients With Upper Urinary Tract Urothelial Carcinoma. Urology 2010;76(2):513.e7-.e12.
16. Scarpini S, Roupret M, Renard-Penna R, Camparo P, Cussenot O, Comperat E. Impact of the expression of Aurora-A, p53, and MIB-1 on the prognosis of urothelial carcinomas of the upper urinary tract. Urol Oncol 2012;30(2):182-7.
17. Feng C, Wang L, Ding G, Ding Q, Zhou Z, Jiang H, et al. Predictive value of clinicopathological markers for the metachronous bladder cancer and prognosis of upper tract urothelial carcinoma. Scientific Reports 2014;4:4015.
18. Garcia-Tello A, Angulo JC, Andres G, Ramon de Fata F, Sanchez-Chapado M, Lopez JI. Impact of p53, MIB-1 and PECAM-1 expression on the prognosis of urothelial carcinoma of the renal pelvis. Actas Urologicas Espanolas 2014;38(8):506-14.
19. Hayashi A, Morikawa T, Kawai T, Kume H, Ishikawa S, Homma Y, et al. Clinicopathological and prognostic significance of EZH2 expression in upper urinary tract carcinoma. Virchows Archiv 2014;464(4):463-71.
20. Krabbe LM, Bagrodia A, Haddad AQ, Kapur P, Khalil D, Hynan LS, et al. 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(5):1486-93.
21. Wu P, Liu S, Zhang W, Zhang Y, Zhu G, Wei D, et al. Low-level Ki-67 expression as an independent predictor of bladder tumour recurrence in patients with primary upper tract urothelial carcinoma after radical nephroureterectomy. Japanese J Clinical Oncol 2015;45(12):1175-81.

22. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ (Clinical research ed) 2009;339:b2535.
23. McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM. Reporting recommendations for tumor marker prognostic studies. J Clinical Oncol 2005;23(36):9067-72.
24. Parmar MK, Torri V, Stewart L. Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Statistics in Medicine 1998;17(24):2815-34.
25. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ (Clinical research ed) 2003;327(7414):557-60.
26. Porten S, Siefker-Radtke AO, Xiao L, Margulis V, Kamat AM, Wood CG, et al. Neoadjuvant chemotherapy improves survival of patients with upper tract urothelial carcinoma. Cancer 2014;120(12):1794-9.
27. Hara T, Fujimoto H, Sakura M, Inokuchi J, Nishiyama H, Miyazaki J, et al. Prognostic factors of recurrent disease in upper urinary tract urothelial cancer after radical nephroureterectomy: Subanalysis of the multi-institutional national database of the Japanese Urological Association. International J Urol 2015;22(11):1013-20.
28. Lumachi F, Orlando R, Marino F, Chiara GB, Basso SM. Expression of p53 and Ki-67 as prognostic factors for survival of men with colorectal cancer. Anticancer Research 2012;32(9):3965-7.
29. Hamilton NA, Liu TC, Cavatiao A, Mawad K, Chen L, Strasberg SS, et al. Ki-67 predicts disease recurrence and poor prognosis in pancreatic neuroendocrine neoplasms. Surgery 2012;152(1):107-13.

30. Mathieu R, Shariat SF, Seitz C, Karakiewicz PI, Fajkovic H, Sun M, et al. Multi-institutional validation of the prognostic value of Ki-67 labeling index in patients treated with radical prostatectomy. World J Urol 2015;33(8):1165-71.
31. Gayed BA, Youssef RF, Bagrodia A, Darwish OM, Kapur P, Sagalowsky A, et al. Ki67 is an independent predictor of oncological outcomes in patients with localized clear-cell renal cell carcinoma. BJU Int 2014;113(4):668-73.
32. Ding W, Gou Y, Sun C, Xia G, Wang H, Chen Z, et al. Ki-67 is an independent indicator in non-muscle invasive bladder cancer (NMIBC); combination of EORTC risk scores and Ki-67 expression could improve the risk stratification of NMIBC. Urologic Oncology 2014;32(1):42.e13-9.
33. Chen J-X, Deng N, Chen X, Chen L-W, Qiu S-P, Li X-F, et al. A Novel Molecular Grading Model: Combination of Ki67 and VEGF in Predicting Tumor Recurrence and Progression in Non-invasive Urothelial Bladder Cancer. Asian Pacific J Cancer Prevention 2012;13(5):2229-34.
34. Lei Y, Li Z, Qi L, Tong S, Li B, He W, et al. The Prognostic Role of Ki-67/MIB-1 in Upper Urinary-Tract Urothelial Carcinomas: A Systematic Review and Meta-Analysis. J Endourol 2015;29(11):1302-8.
35. Tian Y, Ma Z, Chen Z, Li M, Wu Z, Hong M, et al. Clinicopathological and Prognostic Value of Ki-67 Expression in Bladder Cancer: A Systematic Review and Meta-Analysis. PloS one 2016;11(7):e0158891.
36. Sanchez-Munoz A, Navarro-Perez V, Plata-Fernandez Y, Santonja A, Moreno I, Ribelles N, et al. Proliferation Determined by Ki-67 Defines Different Pathologic Response to Neoadjuvant Trastuzumab-Based Chemotherapy in HER2-Positive Breast Cancer. Clinical Breast Cancer 2015;15(5):343-7.

37. Egger M, Zellweger-Zähner T, Schneider M, Junker C, Lengeler C, Antes G. Language bias in randomised controlled trials published in English and German. The Lancet 1997;350(9074):326-9.

Table 1. Main characteristics of the eligible studies

	Year	Country	Recruitment period	Prospective data collection	Inclusion and exclusion criteria	Definition of survival
Chowdhury et al [10]	1996	Japan	NA	No	No	No
Masuda et al [11]	1996	Japan	1983-1993	No	No	No
Kamai et al [12]	2000	Japan	1988-1995	No	No	No
Kamijima et al [13]	2005	Japan	1975-1997	No	No	No
Joung et al [14]	2007	Korea	2001-2005	No	Yes	No
Jeon et al [15]	2010	Korea	1998-2005	No	Yes	Yes
Scarpini et al [16]	2012	France	NA	No	No	Yes
Feng et al [17]	2014	China	2005-1013	No	No	No
García-Tello et al [18]	2014	Spain	2000-2008	Yes	No	No
Hayashi et al [19]	2014	Japan	1996-2012	No	No	No
Krabbe et al [20]	2015	Multination	NA	No	Yes	No
Wu et al [21]	2015	China	2004-2014	No	No	No

*mean

Table 2. Patient characteristics of the eligible studies

	No. of patients	Median age, range (years)	Gender (male/female)	Operative therapy			Adjuvant chemotherapy	Median follow-up, range (months)
				Nephroureterectomy	Nephrectomy	Ureterectomy		
Chowdhury et al [10]	67	67.9* (38-91)	48/19	60	2	5	25	34.5 (7-106)
Masuda et al [11]	70	64.7* (35-83)	52/18	70	0	0	NA	47.2* (6-122)
Kamai et al [12]	37	74.7* (56-90)	19/18	37	0	0	NA	44 (3-105)
Kamijima et al [13]	69	65* (41-80)	50/19	69	0	0	NA	59 (2-252)
Joung et al [14]	38	63.3* (37-87)	26/12	35	0	2	3	37.2* (13-59)
Jeon et al [15]	107	64.1 (36-79)	80/27	107	0	0	NA	53.1 (2-122)
Scarpini et al [16]	42	70.6* (46-100)	31/11	42	0	0	NA	36 (8-110)
Feng et al [17]	78	65.0* (40-83)	59/29	88	0	0	15	28.6* (2-82)
García-Tello et al [18]	82	66.9* (34-89)	65/17	82	0	0	NA	46.8* (4-172)
Hayashi et al [19]	171	NA	119/52	171	0	0	NA	NA
Krabbe et al [20]	475	67.9 (IQR 63-77)	262/213	NA	NA	NA	55	35 (1-196)
Wu et al [21]	115	66.7* (32-85)	57/58	115	0	0	10	54.2* (7-130)

*mean, NA: not available, IQR: interquartile range.

Table 3. Pathologic characteristics of the eligible studies

	Site			pT stage					Grade					pN stage		
	Renal pelvis	ureter	Both	pTcis/a	pT1	pT2	pT3	pT4	G1	G2	G3	CIS	LVI	pNx	pN-	pN+
Chowdhury et al [10]	37	30	0	17	14	13	23	0	11	39	17	NA	NA	NA	NA	NA
Masuda et al [11]	42	23	5	15	14	13	25	3	6	42	22*	NA	NA	NA	NA	NA
Kamai et al [12]	NA	NA	NA	0	8	10	19†	0	4	19	14	NA	NA	0	30	7
Kamijima et al [13]	42	23	4	0	26	13	26	4	3	20	46	NA	50	NA	NA	7
Joung et al [14]	18	14	6	8	7	3	16	1	31‡	0	7‡	3	9	28	7	3
Jeon et al [15]	66	34	7	8	34	17	45	3	36‡	0	71‡	NA	32	50※	0	16
Scarpini et al [16]	24	18	0	16	8	6	10	2	14‡	0	28‡	11	NA	NA	NA	NA
Feng et al [17]	39	39	10	36	14	16	22†	0	31‡	0	57‡	NA	NA	NA	NA	NA
García-Tello et al [18]	56	0	26	3	42	9	24	4	43‡	0	39‡	14	16	64	14	4
Hayashi et al [19]	103	68	0	44	31	18	69	9	19‡	0	152‡	83	74	152※	0	19
Krabbe et al [20]	359	116	0	46	145	85	162	37	0	0	475‡	110	129	225	204	46
Wu et al [21]	55	48	12	35**	0	39	41†	0	47‡	0	67‡	13	NA	109※	0	6

*grade 3-4, **Tis/Ta/T1, †pT3-4, ‡low grade/high grade, ※pNx/N-

CIS: carcinoma *in-situ*, LVI: lymphovascular invasion, NA: not available.

Table 4. Immunohistochemical staining

	Primary antibody	Cut-off	Compartment	Blind assessment
Chowdhury et al [10]	Monoclonal	24%	Nuclei	NA
Masuda et al [11]	NA	13.3% and 21.7%	Nuclei	NA
Kamai et al [12]	Monoclonal	30%	Nuclei	NA
Kamijima et al [13]	Monoclonal	10%	Nuclei	NA
Joung et al [14]	NA	20%	Nuclei	Yes
Jeon et al [15]	Monoclonal	20%	Nuclei	Yes
Scarpini et al [16]	Monoclonal	10%	Nuclei	NA
Feng et al [17]	NA	20%	NA	NA
García-Tello et al [18]	Monoclonal	6%	Nuclei	NA
Hayashi et al [19]	Monoclonal	10%	Nuclei	NA
Krabbe et al [20]	NA	20%	NA	NA
Wu et al [21]	NA	20%	NA	NA

NA: not available.

Table 5. Estimation of the hazard ratio for disease-free survival

	Hazard ratio estimation	Co-factors	Analysis results
Masuda et al [11]	Hazard ratio, 95% confidence interval	Grade, pT stage	Significant
Kamai et al [12]	Hazard ratio, 95% confidence interval	Grade, pT stage, pN stage, p27	Significant
Wu et al [21]	P value, event no. (univariate)	-	Not significant

Table 6. Estimation of the hazard ratio for cancer-specific survival

	Hazard ratio estimation	Co-factors	Analysis results
Chowdhury et al [10]	Hazard ratio, 95% confidence interval	Grade, pT stage	Significant
Kamijima et al [13]	Hazard ratio, 95% confidence interval	Grade, pT stage, pN stage, lymphovascular invasion	Not significant
Jeon et al [15]	Hazard ratio, 95% confidence interval	Age, gender, grade, pT stage, pN stage, lymphovascular invasion, COX-2	Significant
Feng et al [17]	Hazard ratio, 95% confidence interval	Gender, gross hematuria,grade, pT stage, tumor side, tumor location, tumor no. concomitant bladder cancer, non-functioning kidney, p53	Not significant
García-Tello et al [18]	Hazard ratio, 95% confidence interval (univariate)	-	Significant
Hayashi et al [19]	Hazard ratio, 95% confidence interval (univariate)	-	Significant
Krabbe et al [20]	Hazard ratio, 95% confidence interval	Multifocality, pT stage, pN stage, LVI, tumor architecture, necrosis, adjuvant chemotherapy	Significant
Wu et al [21]	P value, event no. (univariate)	-	Significant

Table 7. Estimation of the hazard ratio for overall survival

	Hazard ratio estimation	Co-factors	Analysis results
Masuda et al [11]	Hazard ratio, 95% confidence interval	Grade, pT stage	Significant
Kamai et al [12]	Hazard ratio, 95% confidence interval	Grade, pT stage, pN stage, p27	Significant
Wu et al [21]	P value, event no. (univariate)	-	Not significant

Table 8. Estimation of the hazard ratio for intravesical recurrence-free survival

	Hazard ratio estimation	Co-factors	Analysis results
Joung et al [14]	Hazard ratio, 95% confidence interval	Multiplicity, lymphovascular invasion	Significant
Feng et al [17]	Hazard ratio, 95% confidence interval	Gender, gross hematuria,grade, pT stage, tumor side, tumor location, tumor no. concomitant bladder cancer, non-functioning kidney, p53	Not significant
Wu et al [21]	Hazard ratio, 95% confidence interval	Age, side, grade, pT stage, tumor location, p53	Significant

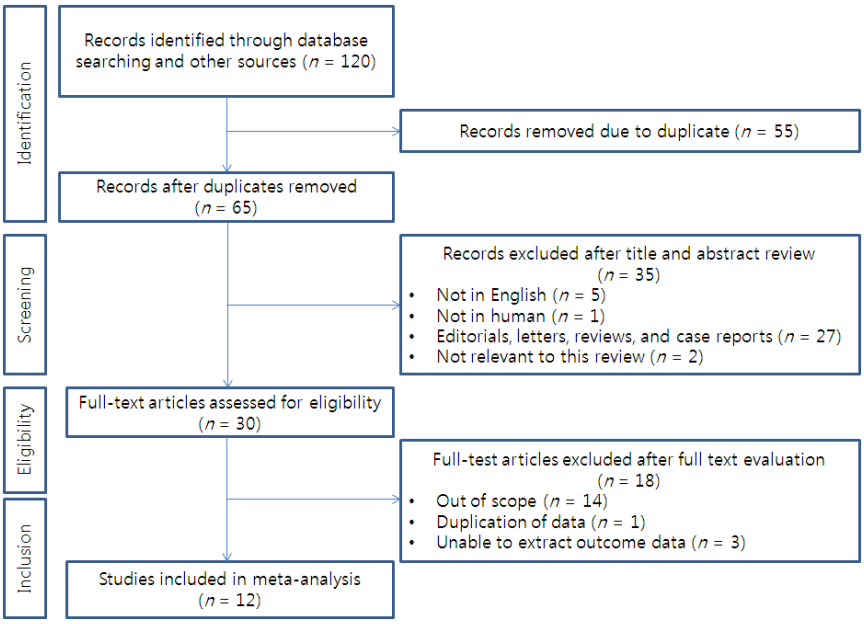

Figure 1. Flow chart of the literature search used in this meta-analysis

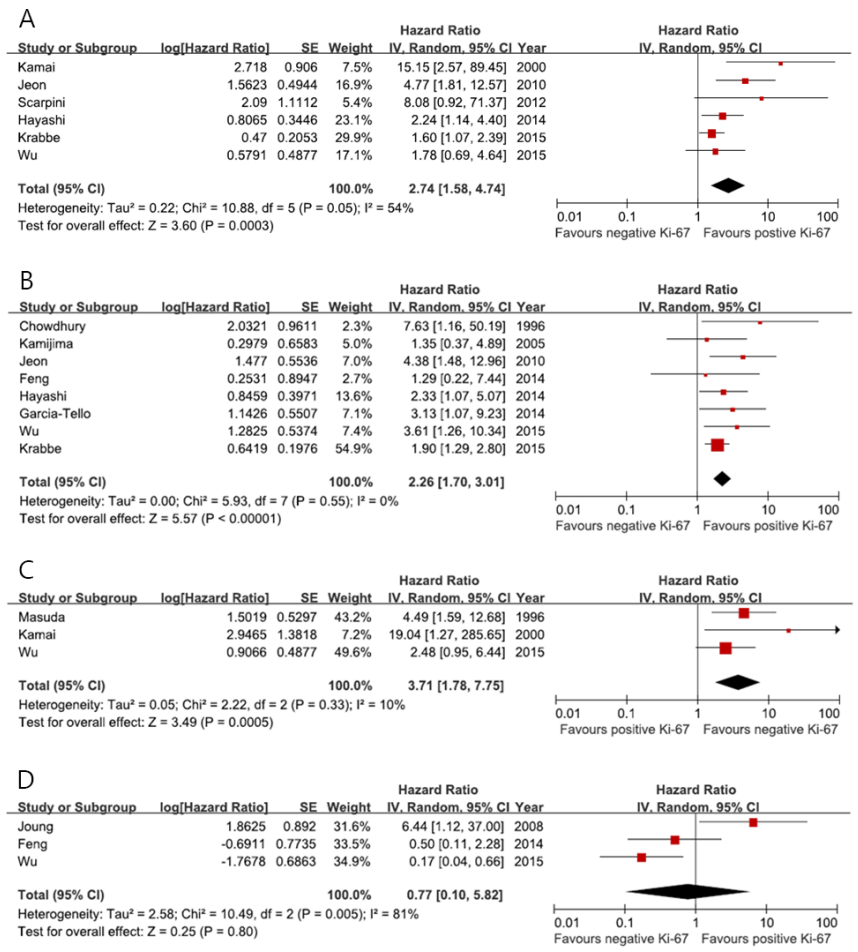

Figure 2. Forest plots of hazard ratios with a random-effects model for ki-67 in patients with upper urinary tract urothelial carcinoma(A: Disease-free survival, B: Cancer-specific survival, C: Overall survival, D: Intravesical recurrence-free survival)

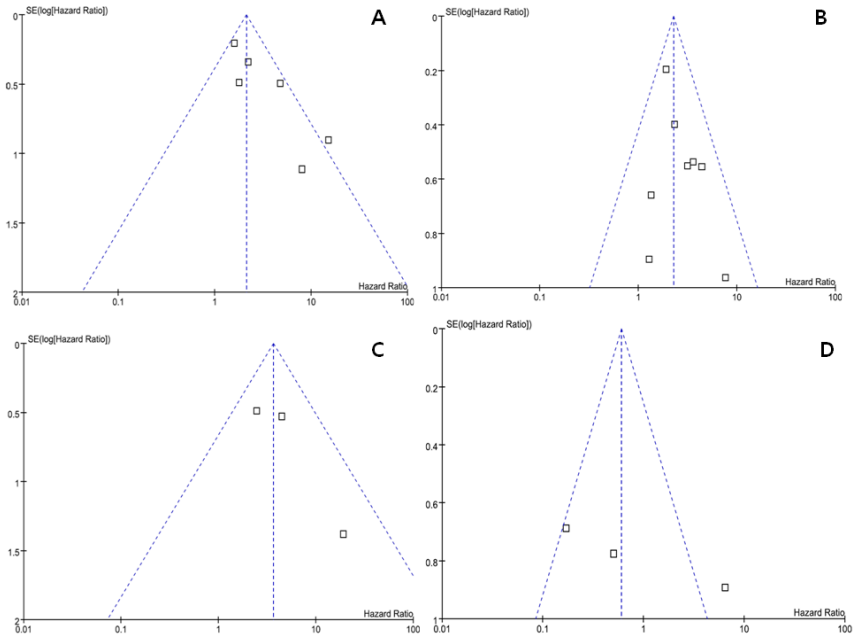

Figure 3. Funnel graphs of the assessment of potential publication bias in studies of ki-67 in patients with upper urinary tract urothelial carcinoma (A: Disease-free survival, B: Cancer-specific survival, C: Overall survival, D: Intravesical recurrence-free survival)

국 문 초 록

서론: 본 연구에서는 상부요로상피암 환자 중 근치적 신장요관절제술을 시행 받은 환자에서 Ki-67 의 예후 예측용 표지자로서의 가치를 평가하기 위해 체계적 문헌고찰 및 발행된 논문의 메타분석을 실시하였다.

대상 및 방법: PRISMA 가이드라인에 따라 Embase, SCOPUS, PubMed 의 2016 년 2 월까지 발표된 논문을 검색하였다. 결과 변수를 방광내 무재발 생존율, 무질병 생존율, 종양특이 생존율, 전반적 생존율로 Ki-67 과 생존 결과의 관계를 위험도와 95% 신뢰구간으로 분석하였다.

결과: 총 1351 명의 환자를 포함하는 12 개의 논문이 체계적 문헌고찰의 기준에 적합하였다. Ki-67 의 과발현은 악화된 무질병 생존률(위험도 2.74; 95% 신뢰구간 1.58-4.74), 종양특이 생존율(위험도 2.26; 95% 신뢰구간 1.70-3.01), 전반적 생존율(위험도 3.71; 95% 신뢰구간 1.78-7.75)과 통계적으로 유의하였다. 그러나 방광내 무재발 생존율(위험도 0.77; 95% 신뢰구간 0.10-5.82)과는 통계적으로 유의하지 않았다. 연구간 이질성 조사에서는 종양특이 생존율(I^2 = 54%, p = 0.05), 방광내 무재발 생존율(I^2 = 81%, p = 0.005)에서 높은 정도의 연구간 이질성이 시사되었다. 출판비뚤림은 funnel plot test 로 배제하였다.

결론: 본 연구의 결과로 Ki-67 의 과발현이 상부요로상피암 환자 중 근치적 신장요관절제술을 받은 환자에서의 유용한 예후 예측용 표지자로 사용될 수 있음을 알 수 있었다. 향후 Ki-67 의

상부요로상피암에서의 예후 예측 표지자로서의 가치를 확립하기 위해 더 큰 규모로 잘 설계된 전향적 연구가 필요하다.

주요어: 상부요로, 요관, 신우, 요로상피암, Ki-67, 예후, 메타분석

학 번: 2016-21946