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SYSTEMATIC REVIEW AND META-ANALYSIS

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# High Ki-67 Immunohistochemical Reactivity Correlates With Poor Prognosis in Bladder Carcinoma

## *A Comprehensive Meta-Analysis with 13,053 Patients Involved*

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**Abstract:** Ki-67 is considered as one of prime biomarkers to reflect cell proliferation and immunohistochemical Ki-67 staining has been widely applied in clinical pathology. To solve the widespread controversy whether Ki-67 reactivity significantly predicts clinical prognosis of bladder carcinoma (BC), we performed a comprehensive meta-analysis by combining results from different literature.

A comprehensive search was conducted in the Chinese databases of WanFang, China National Knowledge Infrastructure and Chinese VIP as well as English databases of PubMed, ISI web of science, EMBASE, Science Direct, and Wiley online library. Independent studies linking Ki-67 to cancer-specific survival (CSS), disease-free survival (DFS), overall survival (OS), progression-free survival (PFS), and recurrence-free survival (RFS) were included in our meta-analysis. With the cut-off values literature provided, hazard ratio (HR) values between the survival distributions were extracted and later combined with STATA 12.0.

In total, 76 studies (n = 13,053 patients) were eligible for the meta-analysis. It was indicated in either univariate or multivariate analysis for survival that high Ki-67 reactivity significantly predicted poor prognosis. In the univariate analysis, the combined HR for CSS, DFS, OS, PFS, and RFS were 2.588 (95% confidence interval [CI]: 1.623–4.127, $P < 0.001$), 2.697 (95%CI: 1.874–3.883, $P < 0.001$), 2.649 (95%CI: 1.632–4.300, $P < 0.001$), 3.506 (95%CI: 2.231–5.508, $P < 0.001$), and 1.792 (95%CI: 1.409–2.279, $P < 0.001$), respectively. The pooled HR of multivariate analysis for CSS, DFS, OS, PFS, and RFS were 1.868 (95%CI: 1.343–2.597, $P < 0.001$), 2.626 (95%CI: 2.089–3.301, $P < 0.001$), 1.104 (95%CI: 1.008–1.209, $P = 0.032$), 1.518 (95%CI: 1.299–1.773, $P < 0.001$), and 1.294 (95%CI: 1.203–1.392, $P < 0.001$), respectively. Subgroup analysis of univariate analysis by origin showed that Ki-67 reactivity significantly correlated with all 5 clinical outcome in Asian and European-American patients ($P < 0.05$). For multivariate analysis, however, the pooled results were only significant for DFS, OS, and RFS in Asian patients, for CSS, DFS, PFS, and RFS in European-American patients ($P < 0.05$). In the subgroup with low cut-off value (<20%), our meta-analysis indicated that high Ki-67 reactivity was significantly correlated with worsened CSS, DFS, OS, PFS, and RFS on univariate analysis ($P < 0.05$). For multivariate analysis, the meta-analysis of literature with low cut-off value (<20%) demonstrated that high Ki-67 reactivity predicted shorter DFS, PFS, and RFS in BC patients ($P < 0.05$). In the subgroup analysis of high cut-off value (≥20%), our meta-analysis indicated that high Ki-67 reactivity, in either univariate or multivariate analysis, significantly correlated with all five clinical outcomes in BC patients ($P < 0.05$).

The meta-analysis indicates that high Ki-67 reactivity significantly correlates with deteriorated clinical outcomes in BC patients and that Ki-67 can be considered as an independent indicator for the prognosis by the meta-analyses of multivariate analysis.

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**Abbreviations**: BC = bladder carcinoma, CI = confidence interval, CSS = cancer-specific survival, DFS = disease-free survival, HR = hazard ratio, IHC = immunohistochemistry, MI = mitotic index, OS = overall survival, PFS = progression-free survival, RFS = recurrence-free survival, UTUC = upper urinary-tract urothelial carcinomas.

## INTRODUCTION

Bladder carcinoma (BC) ranks top as the most common malignant tumor in urinary system, with an estimate of 74,000 new cases and 16,000 deaths in America and 429,800 new cases and 165,100 deaths in 2012 worldwide, respectively.[1,2] Despite the improved therapeutic strategies nowadays, such as transurethral resection or radical cystectomy, there are still an epic number of BC patients suffering from tumor progression and recurrence. In addition, 5-year relative survival trend and prognosis of BC remain obscure and unfavorable.[3,4] According to the National Comprehensive Cancer Network Guidelines of 2013, tumor stages, grades, and metastasis are the major factors to define the individual treatment strategies of BC patients.[5] However, conventional established gauges seem inferior to provide accurate prediction for the prognosis of BC patients with diverse and complicated tumor backgrounds. Detecting technique of protein molecules is now an availably novel approach to evaluate the prognosis of tumors, which immensely contributes to more comprehensive and individualized

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treatments for patients in early stages. Consequently, there is a pressing appeal to identify molecular biomarkers to effectively forecast the clinical outcomes for BC patients.

Ki-67 is a nuclear protein which can be detected during the cell cycles of G1, S, G2, and M stages except G0[6] and now has been widely applied in immunohistochemistry (IHC) to assess the activities of cell proliferation in various cancers. Compared with other well-identified molecular biomarkers, such as p53,[7] survivin,[8] and so on, Ki-67 is a preferably convenient biomarker for the proliferation status of tumor cells. Recently, several studies continuously reported that Ki-67 is an independent indicator to predict poor clinical outcomes of both no-muscle invasive and muscle invasive BC patients.[9–12] Two studies featuring large sample sizes of tissues, by Margulis et al[13] and Wang et al,[14] respectively, have concluded that Ki-67 reactivity correlated significantly with poor cancer-specific survival (CSS) and recurrence-free survival (RFS) of BC patients. Nevertheless, there were also studies demonstrating contradictory results.[9,15] Considering the controversy of literature, we performed the current comprehensive meta-analysis, which combined results from different literature to confirm whether Ki-67 reactivity is an independent indicator to predicting the prognosis of BC patients.

## MATERIALS AND METHODS

### Literature Search and Selection

A comprehensive literature search was completed in the Chinese databases of WanFang, China National Knowledge Infrastructure and Chinese VIP as well as English databases of PubMed, ISI web of science, EMBASE, Science Direct, and Wiley online library with closing date marked on September 15th, 2015. Literature were identified by the combination of the following keywords Ki67 OR Ki-67 OR MKi-67 OR MIB1 OR MIB-1 OR proliferation index OR PI OR proliferation activity OR proliferation marker OR mitotic index OR mitotic figure OR mitotic activity OR mitotic count OR labeling index OR LI OR MI; bladder; tumor OR cancer OR carcinoma OR neoplas* OR malignan*; and prognos* OR predict OR surviv* OR follow-up OR outcome OR mortality.

Two independent investigators (YL and HZ) screened eligible studies by the same multistep procedures. First, investigators reviewed the titles and abstracts of the identified literature prudently. Studies which explored the relationship between Ki-67 reactivity and clinical outcomes of BC patients were deemed to be eligible. Second, full texts of the eligible literature were carefully reviewed and assessed according to the following inclusion criteria: literature must detect the reactivity of Ki-67 by IHC in BC tissues and evaluate the correlation of Ki-67 reactivity with survival outcome of BC patients, and were published in either Chinese or English; hazard ratios (HRs) and its 95% confidence interval (CI) for prognostic marker were provided by authors or could be calculated from available data presented in articles. Accordingly, the following circumstances might result in exclusion for the current meta-analysis: articles investigated the clinical outcomes of BC patients by the combination of Ki-67 with other immunohistochemical marker(s); animals, cell lines, or laboratory studies were not suitable for the meta-analysis; reviews, letters, and case reports were also excluded; and literature failed to estimate HR and its 95%CI by the information offered. Moreover, we tried to contact the authors when survival analysis was conducted in the articles but insufficient data were supplied for HR and 95%CI calculation. To avoid duplications, literature with the most complete data for analysis should be included if the same or overlapping cohorts were used in different studies or the same article published in different journals. Finally, the interesting articles remained were included in our meta-analysis as well. Controversies were solved by discussing with a 3rd investigator (GC).

### Data Extraction

The information of the included studies was extracted carefully by 2 independent researchers (YL and CH) with the same criteria and standardized table. The following data from literature were extracted: first author, published year, number of patients, median or mean age of the patients, fold of antibody dilution, whether blind reading or not, cut-off value, tumor stage, treatments for patients, follow-up period, and data for survival analysis. No quality assessment was performed for each study by any scale, since there existed no universally acknowledged quality scoring systems for meta-analyses of follow-up studies.[16] Controversial viewpoints were solved by discussion with a 3rd investigator (CG).

### Statistical Methods

The HR values were extracted from each study, later synthesized into a meta-analysis, and used to estimate the risk of high Ki-67 reactivity on patients' survival. For each study, we extracted the survival data with the method by Tierney et al.[17] Survival data were either drawn directly or calculated by the calculator of Review Manager 5.3 if required data were available to estimate the HR values and its 95%CI. For the literature only with survival curves, the software, Engauge Digitizer version 4.1 (http://digitizer.sourceforge.net/), was in the charge of calculation. When an $HR > 1$ and its $95\%CI \leq 1$, it indicated that high Ki-67 reactivity was associated with poor survival outcomes in BC patients.

Heterogeneity test for studies included was conducted by $\chi^2$ and inconsistency ($I^2$) test, setting a significant level at 0.05% and 50%.[18,19] To take all heterogeneity, such as different cut-off value and/or methods of measurement, into consideration, random-effect model was employed to calculate the combined HRs.[20–22] Subsequently, subgroup analysis was employed to further analyze the associations of Ki-67 reactivity with clinical outcomes in patients from different origins and under different cut-off values. We have divided the studies into Asian or European-American group by origins and divided the studies into high or low cut-off group with a ''cut-off'' value of 20%, which were used to classify Ki-67 reactivity in 2 large samples studies published by Margulis et al and Wang et al, respectively.[13,14] Sensitivity analysis was conducted to examine the stability of meta-analysis. Moreover, Begg test and funnel plots would be used to examine the publication bias if the numbers of studies were above 10.[23] If significant publication bias was detected, trim and fill method would be applied to retrieve ''missing'' literature.[24] The statistical processes were all conducted by STATA version 12.0, and a 2-sided $P \leq 0.05$ was considered statistically significant. The current study is a meta-analysis and all the data were extracted from published literature, and therefore ethical approval was waived for this meta-analysis. All the procedures of this meta-analysis were performed according to PRISMA guidelines.[25]

## RESULTS

### Summary of Literature' Characteristics

The search strategy of the current meta-analysis identified a total of 3682 literature, including 2825 studies form English

databases and 857 studies from Chinese databases. Overall, 3241 records, identified irrelevant by title and abstract screening, were excluded; and the remained 441 records, which investigated the relationship between Ki-67 reactivity and survival outcomes of BC patients, were evaluated in full text. According to our inclusion criteria, 76 studies (n = 13,053 patients),[9,13–15,26–96] published from 1997 to 2015, were eligible and eventually included in our meta-analysis for analysis of the correlation between Ki-67 reactivity and clinical prognosis of BC patients (Figure 1).

Tables 1 and 2 summarized the main characteristics of the included studies that analyzed the relationships between Ki-67 reactivity and clinical outcome by univariate analysis and multivariate analysis, respectively. For the patient cohorts among 76 studies, 35 were from Asia, 32 from Europe, and 9 from North America. The number of cohorts of each study ranged from 31 to 993, and all the studies detected Ki-67 reactivity by IHC, with an antibody dilution varied from 1:800 to 1:10. Thirty-seven studies assessed the status of immunostaining by pathologist(s) who was/were blind to the clinical outcomes, while another 39 studies did not report whether blind reading was adopted or not. The cut-off value to distinguish high Ki-67 reactivity from low Ki-67 reactivity was set from 5% to 55%. The clinical stage was Ta-T1 in 36 studies and another 40 studies contained tumor stage in T2-T4 stage. As shown in Tables 1 and 2, the main treatments for BC patients were transurethral resection, radical cystectomy or partial cystectomy, and intravesical chemotherapy. The total follow-up periods range from 12 to 266.8 months. Overall, 45 studies provided data by the calculation of univariate analysis, including 13 for CSS, 5 for disease-free survival (DFS), 12 for overall survival (OS), 21 for progression-free survival (PFS), and 23 for RFS. Fifty-eight studies presented results by the calculation of multivariate analysis, including 18 for CSS, 6 for DFS, 14 for OS, 23 for PFS, and 35 for RFS.

## Univariate Analysis of Survival Status

A total of 45 studies (n = 10,083 patients) of univariate analysis for survival status were available for our meta-analysis (Table 3), including 13 studies (n = 4641 patients) for CSS, 5 studies (n = 626 patients) for DFS, 12 studies (n = 1491) for OS, 21 studies (n = 4145) for PFS, and 23 studies (n = 3452) for RFS. Our meta-analysis demonstrated that high Ki-67 reactivity significantly correlated with worsened CSS, DFS, OS, PFS, and RFS in BC patients. The combined HRs were 2.588 (95%CI: 1.623–4.127, $P < 0.001$) (Figure 2A), 2.697 (95%CI: 1.874–3.883, $P < 0.001$) (Figure 2B), 2.649 (95%CI: 1.632–4.300, $P < 0.001$) (Figure 2C), 3.506 (95%CI: 2.231–5.508, $P < 0.001$) (Figure 2D), and 1.792 (95%CI: 1.409–2.279, $P < 0.001$) (Figure 2E) for CSS, DFS, OS, PFS, and RFS, respectively (Table 3). Nevertheless, significant heterogeneity existed in all meta-analyses of CSS ($P < 0.001$, $I^2 = 94.0\%$), DFS ($P = 0.090$, $I^2 = 50.2\%$), OS ($P < 0.001$, $I^2 = 80.2\%$), PFS ($P < 0.001$, $I^2 = 90.2\%$), and RFS ($P < 0.001$, $I^2 = 85.8\%$). Thus, it was urgently required to find the main causes of heterogeneity. Consequently, subgroup analysis was performed in accordance with origins of patients and cut-off values to reveal the main factors contributing to significant heterogeneity.

Subgroup analysis by origin indicated that high Ki-67 reactivity was significantly associated with poor CSS (HR = 2.930, 95%CI: 1.687–5.089, $P < 0.001$), DFS (HR = 3.867, 95%CI: 2.280–6.590), OS (HR = 7.691, 95%CI: 1.832–32.294, $P = 0.005$), PFS (HR = 3.868, 95%CI: 1.983–7.543, $P < 0.001$), and RFS (HR = 2.065, 95%CI: 1.526–2.793, $P < 0.001$) in Asian patients. For European-American patients, the similar results were observed for CSS (HR = 2.930, 95%CI: 1.687–5.089, $P < 0.001$), DFS (HR = 2.373, 95%CI: 1.649–3.416, $P < 0.001$), OS (HR = 1.653, 95%CI: 1.161–2.353, $P < 0.001$), PFS (HR = 3.377, 95%CI: 2.040–5.589, $P < 0.001$), and RFS (HR = 1.528, 95%CI: 1.134–2.060, $P < 0.001$). Subgroup analysis by cut-off value demonstrated that high Ki-67 reactivity was significantly associated with deteriorated CSS, DFS, OS, PFS, and RFS regardless of high (<20%)/low cut-off (≥20%) value cut-off values ($P < 0.05$, Table 4).

## Multivariate Analyses of Survival Status

For the meta-analyses of multivariate analysis, 58 studies with 8128 patients enrolled were included. The concomitant variables corrected in the multivariate analysis of each study have list in Table S1 (Additional file 2, http://links.lww.com/MD/A894). Our meta-analysis represented that high Ki-67 reactivity was still significantly associated with shorter CSS 1.868 (95%CI: 1.343–2.597, $P < 0.001$) (Figure 3A), DFS (HR = 2.626, 95%CI: 2.089–3.301, $P < 0.001$) (Figure 3B), OS (HR = 1.104, 95%CI: 1.008–1.209, $P = 0.032$) (Figure 3C), PFS (HR = 1.518, 95%CI: 1.299–1.773, $P < 0.001$) (Figure 3D), and RFS (HR = 1.294, 95%CI: 1.203–1.392, $P < 0.001$) (Figure 3E) after adjusting concomitant variables. Significant heterogeneity emerged in CSS ($I^2 = 64.1\%$, $P < 0.001$), OS ($I^2 = 77.2\%$, $P < 0.001$), PFS ($I^2 = 78.2\%$, $P < 0.001$), and RFS ($I^2 = 77.7\%$, $P < 0.001$) but not DFS ($I^2 = 0.0\%$, $P = 0.675$). Identically, we conducted subgroup analyses in accordance with origins of patients and cut-off values.

The results of subgroup analyses demonstrated that, in Asian patients, high Ki-67 reactivity significantly correlated with poorer DFS (HR = 2.576, 95%CI: 1.958–3.390, $P < 0.001$), OS (HR = 2.265, 95%CI: 1.012–5.073, $P = 0.047$), and RFS (HR = 1.832, 95%CI: 1.469–2.285, $P < 0.001$) but not for CSS (HR = 1.571, 95%CI: 0.396–6.237, $P = 0.521$) and PFS (HR = 1.489, 0.657–3.375, $P = 0.34$). For European-American patients, significant results were observed in the meta-analyses for CSS (HR = 1.648, 95%CI: 1.279–2.122, $P < 0.001$), DFS (HR = 2.744, 95%CI:

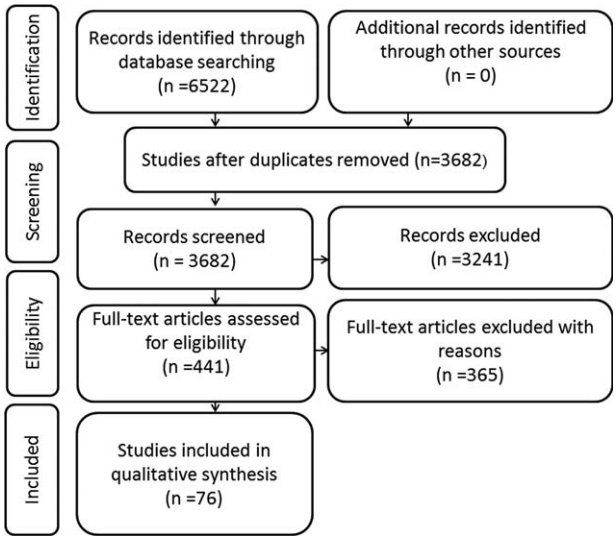


**FIGURE 1.** Flow diagram of literature search and selection.

**TABLE 1.** Main Characteristics of the Included Studies Estimated Prognosis of High Ki-67 Analyzed by Univariate Analysis

| First Author | Year | Origin | No. of Patients | Age | Antibody Dilution | Blind Reading | Cut-off (Method to Decide Cut-off | Tumor Stage | Follow-Up, months | Outcome | HR | LL | UL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tsuji et al[29] | 1997 | Japan | 31 | 66* | NR | NR | 32% (ROC analysis) | T1 11 T2-T4 20 | 71 | CSS | 3.465 | 0.497 | 25.959 |
| Osen et al[31] | 1998 | Norway | 131 | 72† | 1:100 | NR | 25% (NR) | T2-T4 | 140 | OS | 1.240 | 0.770 | 1.990 |
| Nakopoulou et al[30] | 1998 | Greece | 105 | 68* | 1:100 | Yes | 5% (NR) | Ta-T1 | 37.5* | OS | 1.633 | 0.637 | 4.327 |
| Liukkonen et al[32] | 1999 | Finland | 196 | 65.8* | 1:40 | NR | 15% (NR) | pTa-pT1 | 72 | PFS | 3.915 | 1.035 | 14.915 |
| Oosterhuis et al[35] | 2000 | Netherlands | 240 | NR | 1:250 | NR | 13% (median) | Ta-T1 | 60 | OS | 1.970 | 1.367 | 2.840 |
| Oosterhuis et al[35] | 2000 | Netherlands | 240 | NR | 1:250 | NR | 13% (median) | Ta-T1 | 60 | PFS | 1.903 | 1.320 | 2.743 |
| Oosterhuis et al[35] | 2000 | Netherlands | 240 | NR | 1:250 | NR | 13% (median) | Ta-T1 | 60 | RFS | 2.050 | 1.377 | 3.063 |
| Wu et al[36] | 2000 | China | 93 | NR | 1:100 | Yes | 10.6% (median) | pTa-pT1 | 94 | RFS | 0.910 | 0.021 | 4.020 |
| Nocito et al[41] | 2001 | Switzerland | 880 | NR | 1:800 | NR | 11% (NR) | NR | 60 | CSS | 2.420 | 1.510 | 3.880 |
| Nocito et al[41] | 2001 | Switzerland | 911 | NR | 1:800 | NR | 11% (NR) | NR | 60 | CSS | 8.830 | 5.970 | 13.050 |
| Nocito et al[41] | 2001 | Switzerland | 993 | NR | 1:800 | NR | 11% (NR) | NR | 60 | CSS | 12.600 | 7.870 | 20.170 |
| Kamai et al[38] | 2001 | Japan | 145 | 76.1* | NR | NR | 30% (NR) | Ta-T1 86 T2-T4 59 | 124 | DFS | 3.876 | 2.280 | 6.590 |
| Kamai et al[38] | 2001 | Japan | 145 | 76.1* | NR | NR | 30% (NR) | Ta-T1 86 T2-T4 59 | 124 | OS | 23.352 | 6.971 | 78.232 |
| Nocito et al[41] | 2001 | Switzerland | 496 | NR | 1:800 | NR | 11% (NR) | NR | 60 | PFS | 9.480 | 3.690 | 24.370 |
| Nocito et al[41] | 2001 | Switzerland | 518 | NR | 1:800 | NR | 11% (NR) | NR | 60 | PFS | 20.260 | 7.180 | 57.170 |
| Nocito et al[41] | 2001 | Switzerland | 558 | NR | 1:800 | NR | 11% (NR) | NR | 60 | PFS | 10.680 | 4.040 | 28.200 |
| Blanchet et al[37] | 2001 | France | 70 | 62.6* | 1:200 | Yes | 13% (ROC analysis) | pTa-pT1 | 111 | PFS | 5.105 | 1.67 | 14.625 |
| Kamai et al[38] | 2001 | Japan | 145 | 76.1* | NR | NR | 30% (NR) | Ta-T1 86 T2-T4 59 | 124 | RFS | 3.289 | 1.333 | 8.115 |
| Li[44] | 2002 | China | 64 | 53.2† | NR | NR | Score≥4 (NR) | Ta-T1 47 T2-T3 33 | 120 | CSS | 4.243 | 2.285 | 6.631 |
| Sgambato et al[50] | 2002 | Italy | 96 | 68* | 1:100 | Yes | 10% (refer to[33]) | Ta-T1 | 102 | DFS | 2.230 | 1.320 | 3.770 |
| Pich et al[47] | 2002 | Italy | 62 | 62* | 1:100 | NR | 10% (refer to[33]) | pTa | 97 | DFS | 5.026 | 1.950 | 13.045 |
| Li[44] | 2002 | China | 64 | 53.2† | NR | NR | Score≥4 (NR) | Ta-T1 47 T2-T3 33 | 120 | OS | 30.246 | 6.954 | 78.323 |
| Bozlu et al[43] | 2002 | Turkey | 77 | 58.6* | 1:50 | NR | 29.68% (mean) | Ta-T1 43 ≥T2 34 | 72 | RFS | 5.020 | 1.395 | 18.530 |
| van Rhijn et al[53] | 2003 | Netherlands | 246 | 65.7* | 1:200 | Yes | 25% (teaching set) | pTa-pT1 | 220.8 | PFS | 3.305 | 1.255 | 8.775 |
| Frank et al[54] | 2004 | America | 139 | NR | 1:400 | NR | NR | N1 | 120 | CSS | 1.000 | 0.890 | 1.120 |
| Matsumoto et al[56] | 2004 | Japan | 62 | 68† | NR | Yes | 21% (median) | T1 12 T2-T4 49 | 84.2 | CSS | 1.822 | 0.479 | 6.932 |
| Lopez-Beltran et al[55] | 2004 | Spain | 159 | 61* | prediluted | Yes | 13% (NR) | Ta-T1 | 120 | DFS | 2.910 | 1.440 | 5.880 |
| Lopez-Beltran et al[55] | 2004 | Spain | 159 | 61* | prediluted | Yes | 13% (NR) | Ta-T1 | 120 | OS | 2.100 | 0.560 | 7.880 |
| Su et al[52] | 2004 | Japan | 79 | 48.7* | 1:50 | NR | 18% (refer to[35,50]) | pTa-pT1 | 78 | RFS | 2.870 | 1.410 | 5.860 |
| Frank et al[54] | 2004 | America | 139 | NR | 1:400 | NR | NR | N1 | 120 | RFS | 1.070 | 0.880 | 1.300 |
| Mahnken et al[60] | 2005 | Germany | 63 | 68† | 1:20 | Yes | 50% (median) | T1 | 120 | PFS | 1.130 | 0.500 | 2.590 |
| Kruger et al[59] | 2005 | Germany | 73 | NR | 1:20 | Yes | continuous | T1 | NR | PFS | 1.000 | 0.980 | 1.020 |
| Mahnken et al[60] | 2005 | Germany | 63 | 68† | 1:20 | Yes | 50% (median) | T1 | 120 | RFS | 2.040 | 1.110 | 3.750 |
| Kruger et al[59] | 2005 | Germany | 73 | NR | 1:20 | Yes | continuous | T1 | NR | RFS | 1.010 | 1.000 | 1.020 |
| Hilmy et al[65] | 2006 | British | 103 | NR | NR | Yes | NR | pTa-pT1 61 pT2-pT4 42 | 60† | CSS | 1.730 | 1.110 | 2.720 |
| Yin et al[70] | 2006 | China | 101 | NR | 1:100 | NR | 20% (NR) | Ta-T1 | 70.8 | CSS | 7.606 | 1.110 | 52.080 |
| Quintero et al[69] | 2006 | Spain | 164 | 61* | Prediluted | Yes | 13% (refer to[55]) | Ta-T1 | 144 | DFS | 1.786 | 1.219 | 2.616 |
| Qu et al[68] | 2006 | China | 33 | 65† | 1:50 | Yes | 10% (NR) | T1 17 T2-T4 39 | 90 | OS | 2.559 | 0.662 | 9.905 |
| Galmozzi et al[62] | 2006 | Italy | 82 | NR | NR | NR | 55% (median) | pT2-pT4 | 38 | OS | 2.100 | 0.990 | 4.390 |
| Grossman et al[64] | 2006 | America | 94 | 64† | 1:20 | NR | 38.4% (median) | T2-T4 | 204 | OS | 0.740 | 0.440 | 1.240 |
| Quintero et al[69] | 2006 | Spain | 164 | 61* | Prediluted | Yes | 13% (refer to[55]) | Ta-T1 | 144 | OS | 5.882 | 1.219 | 26.794 |
| Grossman et al[64] | 2006 | America | 94 | 64† | 1:20 | NR | 38.4% (median) | T2-T4 | 204 | PFS | 0.620 | 0.370 | 1.030 |

| First Author | Year | Origin | No. of Patients | Age | Antibody Dilution | Blind Reading | Cut-off (Method to Decide Cut-off | Tumor Stage | Follow-Up, months | Outcome | HR | LL | UL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Quintero et al[69] | 2006 | Spain | 164 | 61* | Prediluted | Yes | 13% (refer to[55]) | Ta-T1 | 144 | PFS | 4.348 | 1.301 | 14.536 |
| Yin et al[70] | 2006 | China | 101 | NR | 1:100 | NR | 20% (NR) | Ta-T1 | 70.8 | PFS | 5.190 | 1.420 | 19.147 |
| Shariat et al[72] | 2009 | Canada | 80 | 64.6† | 1:500 | NR | 20% (NR) | pT1 | 195.4 | CSS | 4.360 | 1.270 | 14.910 |
| Margulis et al[13] | 2009 | America | 713 | 67.5* | NR | NR | 20% (optimal) | pT1 89 pT2-pT4 624 | 236 | CSS | 2.330 | 1.820 | 2.980 |
| Shariat et al[72] | 2009 | Canada | 80 | 64.6† | 1:500 | NR | 20% (NR) | pT1 | 195.4 | RFS | 2.800 | 1.040 | 6.340 |
| Margulis et al[13] | 2009 | America | 713 | 67.5* | NR | NR | 20% (optimal) | pT1 89 pT2-pT4 624 | 236 | RFS | 2.760 | 2.040 | 3.750 |
| Seo et al[75] | 2010 | Korea | 129 | 64.2† | 1:50 | NR | 20% (refer to[33,47,54]) | Ta-T1 | 96 | PFS | 4.380 | 1.480 | 13.010 |
| Miyake [74] | 2010 | Japan | 109 | 68.5* | NR | Yes | 25% (refer to[53]) | pTis-pT1 | 99 | RFS | 2.650 | 1.470 | 5.580 |
| Seo et al[75] | 2010 | Korea | 129 | 64.2† | 1:50 | NR | 20% (refer to[33,47,54]) | Ta-T1 | 96 | RFS | 1.190 | 0.710 | 2.000 |
| Maeng et al[73] | 2010 | Korea | 55 | 67* | 1:80 | NR | 25% (refer to[53]) | Ta-T1 | 70 | RFS | 3.108 | 1.245 | 7.759 |
| Youssef et al[81] | 2011 | America | 152 | 51† | NR | NR | 20% (refer to[12,72]) | T1 10 T2-T4 142 | 100 | CSS | 1.330 | 0.470 | 3.740 |
| Sugino et al[79] | 2011 | Japan | 58 | NR | 1:100 | NR | 50% (NR) | pTa-pT1 21 pT2-pT4 37 | 163 | CSS | 1.560 | 0.690 | 3.520 |
| Yan et al[77] | 2011 | China | 82 | 68.3* | 1:100 | NR | 30% (NR) | Ta-T1 48 T2-T4 34 | 184 | OS | 2.160 | 1.030 | 4.530 |
| Yang et al[80] | 2011 | China | 104 | 71† | 1:100 | NR | 10% (NR) | NR | 95 | PFS | 3.093 | 1.385 | 6.892 |
| Yang et al[80] | 2011 | China | 104 | 71† | 1:100 | NR | 10% (NR) | NR | 95 | RFS | 1.372 | 0.235 | 7.937 |
| Youssef et al[81] | 2011 | America | 152 | 51† | NR | NR | 20% (refer to[12,72]) | T1 10 T2-T4 142 | 100 | RFS | 1.090 | 0.390 | 3.080 |
| Laurberg et al[85] | 2012 | Denmark | 162 | 62† | 1:75 | Yes | 20% (refer to[13]) | T1 9 T2-T4 153 | 96 | CSS | 0.830 | 0.530 | 1.290 |
| Chen et al[84] | 2012 | China | 72 | 61.3* | 1:50 | Yes | 25% (NR) | Ta-T1 | 93 | PFS | 10.729 | 1.358 | 84.752 |
| Chen et al[84] | 2012 | China | 72 | 61.3* | 1:50 | Yes | 25% (NR) | Ta-T1 | 93 | RFS | 2.390 | 1.220 | 4.682 |
| Ruan et al[91] | 2012 | China | 126 | 64.52* | 1:50 | NR | 10% (NR) | T1 | 60 | RFS | 1.186 | 0.599 | 2.349 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2* | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | CSS | 3.460 | 1.220 | 9.800 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2* | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | OS | 2.300 | 1.310 | 4.020 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2* | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | PFS | 3.820 | 1.040 | 13.960 |
| Park et al[90] | 2013 | Korea | 61 | 66† | 1:200 | Yes | 10.4% (median) | T1G3 | 217 | PFS | 0.628 | 0.158 | 2.487 |
| Gontero et al[93] | 2013 | Italy | 131 | 66.37* | NR | NR | 20% (median) | T1 | 71.6† | PFS | 4.850 | 1.920 | 12.210 |
| Gontero et al[93] | 2013 | Italy | 131 | 66.37* | NR | NR | 20% (median) | T ≥ 2 | 71.6† | PFS | 6.540 | 1.320 | 32.440 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2* | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | RFS | 0.900 | 0.620 | 1.310 |
| Park et al[90] | 2013 | Korea | 61 | 66† | 1:200 | Yes | 10.4% (median) | T1G3 | 217 | RFS | 0.846 | 0.386 | 1.856 |
| Lotan et al[87] | 2013 | American | 216 | 70† | NR | NR | 10% (NR) | pTa-pT1 86 pT2-pT4 130 | 24 | RFS | 29.080 | 3.907 | 216.455 |
| Okazoe et al[89] | 2013 | Japan | 71 | 72† | 1:100 | Yes | 18% (refer to[51]) | Tis/T1 | 51.8 | RFS | 1.610 | 0.555 | 2.426 |
| Gontero et al[93] | 2013 | Italy | 131 | 66.37* | NR | NR | 20% (median) | NR | 71.6† | RFS | 1.000 | 0.510 | 1.930 |
| Ding et al[10] | 2014 | China | 332 | 67† | NR | NR | 25% (refer to[53]) | Ta-T1 | 124 | PFS | 7.177 | 3.700 | 13.910 |
| Ding et al[10] | 2014 | China | 332 | 67† | NR | NR | 25% (refer to[53]) | Ta-T1 | 124 | RFS | 3.334 | 2.270 | 4.890 |
| Poyet et al[15] | 2015 | Switzerland | 174 | 69.5† | 1:50 | NR | 10% (refer to[53]) | pTa-pT1 158 pT2-pT4 16 | 266.8 | PFS | 3.360 | 1.428 | 7.904 |

CSS = cancer specific survival, DFS = disease free survival, HR = hazard ratio, LL = lower limit, NR = not report, OS = overall survival, PFS = progression free survival, RFS = recurrence free survival, UL = upper limit.

*Mean.

†Median.

**TABLE 2.** Main Characteristics of the Included Studies Estimated Prognosis of High Ki-67 Analyzed by Multivariate Analysis

| First Author | Year | Origin | No. of Patients | Age | Antibody Dilution | Blind Reading | Cut-off (Method to Decide Cut-off) | Tumor Stage | Follow-Up, months | Outcome | HR | LL | UL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Suwa et al[28] | 1997 | Japan | 75 | 63.5* | NR | NR | 32.1% (NR) | Ta-T1 30 T2-T4 45 | 164.4 | OS | 3.760 | 1.281 | 11.032 |
| Asakura et al[26] | 1997 | Japan | 104 | 63† | 1:200 | NR | 5.35% (median) | pTa-pT1 | 134 | RFS | 2.557 | 1.427 | 4.583 |
| Korkolopoulou et al[27] | 1997 | Greece | 106 | 70† | prediluted | Yes | 14% (mean) | Ta-T1 36 T2-T4 70 | 65 | RFS | 1.101 | 1.040 | 1.166 |
| Nakopoulou et al[30] | 1998 | Greece | 105 | 68† | 1:100 | Yes | 5% (NR) | Ta-T1 | 37.5† | OS | 0.850 | 0.360 | 1.990 |
| Liukkonen et al[32] | 1999 | Finland | 196 | 65.8† | 1:40 | NR | 15% (NR) | pTa-pT1 | 72 | CSS | 5.150 | 1.000 | 26.530 |
| Liukkonen et al[32] | 1999 | Finland | 196 | 65.8† | 1:40 | NR | 15% (NR) | pTa-pT1 | 72 | PFS | 5.410 | 2.080 | 14.080 |
| Pfister et al[33] | 1999 | Canada | 287 | 65.1† | 1:50 | Yes | 10% (optimal among 10%, 15%, 25%) | pTa-pT1 | 92.2 | RFS | 1.110 | 0.790 | 1.540 |
| Tomobe et al[34] | 1999 | Japan | 50 | 63.9† | 1:200 | Yes | NR | pTa-pT1 | 80 | RFS | 2.801 | 0.723 | 10.849 |
| Oosterhuis et al[35] | 2000 | Netherlands | 240 | NR | 1:250 | NR | 13% (median) | Ta-T1 | 200 | RFS | 1.500 | 1.036 | 2.172 |
| Wu et al[36] | 2000 | Taiwan | 93 | NR | 1:100 | Yes | 10.60% (median) | pTa-T1 | 94 | RFS | 2.470 | 1.100 | 5.570 |
| Nakopoulou et al[40] | 2001 | Greece | 94 | NR | NR | Yes | NR | Ta-T3 | 88 | CSS | 1.218 | 0.757 | 1.960 |
| Kamai et al[38] | 2001 | Japan | 145 | 76.1† | NR | NR | 30% (NR) | Ta-T1 86 T2-T4 59 | 124 | DFS | 2.914 | 1.435 | 5.916 |
| Korkolopoulou et al[39] | 2001 | Greece | 139 | 69.3† | prediluted | Yes | 10% (NR) | Ta-T1 47 T2-T4 92 | 192 | OS | 1.064 | 1.030 | 1.098 |
| Kamai et al[38] | 2001 | Japan | 145 | 76.1† | NR | NR | 30% (NR) | Ta-T1 86 T2-T4 59 | 124 | OS | 12.971 | 3.232 | 52.061 |
| Tomobe et al[42] | 2001 | Japan | 44 | 69.1† | NR | Yes | NR | T2-T4 | 103 | OS | 0.613 | 0.192 | 1.954 |
| Blanchet et al[37] | 2001 | France | 70 | 62.6† | 1:200 | Yes | 13% (ROC analysis) | pTa-pT1 | 111 | PFS | 4.610 | 1.628 | 13.052 |
| Kamai et al[38] | 2001 | Japan | 145 | 76.1† | NR | NR | 30% (NR) | Ta-T1 86 T2-T4 59 | 124 | RFS | 3.586 | 1.247 | 10.313 |
| Li[44] | 2002 | China | 64 | 53.2* | NR | NR | Score≥4 (NR) | Ta-T1 47 T2-T3 33 | 120 | CSS | 3.768 | 1.454 | 5.976 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 5% (quartiles) | T1 175 T2 35 | 79.95† | CSS‡ | 0.790 | 0.200 | 3.010 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 12% (quartiles) | T1 175 T2 35 | 79.95† | CSS‡ | 1.170 | 0.330 | 4.100 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 27% (quartiles) | T1 175 T2 35 | 79.95† | CSS‡ | 1.170 | 0.310 | 4.420 |
| Sgambato et al[50] | 2002 | Italy | 96 | 68† | 1:100 | Yes | 10% (refer to[33]) | Ta-T1 | 102 | DFS | 1.932 | 1.007 | 3.707 |
| Liu[45] | 2002 | China | 64 | 61.3† | NR | NR | 10% (NR) | Ta-T1 30 T2-T3 34 | 24 | DFS | 3.266 | 1.365 | 7.816 |
| Nomura et al[46] | 2002 | Japan | 54 | 69† | NR | Yes | NR | pTa-pT130 pT2-pT4 24 | 89 | OS | 1.035 | 0.996 | 1.075 |
| Li[44] | 2002 | China | 64 | 53.2* | NR | NR | Score≥4 (NR) | Ta-T1 47 T2-T3 33 | 120 | OS | 25.174 | 3.431 | 54.721 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 5% (quartiles) | T1 175 T2 35 | 79.95† | PFS‡ | 1.820 | 0.510 | 6.470 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 12% (quartiles) | T1 175 T2 35 | 79.95† | PFS‡ | 2.690 | 0.780 | 9.260 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 27% (quartiles) | T1 175 T2 35 | 79.95† | PFS‡ | 3.450 | 0.950 | 12.570 |
| Rao et al[48] | 2002 | America | 68 | NR | NR | Yes | NR | T1-T4 | NR | RFS | 1.210 | 0.580 | 2.510 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 5% (quartiles) | T1 175 T2 35 | 79.95† | RFS‡ | 1.860 | 0.990 | 3.520 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 12% (quartiles) | T1 175 T2 35 | 79.95† | RFS‡ | 0.940 | 0.460 | 1.950 |
| Rodriguez-Alonso et al[49] | 2002 | Spain | 210 | NR | 1:100 | NR | 27% (quartiles) | T1 175 T2 35 | 79.95† | RFS‡ | 1.880 | 0.880 | 4.040 |
| Santos et al[51] | 2003 | Portugal | 159 | 66* | 1:50 | NR | 18% (median) | pTa-pT1 | 123 | PFS | 6.270 | 2.570 | 15.300 |
| Santos et al[51] | 2003 | Portugal | 159 | 66* | 1:50 | NR | 18% (median) | pTa-pT1 | 123 | RFS | 2.400 | 1.500 | 4.130 |
| Matsumoto et al[56] | 2004 | Japan | 62 | 68* | NR | Yes | 21% (median) | T1 12 T2-T4 49 | 84.2 | CSS | 5.760 | 1.420 | 38.440 |
| Lopez-Beltran et al[55] | 2004 | Spain | 159 | 61† | prediluted | Yes | 13% (NR) | Ta-T1 | 120 | DFS | 5.097 | 1.801 | 14.326 |
| Lopez-Beltran et al[55] | 2004 | Spain | 159 | 61† | prediluted | Yes | 13% (NR) | Ta-T1 | 120 | OS | 3.665 | 1.074 | 12.499 |
| Mhawech et al[57] | 2004 | Switzerland | 101 | 70.3† | 1:50 | Yes | 10% (NR) | Ta-T1 71 T2 30 | 77 | PFS | 0.790 | 0.240 | 2.560 |
| Popov et al[58] | 2004 | Macedonia | 113 | 65† | 1:50 | Yes | 28% (median) | Ta-T1 62 T2-T4 51 | 136 | PFS | 9.800 | 2.800 | 34.800 |
| Su et al[52] | 2004 | Japan | 79 | 48.7† | 1:50 | NR | 18% (refer to[35,50]) | pTa-pT1 | 78 | RFS | 2.680 | 1.230 | 5.840 |
| Mahnken et al[60] | 2005 | Germany | 63 | 68* | 1:20 | Yes | 50% (median) | T1 | 120 | RFS | 1.490 | 0.680 | 3.240 |

| First Author | Year | Origin | No. of Patients | Age | Antibody Dilution | Blind Reading | Cut-off (Method to Decide Cut-off) | Tumor Stage | Follow-Up, months | Outcome | HR | LL | UL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Theodoropoulos et al[61] | 2005 | Greece | 140 | 69† | prediluted | Yes | 8.60% (median) | Ta-T1 | 131 | RFS | 2.350 | 1.140 | 4.840 |
| Kruger et al[59] | 2005 | Germany | 73 | NR | 1:20 | Yes | Continuous | T1 | NR | RFS | 1.010 | 1.000 | 1.020 |
| Kruger et al[59] | 2006 | Germany | 132 | 64* | 1:20 | Yes | 52% (median) | pT2-pT4 | 120 | CSS | 10.180 | 2.810 | 37.000 |
| Yin et al[70] | 2006 | China | 101 | NR | 1:100 | NR | 20% (NR) | Ta-T1 | 70.8 | CSS | 1.138 | 0.348 | 3.725 |
| Quintero et al[69] | 2006 | Spain | 164 | 61† | prediluted | Yes | 13% (refer to[55]) | Ta-T1 | 144 | DFS | 0.330 | 0.176 | 0.618 |
| Hu[66] | 2006 | China | 54 | 65.3† | NR | Yes | 16% (ROC analysis) | Ta-T1 37 T2-T4 17 | 83 | DFS | 2.436 | 1.775 | 3.344 |
| Galmozzi et al[62] | 2006 | Italy | 82 | NR | NR | NR | 55% (median) | pT2-pT4 | 38 | OS | 2.330 | 0.990 | 5.430 |
| Quintero et al[69] | 2006 | Spain | 164 | 61† | prediluted | Yes | 13% (refer to[55]) | Ta-T1 | 144 | OS | 0.314 | 0.085 | 0.806 |
| Yurakh et al[71] | 2006 | Spain | 84 | NR | 1:50 | NR | 50% (NR) | pTa-pT1 63 pT2-pT4 21 | 49.2 | OS | 1.030 | 1.000 | 1.070 |
| Gonzalez-Campora et al[63] | 2006 | Spain | 147 | 66† | 1:20 | Yes | 10% (preliminary teaching set) | Ta-T1 | 120 | PFS | 1.033 | 1.001 | 1.067 |
| Quintero et al[69] | 2006 | Spain | 164 | 61† | Prediluted | Yes | 13% (refer to[55]) | Ta-T1 | 144 | PFS | 0.296 | 0.109 | 0.799 |
| Yin et al[70] | 2006 | China | 101 | NR | 1:100 | NR | 20% (NR) | Ta-T1 | 70.8 | PFS | 1.236 | 0.434 | 3.520 |
| Shariat et al[72] | 2009 | Canada | 80 | 64.6* | 1:500 | NR | 20% (NR) | pT1 | 195.4 | CSS | 6.230 | 1.580 | 24.480 |
| Margulis et al[13] | 2009 | America | 713 | 67.5† | NR | NR | 20% (optimal) | pT1 89 pT2-pT4 624 | 236 | CSS | 1.710 | 1.330 | 2.210 |
| Shariat et al[72] | 2009 | Canada | 80 | 64.6* | 1:500 | NR | 20% (NR) | pT1 | 195.4 | RFS | 3.960 | 1.230 | 12.790 |
| Margulis et al[13] | 2009 | America | 713 | 67.5† | NR | NR | 20% (optimal) | pT1 89 pT2-pT4 624 | 236 | RFS | 2.370 | 1.720 | 3.250 |
| van Rhijn et al[76] | 2010 | Netherlands | 230 | NR | NR | NR | 25% (refer to[53]) | pTa-pT1 | 141.6 | CSS | 2.010 | 0.660 | 4.810 |
| van Rhijn et al[76] | 2010 | Netherlands | 230 | NR | NR | NR | 25% (refer to[53]) | pTa-pT1 | 141.6 | PFS | 1.350 | 0.620 | 4.850 |
| Miyake et al[74] | 2010 | Japan | 109 | 68.5† | NR | Yes | 25% (refer to[53]) | pTis-pT1 | 99 | RFS | 2.510 | 1.310 | 4.780 |
| Seo et al[75] | 2010 | Korea | 129 | 64.2* | 1:50 | NR | 20% (refer to[33,47,54]) | Ta-T1 | 96 | RFS | 3.400 | 1.040 | 11.050 |
| van Rhijn et al[76] | 2010 | Netherlands | 230 | NR | NR | NR | 25% (refer to[53]) | pTa-pT1 | 141.6 | RFS | 1.230 | 0.430 | 3.570 |
| Yan et al[77] | 2011 | China | 82 | 68.3† | 1:100 | NR | 30% (NR) | Ta-T1 48 T2-T4 34 | 184 | OS | 1.257 | 0.334 | 4.734 |
| Bertz et al[92] | 2012 | Germany | 261 | NR | 1:50 | Yes | 15% (NR) | pT1 | 172 | CSS | 3.830 | 1.590 | 9.260 |
| Shan et al[86] | 2012 | China | 96 | 55† | NR | NR | 41% (median) | pTa-pT1 64 pT2-pT4 32 | 76 | OS | 0.770 | 0.360 | 1.610 |
| Shan et al[86] | 2012 | China | 96 | 55† | NR | NR | 41% (median) | pTa-pT1 64 pT2-pT4 32 | 76 | PFS | 0.570 | 0.220 | 1.490 |
| Bertz et al[92] | 2012 | Germany | 261 | NR | 1:50 | Yes | 15% (NR) | pT1 | 172 | PFS | 2.800 | 1.450 | 5.430 |
| Chen et al[84] | 2012 | China | 72 | 61.3† | 1:50 | Yes | 25% (NR) | Ta-T1 | 93 | PFS | 12.182 | 1.527 | 97.151 |
| Bi et al[83] | 2012 | China | 111 | 66* | 1:400 | Yes | 5% (NR) | <pT1 53 ≥pT1 58 | 26 | RFS | 1.405 | 0.976 | 2.023 |
| Shan et al[86] | 2012 | China | 96 | 55† | NR | NR | 41% (median) | pTa-pT1 64 pT2-pT4 32 | 76 | RFS | 0.930 | 0.410 | 2.110 |
| Bertz et al[92] | 2012 | Germany | 261 | NR | 1:50 | Yes | 15% (NR) | pT1 | 172 | RFS | 1.750 | 0.980 | 3.150 |
| Chen et al[84] | 2012 | China | 72 | 61.3† | 1:50 | Yes | 25% (NR) | Ta-T1 | 93 | RFS | 2.021 | 1.018 | 4.010 |
| Ruan et al[91] | 2012 | China | 126 | 64.52† | 1:50 | NR | 10% (NR) | T1 | 60 | RFS | 0.690 | 0.321 | 1.484 |
| Acikalin et al[82] | 2012 | Turkey | 68 | 63† | 1:50 | Yes | 10% (NR) | T1 | 132 | RFS | 2.059 | 0.759 | 5.585 |
| Acikalin et al[82] | 2012 | Turkey | 68 | 63† | 1:50 | Yes | 10% (NR) | T1 | 132 | PFS | 1.883 | 0.391 | 9.064 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2† | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | CSS | 1.980 | 0.560 | 6.930 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2† | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | OS | 1.720 | 0.890 | 3.310 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2† | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | PFS | 1.470 | 0.330 | 6.490 |
| Park et al[90] | 2013 | Korea | 61 | 66* | 1:200 | Yes | 10.40% (median) | T1G3 | 217 | PFS | 0.421 | 0.084 | 2.118 |
| Gontero et al[93] | 2013 | Italy | 131 | 66.37† | NR | NR | 20% (median) | T1 | 71.6* | PFS | 5.250 | 1.880 | 14.670 |
| Gontero et al[93] | 2013 | Italy | 131 | 66.37† | NR | NR | 20% (median) | T≥2 | 71.6* | PFS | 6.170 | 1.150 | 33.100 |
| Oderda et al[88] | 2013 | Italy | 192 | 73.2† | 1:10 | Yes | 20% (median) | Ta-T1 | 229 | RFS | 1.170 | 0.740 | 1.870 |
| Park et al[90] | 2013 | Korea | 61 | 66* | 1:200 | Yes | 10.40% (median) | T1G3 | 217 | RFS | 0.743 | 0.295 | 1.871 |
| Gontero et al[93] | 2013 | Italy | 131 | 66.37† | NR | NR | 20% (median) | NR | 71.6* | RFS | 1.010 | 0.500 | 2.060 |

| First Author | Year | Origin | No. of Patients | Age | Antibody Dilution | Blind Reading | Cut-off (Method to Decide Cut-off) | Tumor Stage | Follow-Up, months | Outcome | HR | LL | UL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wang et al[14] | 2014 | America | 588 | 65* | 1:500 | NR | 20% (NR) | pTa-pT1 93 pT2-pT4 495 | NR | CSS | 1.500 | 1.200 | 2.100 |
| Ding et al[10] | 2014 | China | 332 | 67* | NR | NR | 25% (refer to[53]) | Ta-T1 | 124 | PFS | 2.970 | 1.420 | 6.220 |
| Wang et al[94] | 2014 | China | 268 | 65.71† | 1:100 | NR | 20% (NR) | Ta-T1 | 46 | RFS | 1.756 | 0.930 | 3.318 |
| Ding et al[10] | 2014 | China | 332 | 67* | NR | NR | 25% (refer to[53]) | Ta-T1 | 124 | RFS | 2.140 | 1.450 | 3.160 |
| Wang et al[14] | 2014 | America | 588 | 65* | 1:500 | NR | 20% (NR) | pTa-pT1 93 pT2-pT4 495 | NR | RFS | 1.600 | 1.200 | 2.000 |
| Tanabe et al[9] | 2015 | Japan | 94 | 69* | 1:200 | Yes | 20% (optimal) | T2-T4 | 171 | CSS | 0.303 | 0.122 | 0.752 |
| Makboul et al[95] | 2015 | Germany | 104 | NR | 1:200 | NR | 20% (refer to[12]) | Ta-T1 21 T2-T4 83 | 12 | PFS | 1.075 | 1.040 | 1.111 |
| Poyet et al[15] | 2015 | Switzerland | 174 | 69.5 | 1:50 | NR | 10% (refer to[53]) | pTa-pT1 158 pT2-pT4 16 | 266.8 | PFS | 1.760 | 0.627 | 4.941 |
| Makboul et al[95] | 2015 | Germany | 104 | NR | 1:200 | NR | 20% (refer to[12]) | Ta-T1 21 T2-T4 83 | 12 | RFS | 1.008 | 0.986 | 1.030 |
| Ozyalvacli et al[96] | 2015 | Turkey | 90 | NR | NR | NR | 10% (NR) | pTa-pT1 | 32.8† | RFS | 1.790 | 1.010 | 33.560 |

CSS = cancer specific survival, DFS = disease free survival, HR = hazard ratio, LL = lower limit, NR = not report, OS = overall survival, PFS = progression free survival, RFS = recurrence free survival, UL = upper limit.
*Median.
†Mean.
‡The same patients sources in different cut-off values.

1.813–4.153, $P < 0.001$), PFS (HR = 1.475, 95%CI: 1.261–1.726, $P < 0.001$), and RFS (HR = 1.201, 95%CI: 1.120–1.289, $P < 0.001$) (Table 4). In terms of both low and high cut-off values to assess high Ki-67 reactivity, subgroup analysis uncovered the significant correlation of high Ki-67 reactivity with worse DFS, PFS, and RFS ($P < 0.05$, Table 4). For CSS and OS, no significant correlation was observed between Ki-67 reactivity and clinical outcomes when the cut-off value was <20% (HR = 2.097, 95%CI: 0.889–4.950, $P = 0.091$; HR = 1.576, 95%CI: 0.813–3.056, $P = 0.178$). However, an increased cut-off value (≥20%) would lead to statistically significant results (HR = 1.767, 95%CI: 1.154–2.708, $P = 0.009$; HR = 1.801, 95%CI: 1.053–3.097, $P = 0.032$) (Table 4).

## Sensitivity Analysis and Publication Bias

Sensitivity analysis was performed to examine the stability of the current meta-analysis by removing 1 study each time and later pooling the rest. The result of sensitivity analysis indicated that the study published by Frank et al[54] in CSS and Kruger et al[59] in PFS of univariate analysis were not stable and significantly influenced the pooled HR (Additional file 1: Figures S1–S2, http://links.lww.com/MD/A894). After excluding the study by Frank et al[54] and Kruger et al,[59] the meta-analyses of the remained studies were stable (Additional file 1: Figures S3–S4, http://links.lww.com/MD/A894). The pooled HRs (random effect model) for CSS of univariate analysis changed from 2.588 (95%CI: 1.623–4.127, $P < 0.001$) to 2.798 (95%CI: 1.783–4.391, $P < 0.001$), and the pooled HR (random effect model) for PFS of univariate analysis altered from 3.084 (95%CI: 1.962–4.846, $P < 0.001$) to 3.331 (95%CI: 2.068–5.365, $P < 0.001$). For the others meta-analyses, the combined HRs were similar after the exclusion of studies, with the stability of meta-analyses confirmed.

Begg test and funnel plot were conducted to estimate the publication bias of the included studies. For univariate analysis, the results of Begg test and the shape of funnel plot presented us with no significant publication bias for CSS ($P = 0.857$), OS ($P = 0.100$), PFS ($P = 0.227$), and RFS ($P = 0.771$) (Table 3, Figure 4A–D). As to multivariate analysis, no evidence of publication bias for meta-analyses ($P > 0.05$) was observed except for OS ($P = 0.033$) (Table 3, Figure 5A–D). After refilling ''missing'' studies by trim and fill method, the adjusted pooled HR was not significant for OS (HR = 1.058, 95%CI: 0.940–1.191, $P = 0.347$) (Additional file 1: Figure S5, http://links.lww.com/MD/A894).

## Systematic Review

According to the criteria of our meta-analysis, some studies, which investigated the relationship between Ki-67 reactivity and survival status of BC patients, were excluded for failing to estimate HRs and its 95% CIs. The main information of 23 excluded studies[97–119] was listed in Table S2 (Additional file 2, http://links.lww.com/MD/A894), including 5 studies for CSS, 5 for DFS, 9 for OS, 9 for PFS, and 10 for RFS. For all 5 survival outcome (CSS, DFS, OS, RFS, and PFS), it can be concluded from 5 studies[97,101,109–111] that Ki-67 is an independent indicator to predict prognosis by multivariate analysis, while 7 studies[104,105,110,112,113,116,117] reported negative results.

# DISCUSSION

Ki-67 is among the well-established proliferation biomarkers, which can be detected by monoclonal antibodies of Ki-67 with IHC.[120] Recently, proliferation biomarkers have

**TABLE 3.** Meta-Analysis of Single HR Evaluating the Prognostic Function of Ki-67 Reactivity in BC Patients

| Groups | No. of Studies | Pooled HR | 95%CI | *P* | Heterogeneity Test | | | Statistical Method | Publication Bias (*P*) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Q* | *P* | $I^2$, % | | |
| Univariate | | | | | | | | | |
| CSS | 16 | 2.588 | 1.623–4.127 | <0.001 | 252.04 | <0.001 | 94.0 | Random | 0.857 |
| DFS | 5 | 2.697 | 1.874–3.883 | <0.001 | 8.04 | 0.090 | 50.2 | Random | – |
| OS | 12 | 2.649 | 1.632–4.300 | <0.001 | 55.43 | <0.001 | 80.2 | Random | 0.100 |
| PFS | 21 | 3.506 | 2.231–5.508 | <0.001 | 203.86 | <0.001 | 90.2 | Random | 0.227 |
| RFS | 23 | 1.792 | 1.409–2.279 | <0.001 | 155.38 | <0.001 | 85.8 | Random | 0.771 |
| Multivariate | | | | | | | | | |
| CSS | 18 | 1.868 | 1.343–2.597 | <0.001 | 41.77 | <0.001 | 64.1 | Random | 0.322 |
| DFS | 6 | 2.626 | 2.089–3.301 | <0.001 | 3.16 | 0.675 | 0.0 | Random | – |
| OS | 14 | 1.104 | 1.008–1.209 | 0.032 | 56.95 | <0.001 | 77.2 | Random | 0.033 |
| PFS | 23 | 1.518 | 1.299–1.773 | <0.001 | 100.22 | <0.001 | 78.2 | Random | 0.509 |
| RFS | 35 | 1.294 | 1.203–1.392 | <0.001 | 152.70 | <0.001 | 77.7 | Random | 0.206 |

BC = bladder carcinoma, CI = confidence interval, CSS = cancer specific survival, DFS = disease free survival, HR = hazard ratio, LL = lower limit, OS = overall survival, PFS = progression free survival, RFS = recurrence free survival, UL = upper limit.

attracted increasing attention in malignant neoplasms as prognostic indicators. A meta-analysis has demonstrated that Ki-67, MI, proliferating cell nuclear antigen, and thymidine or bromodeoxyuridine labeling index (LI) are significantly associated with worse survival outcomes in early-staged breast cancer.[121] Significant correlations of proliferating cell nuclear antigen with poor outcomes were also discovered in BC.[27,39,122] It is also reported that minichromosome maintenance 2, a prerequisite protein for initiation and elongation of DNA, is an independent prognostic factor for OS in gastric cancer and nonsmall-cell lung cancer.[123,124] In addition, several meta-analyses claimed that Ki-67 exerts its vital influence on the prognosis of hepatocellular carcinoma, breast cancer, lung cancer, upper urinary-tract urothelial carcinomas (UTUC), cervical cancer, gliomas, and lymphoma.[125–133] However, conflicting conclusions exist as to the prognostic role of Ki-67 reactivity in BC patients. Considering that a single study might seem unconvincing, we performed the current meta-analysis by combining the results from multiple studies to reach a reasonable conclusion.

The prognostic value of Ki-67 reactivity for tumors in urinary system has been reported in previous meta-analysis published by Lei et al,[134] which demonstrated that high Ki-67 reactivity displays significantly higher cancer-specific mortality and shorter 5-year DFS and OS in UTUC. Compared with the previous meta-analysis for UTUC,[134] our meta-analysis highlighted a larger cohort of patients and a more comprehensive report for clinical outcomes. Our results have drawn a similar conclusion that high Ki-67 reactivity seems to be an unexceptionable biomarker to predict an unfavorable prognosis for patients with urinary system tumors. The results of univariate analysis strongly supported that Ki-67 reactivity, assessed by IHC, is significantly associated with worse CSS (HR = 2.588, 95%CI: 1.623–4.127), DFS (HR = 2.697, 95%CI: 1.874–3.883), OS (HR = 2.649, 95%CI: 1.632–4.300), PFS (HR = 3.506, 95%CI: 2.231–5.508), and RFS (HR = 1.792, 95%CI: 1.409–2.279). After adjusting the effect of sex, age, tumor stage, and grade as well as other biological variables by multivariate analysis, the pooled HRs were slightly declined but still significant for CSS 1.868 (95%CI: 1.343–2.597), DFS (HR = 2.626, 95%CI: 2.089–3.301), PFS (HR = 1.518, 95%CI: 1.299–1.773), and RFS (HR = 1.294, 95%CI: 1.203–1.392). For multivariate analysis of OS, the pooled HR was of inferior significance with a narrower 95% CI (HR = 1.104, 95%CI: 1.008–1.209), but no significant results were observed after refilling 5 missed studies estimated by trim and fill method (HR = 1.058, 95%CI: 0.940–1.191).

Between-study heterogeneity may be a potential factor which influenced the pooled results, and significant heterogeneity was observed in the major meta-analyses. Although we have established rigorous inclusion criteria, such as that all the included studies should detect Ki-67 reactivity by IHC, there were still a few substantial differences among included studies, which might influence the pooled results. First, sufficient follow-up period is one of the vital factors for a prospective study, but not all the included studies were strictly designed to suit the condition. A case in point would be that 1 study included subjected to a follow-up period of merely 12 months.[95] Second, the tumor staging systems adopted for each study were also different, which might swing the clinical outcomes, since patients with more advanced stages are more vulnerable to unfavorable prognosis. Third, the most accurate HR should be calculated by multivariate analysis with original data. In the current meta-analysis, however, some studies failed to provide results of multivariate analysis. Hence, HR values would need to be extracted the from survival curves whenever possible, which is less reliable than data directly provided in the articles. Fourth, the differences in adjusting concomitant variables of each study might also contribute to heterogeneity, though we have pooled the multivariate HRs. Apart from the circumstances mentioned above, there might be other factors, such as scales of the cohort, ages of patients, dilution folds of antibody, and treatment methods, which would contribute to heterogeneity, so a random-effect model was employed to combine the HR values to take all the between-study heterogeneity into consideration.

Subgroup analysis by origin showed that Ki-67 reactivity significantly correlated with unfavorable clinical outcomes in Asian and European-American patients in the univariate analysis. However, after adjusting concomitant variables, such as sex, age, tumor stage, and so on, different impacts of Ki-67

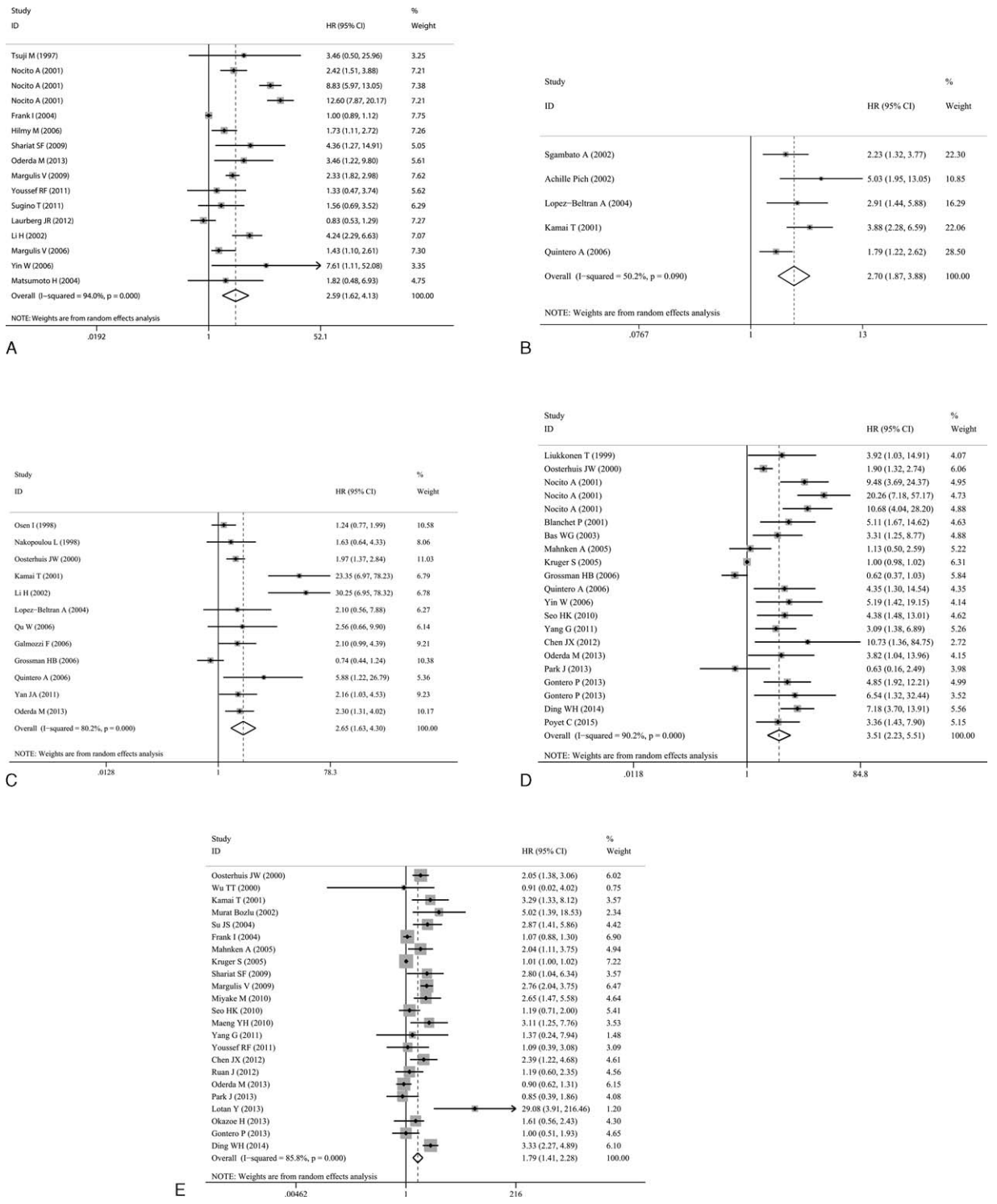


**FIGURE 2.** Meta-analyses of studies estimating the correlation of Ki-67 reactivity with survival status by univariate analysis. (A) CSS, (B) DFS, (C) OS, (D) PFS, and (E) RFS. CSS = cancer-specific survival, DFS = disease-free survival, OS = overall survival, PFS = progression-free survival, RFS = recurrence-free survival.

reactivity on clinical outcomes emerged in BC patients of different races. This meta-analysis demonstrated that the impacts of Ki-67 reactivity on survival status vary considerably among Asian patients and European-American patients, which implies that Ki-67 reactivity might exert different effects on tumor variations in patients with diverse ethnic backgrounds. Further perspective researches among different ethnicities would be required to confirm the aforementioned hypothesis.

**TABLE 4.** Subgroup Analysis Evaluating the Prognostic Function of Ki-67 Reactivity in BC Patients

| Groups | | No. of Studies | Pooled HR | 95%CI | *P* | Heterogeneity Test | | | Statistical Method |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *Q* | *P* | $I^2$, % | |
| Univariate | | | | | | | | | |
| CSS | Origin | | | | | | | | |
| | Asian | 5 | 2.930 | 1.687–5.089 | <0.001 | 5.52 | 0.238 | 27.5 | Random |
| | Non-Asian | 11 | 2.482 | 1.433–4.299 | 0.001 | 234.14 | <0.001 | 95.7 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 3 | 6.476 | 2.541–16.504 | <0.001 | 26.72 | <0.001 | 92.5 | Random |
| | ≥20% | 10 | 1.843 | 1.260–2.697 | 0.002 | 23.44 | 0.005 | 61.6 | Random |
| DFS | Origin | | | | | | | | |
| | Asian | 1 | 3.867 | 2.280–6.590 | | | | | |
| | Non-Asian | 4 | 2.373 | 1.649–3.416 | <0.001 | 4.66 | 0.199 | 35.6 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 4 | 2.373 | 1.649–3.416 | <0.001 | 4.66 | 0.199 | 35.6 | Random |
| | ≥20% | 1 | 3.867 | 2.280–6.590 | | | | | |
| OS | Origin | | | | | | | | |
| | Asian | 4 | 7.691 | 1.832–32.294 | 0.005 | 20.46 | <0.001 | 85.3 | Random |
| | Non-Asian | 8 | 1.653 | 1.161–2.353 | 0.005 | 15.87 | 0.026 | 55.9 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 5 | 2.056 | 1.501–2.816 | <0.001 | 2.15 | 0.707 | 0.0 | Random |
| | ≥20% | 6 | 2.155 | 1.112–4.731 | 0.023 | 31.26 | <0.001 | 84.0 | Random |
| PFS | Origin | | | | | | | | |
| | Asian | 6 | 3.868 | 1.983–7.543 | <0.001 | 11.27 | 0.046 | 55.6 | Random |
| | Non-Asian | 15 | 3.377 | 2.040–5.589 | <0.001 | 144.12 | <0.001 | 90.3 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 10 | 4.344 | 2.437–7.742 | <0.001 | 37.33 | <0.001 | 75.9 | Random |
| | ≥20% | 10 | 3.366 | 1.638–6.914 | 0.001 | 49.65 | <0.001 | 81.9 | Random |
| RFS | Origin | | | | | | | | |
| | Asian | 13 | 2.065 | 1.526–2.793 | <0.001 | 23.85 | 0.021 | 49.1 | Random |
| | Non-Asian | 10 | 1.528 | 1.134–2.060 | <0.001 | 75.29 | <0.001 | 88.0 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 8 | 1.782 | 1.135–2.796 | 0.012 | 14.86 | 0.038 | 52.9 | Random |
| | ≥20% | 13 | 2.047 | 1.488–2.816 | <0.001 | 43.46 | <0.001 | 72.4 | Random |
| Multivariate | | | | | | | | | |
| CSS | Origin | | | | | | | | |
| | Asian | 4 | 1.571 | 0.396–6.237 | <0.001 | 21.14 | <0.001 | 85.8 | Random |
| | Non-Asian | 12 | 1.885 | 1.403–2.532 | 0.521 | 20.56 | 0.038 | 46.5 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 4 | 2.097 | 0.889–4.950 | 0.091 | 5.711 | 0.127 | 47.4 | Random |
| | ≥20% | 10 | 1.767 | 1.154–2.708 | 0.009 | 28.17 | 0.001 | 68.0 | Random |
| DFS | Origin | | | | | | | | |
| | Asian | 3 | 2.576 | 1.958–3.390 | <0.001 | 0.52 | 0.771 | 0.0 | Random |
| | Non-Asian | 3 | 2.795 | 1.728–4.521 | <0.001 | 2.58 | 0.275 | 22.4 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 5 | 2.595 | 2.037–3.304 | <0.001 | 3.07 | 0.546 | 0.0 | Random |
| | ≥20% | 1 | 2.914 | 1.435–5.917 | | | | | |
| OS | Origin | | | | | | | | |
| | Asian | 7 | 2.265 | 1.012–5.073 | 0.047 | 40.04 | <0.001 | 85.0 | Random |
| | Non-Asian | 7 | 1.071 | 0.991–1.158 | 0.084 | 16.72 | 0.010 | 64.1 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 4 | 1.576 | 0.813–3.056 | 0.178 | 9.12 | 0.028 | 67.1 | Random |
| | ≥20% | 7 | 1.801 | 1.053–3.097 | 0.032 | 24.78 | <0.001 | 75.8 | Random |
| PFS | Origin | | | | | | | | |
| | Asian | 6 | 1.489 | 0.657–3.375 | 0.34 | 13.71 | 0.018 | 63.5 | Random |
| | Non-Asian | 17 | 1.475 | 1.261–1.726 | <0.001 | 83.98 | <0.001 | 80.9 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 12 | 2.213 | 1.316–3.722 | 0.003 | 55.03 | <0.001 | 80.0 | Random |
| | ≥20% | 11 | 2.315 | 1.336–4.013 | 0.003 | 42.73 | <0.001 | 76.6 | Random |
| RFS | Origin | | | | | | | | |
| | Asian | 16 | 1.832 | 1.469–2.285 | <0.001 | 21.91 | 0.110 | 31.5 | Random |
| | Non-Asian | 19 | 1.159 | 1.086–1.238 | <0.001 | 85.64 | <0.001 | 79.0 | Random |
| | Cut-off | | | | | | | | |
| | <20% | 16 | 1.516 | 1.239–1.856 | <0.001 | 41.04 | <0.001 | 63.5 | Random |
| | ≥20% | 16 | 1.705 | 1.310–2.219 | <0.001 | 86.42 | <0.001 | 82.6 | Random |

BC = bladder carcinoma, CI = confidence interval, CSS = cancer specific survival, DFS = disease free survival, HR = hazard ratio, LL = lower limit, OS = overall survival, PFS = progression free survival, RFS = recurrence free survival, UL = upper limit.

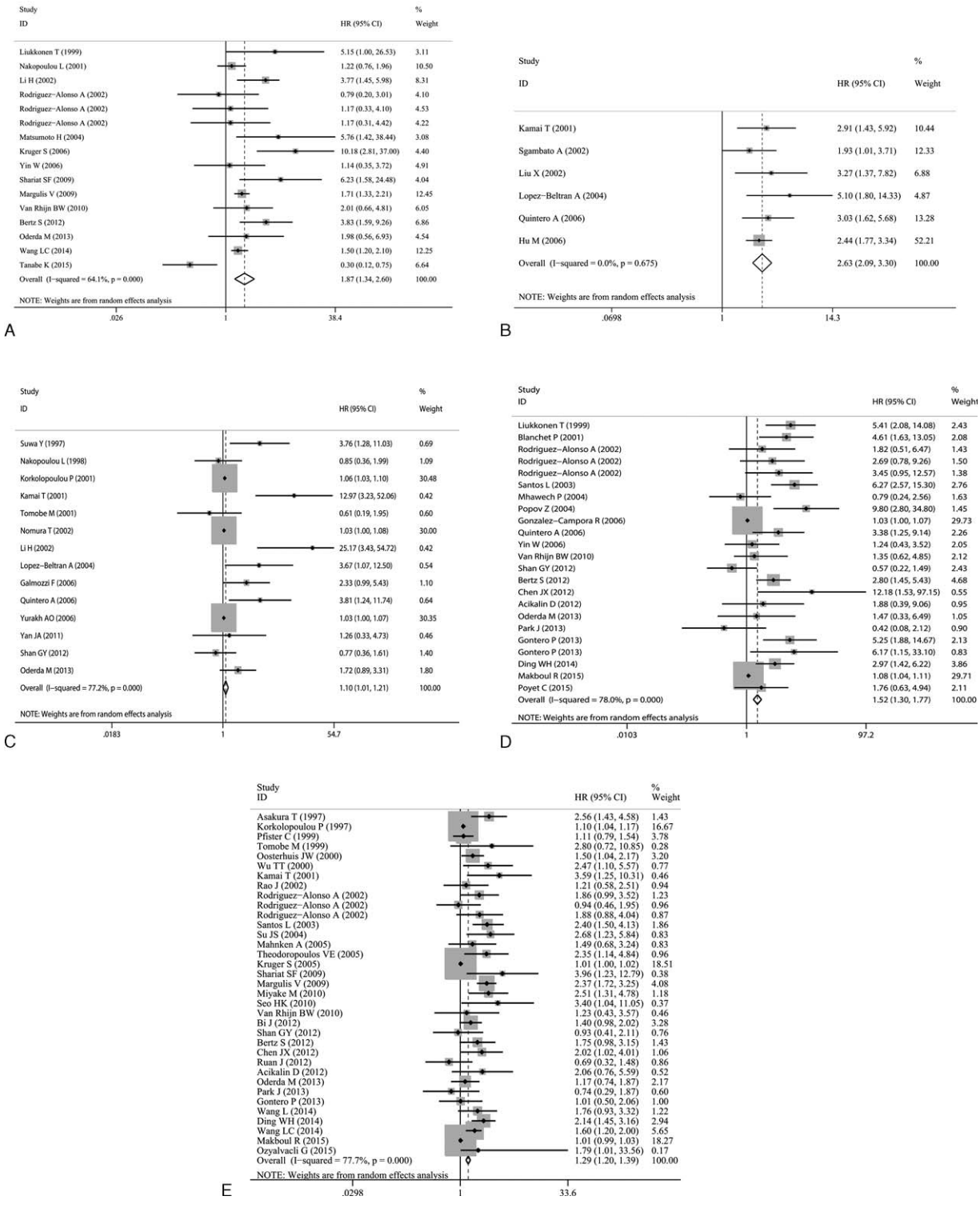


**FIGURE 3.** Meta-analyses of studies estimating the correlation of Ki-67 reactivity with survival status by multivariate analysis. (A) CSS, (B) DFS, (C) OS, (D) PFS, and (E) RFS. CSS = cancer-specific survival, DFS = disease-free survival, OS = overall survival, PFS = progression-free survival, RFS = recurrence-free survival.

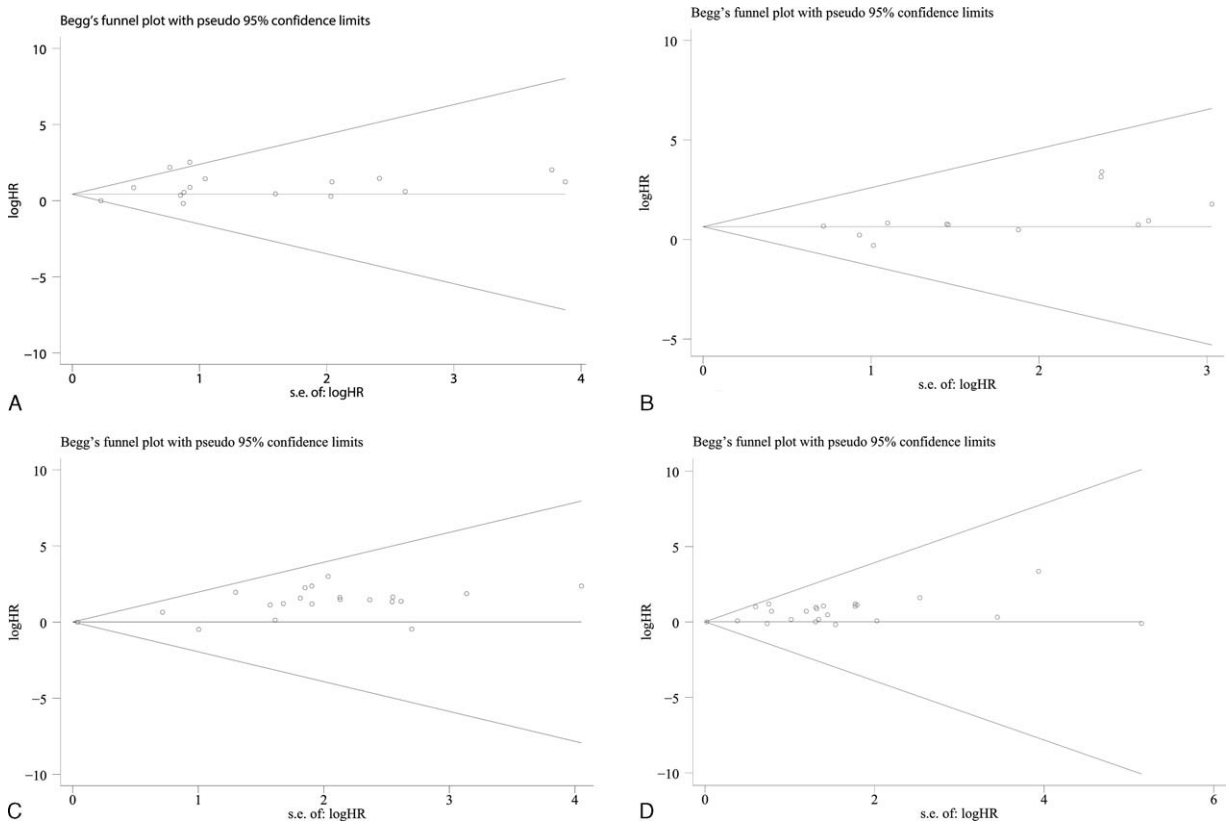


**FIGURE 4.** Funnel plots were applied to show potential publication bias in studies estimating the correlation of Ki-67 reactivity with survival status by univariate analysis. (A) CSS, (B) OS, (C) PFS, and (D) RFS. CSS = cancer-specific survival, OS = overall survival, PFS = progression-free survival, RFS = recurrence-free survival.

The cut-off values to define ''high'' Ki-67 reactivity also differed slightly among studies because of the lack of unified standards, which might potentially contribute to heterogeneity. Apart from this, using optimal cut-points might lead to discrepancy and influence the accurate HRs.[135] In the current meta-analysis, we set a threshold at 20% to define low or high cut-off values according to 2 large sample studies published by Margulis et al and Wang et al, respectively.[13,14] In the subgroup of low cut-off value (<20%), the meta-analysis identified that high Ki-67 reactivity significantly correlated with worsened CSS, DFS, OS, PFS, and RFS in univariate analysis and was associated with shorter CSS, DFS, PFS, and RFS in multivariate analysis. As to the subgroup analysis of high cut-off value (≥20%), it was well indicated that high Ki-67 reactivity, regardless of univariate or multivariate analysis, was significantly related to all 5 clinical outcomes in BC patients. Therefore, according to the results of our meta-analysis, a higher cut-off value (≥20%) might be more appropriate and a well-established, orthodox threshold would be required to define high Ki-67 reactivity.

Publication bias is also a potential factor which influenced the pooled results. To minimize publication bias, we have conducted a comprehensive search and screening in different databases in English and Chinese. The conflicting conclusions of different literature may impel investigators to publish their research data, whether positive or negative, which will partially limit publication bias. However, publication bias was still detected in the studies of multivariate analysis for DFS and OS. The fact that not all relevant reports were retrieved might attribute to the mentioned bias, since quite a few studies were only included in systematic review due to the lack of proper data to estimate HR. Hence, in order to make results more creditable and reliable, trim and fill method was used for ''missing'' studies retrieving to minimize publication bias.

The meta-analysis featured several strengths, which are of great significance to clinical practices. First, we have conducted a comprehensive meta-analysis with a relatively large cohort size included (n = 13,053 patients). Second, results of univariate and multivariate analyses were carefully studied and similar results of associations between Ki-67 reactivity and clinical outcomes were observed, which enhanced the reliability of our meta-analysis. Moreover, we have investigated 5 clinical outcomes of tumor prognosis in multiple dimensions, including CSS, DFS, OS, PFS, and RFS, and the results strongly indicated that high Ki-67 reactivity is significantly associated with poor clinical outcomes. Therefore, it is of primal importance to secure patients' access to personalized treatments in accordance with their individual conditions. A case in point would be for high-risk patients, especially those with high Ki-67 expression, to receive postoperative chemo-/radiotherapy. Meanwhile, the meta-analysis has a few limitations. To begin with, heterogeneity and publication bias still existed in the meta-analysis, which unfavorably influenced the results. Besides, the estimated HRs were extracted from survival curves when the accurate data

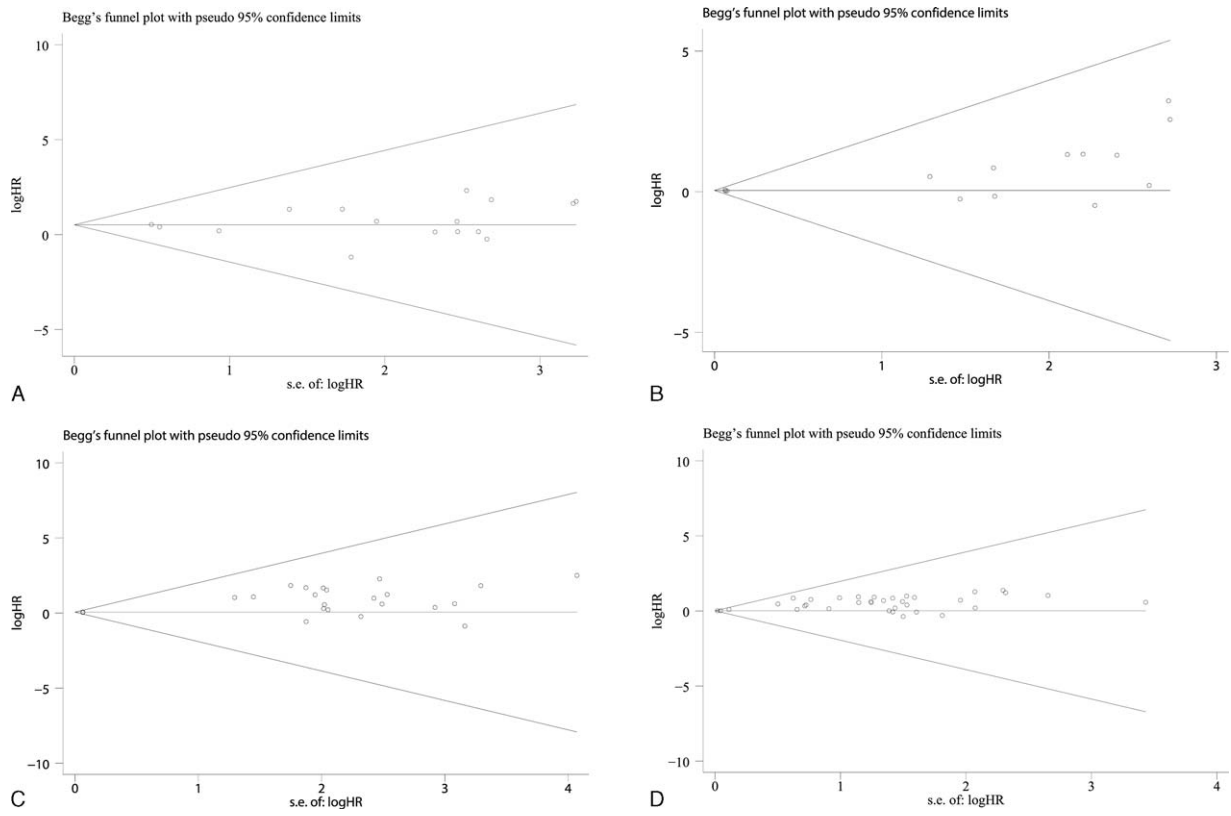


**FIGURE 5.** Funnel plots were applied to show potential publication bias in studies estimating the correlation of Ki-67 reactivity with survival status by multivariate analysis. (A) CSS, (B) OS, (C) PFS, and (D) RFS. CSS = cancer-specific survival, OS = overall survival, PFS = progression-free survival, RFS = recurrence-free survival.

were not immediately available in articles, which could be easily influenced by investigators despite the same methods applied.

Enlightened by the data extracted and results of the meta-analysis, we would like to kindly provide forthcoming researchers with the following recommendations: provide the full information of patients, such as age, tumor stage, and so on; use blind-reading and a well-received common cut-off value to assess the results of IHC; set aside a more sufficient follow-up period to allow for long-range outcomes; provide the results of univariate and multivariate analysis whether they are positive or not; and design the studies as prospective ones in the setting.

To conclude, it is demonstrated, by the current comprehensive meta-analysis on 82 studies with 13,053 patients involved, that high Ki-67 reactivity is significantly associated with deteriorated clinical outcomes, and that Ki-67 is an independent indicator for the prognosis of BC patients. Prospective studies with a large cohort of patients are demanded to further strengthen our findings on the correlation between Ki-67 and prognosis in BC patients.

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