ORIGINAL RESEARCH

Prognostic and clinicopathological value of Ki-67/MIB-1 expression in renal cell carcinoma: a meta-analysis based on 4579 individuals

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Background: Previous studies have investigated the prognostic significance of Ki-67/MIB-1 expression in renal cell carcinoma (RCC), however, the reports are controversial and inconsistent. This study aimed to investigate Ki-67/MIB-1 expression in RCC and its correlation with prognosis and clinicopathological features.

Methods: We searched relevant studies that reported associations between Ki-67/MIB-1 expression and prognosis in RCC from PubMed, Embase, Web of Science, and Cochrane Library studies published until April 14, 2017. Hazard ratios (HRs) and 95% confidence intervals (CIs) were extracted from eligible studies. Fixed and random effects models were used to calculate pooled HRs and 95% CIs according to heterogeneity.

Results: A total of 4579 participants from 23 eligible studies were included in this analysis. The results showed that Ki-67/MIB-1 expression was associated with poor overall survival (HR=2.06, 95% CI: 1.64–2.57) and cancer specific survival (HR=2.01, 95% CI: 1.66–2.44). In addition, Ki-67/MIB-1 expression was also correlated with TNM stage (III/IV vs I/II: OR=1.92, 95% CI: 1.61–2.28), pathological T stage (pT3/pT4 vs pT1/pT2: OR=1.56, 95% CI: 1.21–2.02), distant metastasis (M1 vs M0: OR=1.81, 95% CI: 1.34–2.43), and Fuhrman grade (III/IV vs I/II: OR=1.94, 95% CI: 1.21–3.10).

Conclusion: Our study demonstrates that the presence of high Ki-67/MIB-1 expression and advanced clinicopathological features were correlated with poor prognosis in RCC patients.

Keywords: Ki-67/MIB-1, renal cell carcinoma, prognosis, meta-analysis

Introduction

Renal cell carcinoma (RCC) ranks the seventh most prevalent cancer type in men and ninth in women[1]. Each year, about three hundred thousand cases of RCC are diagnosed, and about 134 thousand deaths are reported worldwide.[2,3] There are multiple treatment methods that could be applied to treat localized RCC; surgery treatment is the most effective, followed by chemotherapy and radiotherapy. Patients with RCC at an early stage may receive complete surgical resection to achieve the purpose of cure; about half of the patients experience disease recurrence after curative resection, and about 30% of RCC patients have metastases at the time of the initial diagnosis.[4] Metastatic RCC is a treatment-resistant malignant tumor, which is usually treated with targeted drugs or immunosuppressive points for systemic therapy;[5] however, it has limited effect. Therefore, reliable prognostic biomarkers are needed to distinguish high-risk patients with RCC and improve clinical outcomes of RCC.

MIB-1, also known as Ki-67, is a marker for cell proliferation and tumor growth, which is present during all active phases of the cell cycle, ie, G1, S, G2, and mitosis,

but is absent in resting cells (G0 phase).[6] High Ki-67/MIB-1 expression is often correlated with the clinical course of the disease, and its coexpression with other well-known markers of proliferation indicates a pivotal role in cell division. It is reported that Ki-67/MIB-1 expression predicts poor prognosis in various multiple solid tumor types, including breast cancer,[7] prostate cancer,[8] cervical cancer,[9] gliomas,[10] and hepatocellular carcinoma.[11] Many studies have reported the prognostic value of p53 expression in RCC, but the results were conflicting.[12–34] Therefore, it is necessary to conduct a comprehensive meta-analysis to evaluate the prognostic and clinicopathological value of Ki-67/MIB-1 expression in patients with RCC.

We retrieved relevant literature and extracted data from eligible studies to perform a meta-analysis. We aim to reveal the association between Ki-67/MIB-1 expression and prognosis and clinicopathological features in patients with RCC.

Materials and methods
Search strategy
We did this meta-analysis using a predefined protocol in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[35] We searched PubMed, Embase, Web of Science, and the Cochrane electronic databases for studies published before April 14, 2017. The keywords were searched as follows: "renal cell carcinoma" or "renal cell cancer" or "renal cell adenocarcinoma" or "kidney tumor" and "Ki-67" or "MIB-1" and "prognosis" or "survival" or "outcome" in humans, and the language of publications was restricted to English.

Two reviewers (ZW and HX) independently screened the titles and abstracts of all initially identified studies according to the selection criteria. Full-text articles of studies that met all selection criteria were retrieved.

Inclusion and exclusion criteria
To be eligible for inclusion in this meta-analysis, a study must meet the following criteria: 1) the prognostic value of Ki-67/MIB-1 expression for overall survival (OS) and/or cancer-specific survival (CSS) were reported; 2) all patients were diagnosed with histologically confirmed RCC; 3) hazard ratios (HRs) and their 95% CIs for survival analysis were reported in text or could be computed from given data; and 4) the expression of Ki-67 was measured by immunohistochemistry (IHC).

The exclusion criteria were as follows: 1) non-human studies, non-English articles; 2) abstract, case reports, review articles, or comment letters; 3) duplicate publications; 4) with insufficient data to calculate the HR and its 95% CIs, or the Kaplan–Meier curve in the article could not be extracted; and 5) with no >30 eligible RCC patients.

Data extraction and quality
Data was independently extracted by ZW and Shuanghe Peng (The Second Hospital of Tianjin Medical University), and in case of any inconsistency, a consensus was reached with the involvement of QLC. The quality of the selected articles was assessed according to the Newcastle–Ottawa Scale.[36] Study with a score of 6 or higher was considered as a high quality study. We used a predesigned data extraction form to obtain relevant information. The data extracted from the eligible studies including the following items: first author, year of publication, country of origin, the number of patients, histopathological stage, detection method, cut-off value, antibody staining for Ki-67/MIB-1, the number of patients with positive Ki-67/MIB-1 expression, HR for survival (OS and/or CSS), and follow-up time. For articles that only provided survival data in a Kaplan–Meier curve, software designed by Tierney et al was used to digitize and extract the relative risk and its 95% CI.[37]

Statistical analysis
Data were analyzed by using Stata SE12.0 (Stata Corp LP, College Station, TX, USA). According to the Meta-analysis Of Observational Studies in Epidemiology guidelines,[38] the associations between clinical factors and Ki-67/MIB-1 expression were presented by odds ratio (OR) and 95% CI. HR with a 95% CI was computed to reveal the correlation between Ki-67/MIB-1 expression and prognosis (OS and CSS). Inter-study heterogeneity was evaluated using the chi-square test and I^2 statistic ($100\% \times [(Q\text{-}df)/Q]$),[39,40] the value of $P_{heterogeneity} < 0.1$ and $I^2 > 50\%$ represents the existence of significant heterogeneity. A fixed effects model was used when the value of $P_{heterogeneity}$ was >0.05 and $I^2 < 50\%$; otherwise, a random effects model was applied. Subgroup analysis was performed for OS and CSS analysis. Begg's funnel plot and Egger's linear regression test evaluated the potential for publication bias. Two-tailed value of $P < 0.05$ was considered statistically significant.

Results
Features of included studies
The work flowchart for this study is shown in Figure 1. Three hundred and one potentially relevant citations were initially retrieved through initial search of relevant databases. After title and abstract screening, 38 articles
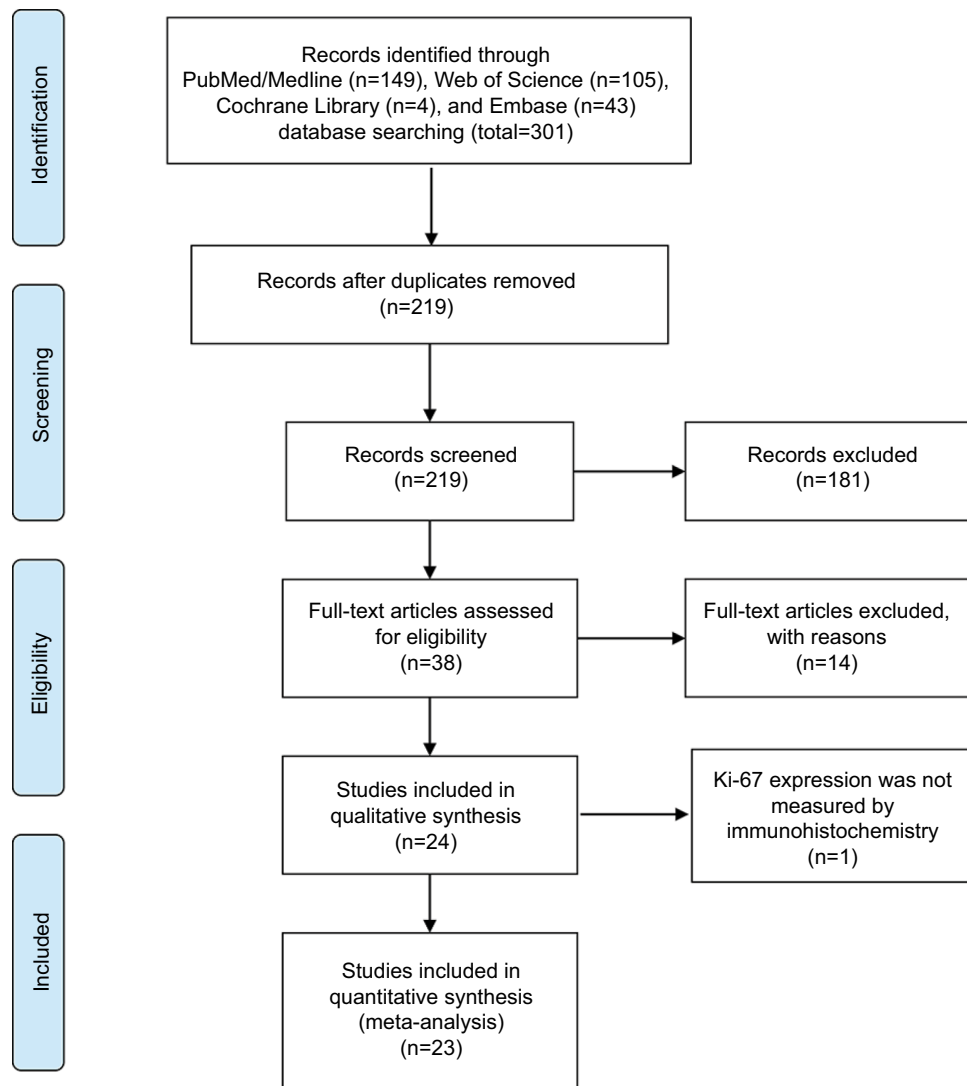
Figure 1 Flow diagram of the study selection process.

remained for full-text assessment. Then 15 articles were excluded (2 articles were duplicate studies, 12 lacked key information, and 1 did not measure Ki-67 expression by IHC). At last, 23 studies[12–34] published from 2000 to 2016 with 4579 patients met our inclusion criteria and were included in the meta-analysis.

Summary of major characteristics of these studies are shown in Table 1. All the studies were of retrospective study design and detected Ki-67/MIB-1 expression using IHC. The sample size ranged from 43 to 741. Nineteen studies were conducted in non-Asian countries, including France,[12] Finland,[22,28,33,34] Germany,[18,29,30] Italy,[25] Sweden,[27] and USA.[13,15–17,20,21,24,26,31] Four studies were conducted in Asian countries, including China,[32] Turkey,[19] and Japan.[14,23] For the prognostic indicator of Ki-67/MIB-1 expression in RCC, 1 article reported both OS and CSS, 6 articles only reported OS, and 16 articles only reported CSS.

Prognostic value of Ki-67/MIB-1 expression for OS and CSS

The association between Ki-67/MIB-1 expression and prognosis for OS and CSS in RCC patients were estimated; pooled HRs and 95% CIs are shown in Table 2 and Figure 2.

OS values were available from 7 studies.[13,21,22,25,28,33,34] The Ki-67/MIB-1 expression had a significant association with poor OS (HR=2.06, 95% CI: 1.64–2.57, $P<0.001$; $I^2=0.0\%$, $P_{heterogeneity}=0.4.73$, Table 2, Figure 2A). Seventeen studies[12,14–21,23,24,26,27,29–32] evaluated CSS outcome. The pooled results indicated that Ki-67/MIB-1 expression was significantly related to poor CSS (HR=2.01, 95% CI: 1.66–2.44, $P<0.001$; $I^2=41\%$, $P_{heterogeneity}=0.04$, Table 2, Figure 2B).

Subgroup analysis

Subgroup analyses were stratified by nation, HR estimate, and pathological types (Table 2). Subgroup analysis according to

Table 1 Main characteristics of included studies

Reference	Year	Country	Ethnicity	Histological type	Tumor stage	No of patients	Gender (M/F)	Mean age (range), (years)
Rioux et al[12]	2000	France	Non-Asian	ccRCC	T1–4	73	47/26	64 (37–86)
Olumi et al[13]	2001	USA	Non-Asian	ccRCC	T1–3	43	26/17	52* (2.5–178)
Yuba et al[14]	2001	Japan	Asian	RCC	T1–4	52	43/9	58.4 (23–77)
Cheville et al[15]	2002	USA	Non-Asian	ccRCC	T1	232	NA	NA
Bui et al[16]	2004	USA	Non-Asian	RCC	T1–4	224	149/75	60.7 (27–89)
Kim et al[17]	2004	USA	Non-Asian	ccRCC	T1–4	318	215/103	60 (27–88)
Lehmann et al[18]	2004	Germany	Non-Asian	ccRCC	T1–3	48	27/25	63* (35–82)
Yildiz et al[19]	2004	Turkey	Asian	RCC	T1–4	48	24/24	54* (20–82)
Kim et al[20]	2005	USA	Non-Asian	m-ccRCC	T3–4	150	107/43	58.1 (30–77)
Kramer et al[21]	2005	USA	Non-Asian	RCC	T1–4	117	78/39	57.24 (36–82)
Kankuri et al[22]	2006	Finland	Non-Asian	RCC	T1–4	117	63/54	61.5 (37–83)
Phuoc et al[23]	2007	Japan	Asian	ccRCC	T1–4	119	78/41	61 (23–86)
Tollefson et al[24]	2007	USA	Non-Asian	ccRCC	T1–4	741	475/266	358/383 (≥65 y/<65 y)
Gontero et al[25]	2008	Italy	Non-Asian	non-ccRCC	T1–4	46	37/9	28/18 (≥65 y/<65 y)
Parker et al[26]	2009	USA	Non-Asian	ccRCC	T1–4	634	413/221	312/322 (≥65 y/<65 y)
Zubac et al[27]	2009	Sweden	Non-Asian	ccRCC	T1–4	160	NA	NA
Kankuri et al[28]	2010	Finland	Non-Asian	RCC	T1–4	57	NA	61 (NA)
Toma et al[29]	2011	Germany	Non-Asian	ccRCC	T1–4	129	82/47	62* (32–88)
Weber et al[30]	2013	Germany	Non-Asian	ccRCC	T1–3	132	80/52	63.5 (57–71)
Gayed et al[31]	2014	USA	Non-Asian	ccRCC	T1–4	401	239/162	58* (17–85)
Teng et al[32]	2014	China	Asian	ccRCC	T1–4	378	272/106	53.4 (NA)
Rautiola et al[33]	2016	Finland	Non-Asian	m-RCC		136	79/57	NA
Virman et al[34]	2016	Finland	Non-Asian	RCC	T1–4	224	132/92	65 (NA)

Notes: *Median follow-up. **The quality of the included studies was evaluated using the Newcastle-Ottawa scale (NOS). ᵃNumber of patients with Ki-67/MIB-1 positive expression.
Abbreviations: ccRCC, clear cell renal cell carcinoma; CSS, cancer specific survival; F, female; IHC, immunohistochemistry; M, male, m-RCC, metastasis RCC; NA, not available; OS, overall survival; RCC, renal cell carcinoma.

Table 2 Ki-67 pooled HRs and 95% CIs in meta-analysis for OS and CSS

Stratified analysis	OS							
	No of studies	Chi-squared	$P_{heterogeneity}$	I^2 (%)	Pooled HR (95% CI)			
					Fixed effect	*P*-value	Random effect	*P*-value
Overall	7	5.57	0.473	0	2.06 (1.64–2.57)	<0.001	2.06 (1.64–2.57)	<0.001
Ethnicity								
Asian	0	–	–	–	–	–	–	–
Non-Asian	7	5.57	0.473	0	2.06 (1.64–2.57)	<0.001	2.06 (1.64–2.57)	<0.001
HR estimate								
Calculated	2	3.4	0.065	71	2.23 (1.51–3.29)	<0.001	4.09 (0.70–23.81)	0.117
Directly	3	0.6	0.741	0	1.85 (1.33–2.56)	<0.001	1.85 (1.33–2.56)	<0.001
Curves	2	0.79	0.373	0	2.29 (1.41–3.73)	0.001	2.29 (1.41–3.73)	0.001
Histopathological subtype								
ccRCC	2	3.75	0.053	73	2.40 (1.01–5.68)	0.047	3.86 (0.49–30.66)	0.202

Note: Bold values in the table indicate the result of pooled HR from a fixed effect model or a random effect model.
Abbreviations: OS, overall survival; CSS, cancer-specific survival; HR, hazard ratio; CI, confidence interval; ccRCC, clear cell renal cell carcinoma; OS, overall survival.

nation showed that Ki-67/MIB-1 expression predicted worse CSS (n=4, HR=3.13, 95% CI: 1.60–6.11, *P*=0.001; I^2=0.0%, pheterogeneity =0.67) in Asian studies. In subgroup analysis according to HR estimate, all the 3 HR estimate methods suggested that Ki-67/MIB-1 expression was significantly associated with poor OS and CSS (Table 2). With regard to histology, Ki-67/MIB-1 expression was significantly correlated with poor CSS (n=13, HR=2.08, 95% CI: 1.67–2.59, *P*<0.001; I^2=45%, $P_{heterogeneity}$ =0041) and poor OS (n=2, HR=3.86, 95% CI: 0.49–30.66, *P*<0.001; I^2=73%, $P_{heterogeneity}$ =0053) in clear cell renal cell carcinoma patients, although a significant heterogeneity exists.

Mean (range) follow-up (months)	(+)a No of patients	Antibody for staining	Method	Cut-off (positive/ high expression)	Survival analysis	NOS**
57 (51–75)	23/66 (34.8%)	MIB-1	IHC	>20%	CSS	8
52 (2.5–178)	14 (32.6%)	MIB-1/Ki-67	IHC	≥10%	OS	8
39 (10–94)	11 (21.2%)	Ki-67	IHC	≥5.6%	CSS	7
126 (0.1–360)	63 (27.2%)	MIB-1	IHC	≥5%	CSS	8
38 (0.3–117)	169 (75%)	MIB-1	IHC	>10%	CSS	8
28 (55)	NA	MIB-1	IHC	>15%	CSS	8
91* (75–120)	12 (25%)	Ki-S5	IHC	≥6%	CSS	6
48* (1–168)	18/42 (43%)	Ab2 Clone MB67	IHC	≥15%	CSS	8
14.8* (NA)	NA	MIB-1	IHC	20%	CSS	6
21.17 (~170)	45/110 (40.9%)	MIB-1	IHC	≥10%	OS, CSS	7
76* (15–177)	21/101 (20.8%)	MIB-1	IHC	10%	OS	6
69.3 (3.6–215.2)	67 (56.3%)	MIB-1	IHC	≥10%	CSS	8
110.4 (1.2–189.6)	281 (40%)	MIB-1	IHC	≥50% positive tumor cells per mm^2	CSS	9
84.5* (NA)	15 (32.6%)	MIB-1	IHC	14%	OS	8
123.6 (1.2–206.4)	245 (38.6%)	MIB-1	IHC	≥50% positive tumor cells per mm^2	CSS	8
85.2 (0.96–232.8)	54 (33.8%)	Dako	IHC	>10%	CSS	8
97* (NA)	NA	MIB-1	IHC	10%	OS	6
87* (2–152)	49 (38%)	Ki67	IHC	5%	CSS	8
123.6 (97.2–156)	8 (6.1%)	MIB-1	IHC	>15%	CSS	8
22 (0–150)	26 (6.5%)	Ki-67	IHC	10%	CSS	8
60* (2–97)	14 (3.7%)	NA	IHC	50%	CSS	8
7.2* (0.23–65.3)	34 (25%)	MIB-1	IHC	10%	OS	7
64.8* (0–260.4)	114 (50.9%)	MIB-1	IHC	1.36 (median value)	OS	9

CSS

No of studies	Chi-squared	P$_{heterogeneity}$	I^2 (%)	HR (95% CI)			
				Fixed effect	P-value	Random effect	P-value
17	27.11	0.04	41	1.81 (1.61–2.03)	<0.001	**2.01 (1.66–2.44)**	<0.001
4	1.57	0.67	0	3.13 (1.60–6.11)	0.001	3.13 (1.60–6.11)	0.001
13	22.88	0.029	48	1.77 (1.58–2.00)	<0.001	**1.95 (1.60–2.38)**	<0.001
2	9.91	0.003	89	1.88 (1.22–2.91)	0.004	**2.24 (0.58–8.66)**	0.24
13	17.27	0.14	31	1.79 (1.58–2.02)	<0.001	**1.94 (1.61–2.32)**	<0.001
2	0.23	0.632	0	2.56 (1.09–6.04)	0.032	2.56 (1.09–6.04)	0.032
13	21.69	0.041	45	1.79 (1.57–2.03)	<0.001	**2.08 (1.67–2.59)**	<0.001

Evaluation of Ki-67/MIB-1 expression and clinicopathological characteristics
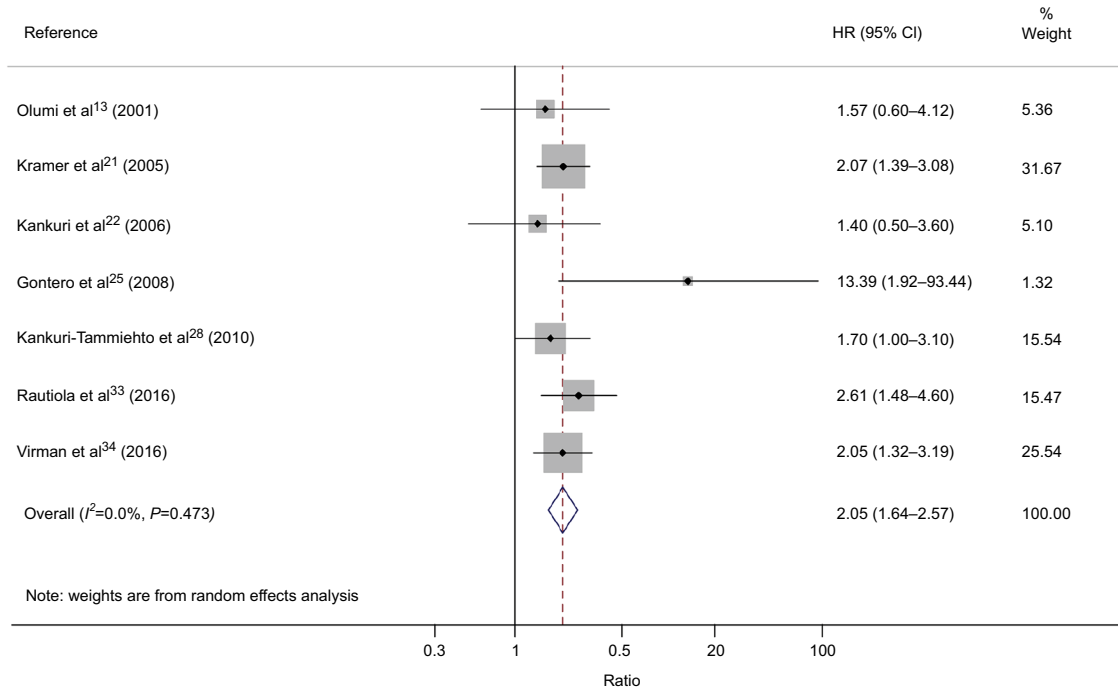
We also estimated the association between Ki-67/MIB-1 expression and clinicopathological characteristics in RCC patients. Ki-67/MIB-1 expression was significantly associated with TNM (III/IV vs I/II, OR=1.92, 95% CI: 1.61–2.28), grade (3/4 vs 1/2, OR=1.94, 95% CI: 1.21–3.10), M (M1 vs M0, OR=1.81, 95% CI: 1.34–2.43), N (N1 vs N0, OR=1.67, 95% CI: 1.33–2.12), and tumor stage (pT3/4 vs pT1/2, OR=1.56, 95% CI: 1.21–2.02) (Figure 3 and Table 3).

Publication bias

Funnel plots for meta-analysis of Ki-67/MIB-1 expression, OS, and CSS are shown in Figure 4. Both the Begg's funnel
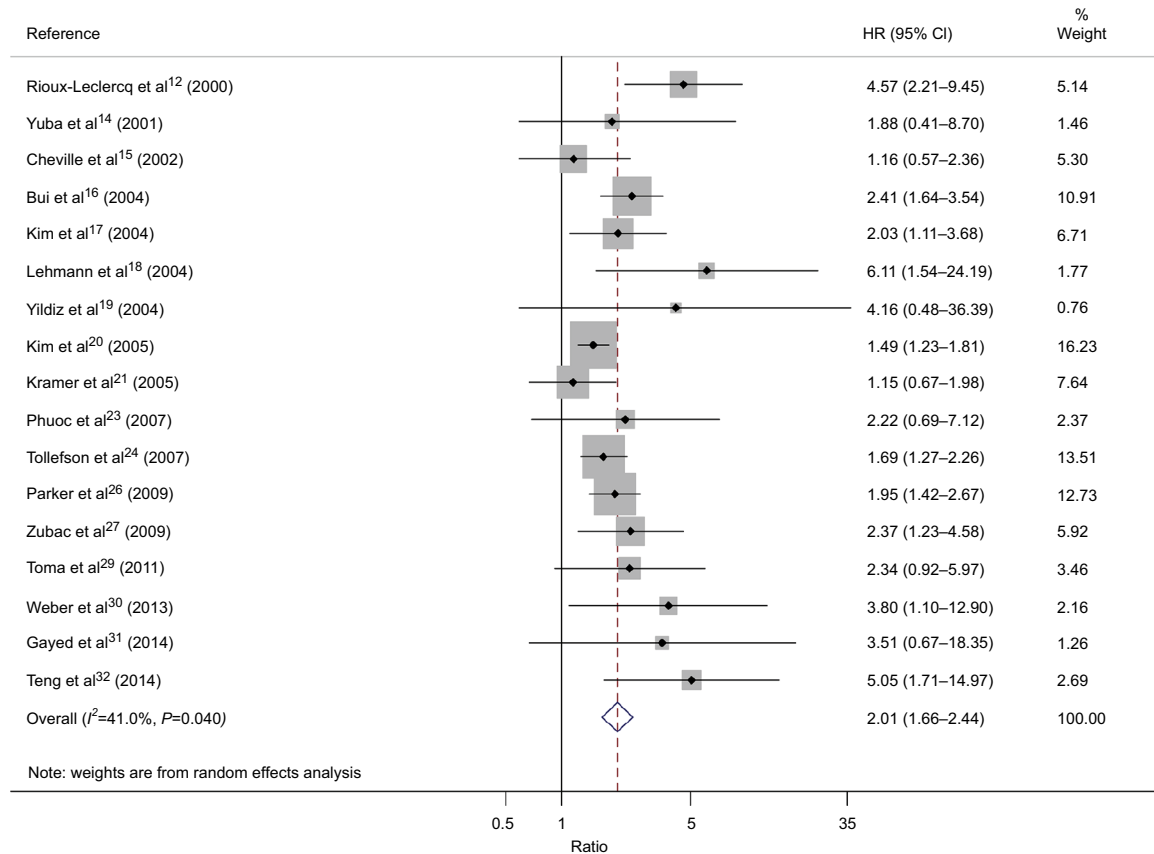
A

Reference		HR (95% CI)	% Weight
Olumi et al[13] (2001)		1.57 (0.60–4.12)	5.36
Kramer et al[21] (2005)		2.07 (1.39–3.08)	31.67
Kankuri et al[22] (2006)		1.40 (0.50–3.60)	5.10
Gontero et al[25] (2008)		13.39 (1.92–93.44)	1.32
Kankuri-Tammiehto et al[28] (2010)		1.70 (1.00–3.10)	15.54
Rautiola et al[33] (2016)		2.61 (1.48–4.60)	15.47
Virman et al[34] (2016)		2.05 (1.32–3.19)	25.54
Overall (I^2=0.0%, P=0.473)		2.05 (1.64–2.57)	100.00

Note: weights are from random effects analysis

0.3 1 0.5 20 100
Ratio

B

Reference		HR (95% CI)	% Weight
Rioux-Leclercq et al[12] (2000)		4.57 (2.21–9.45)	5.14
Yuba et al[14] (2001)		1.88 (0.41–8.70)	1.46
Cheville et al[15] (2002)		1.16 (0.57–2.36)	5.30
Bui et al[16] (2004)		2.41 (1.64–3.54)	10.91
Kim et al[17] (2004)		2.03 (1.11–3.68)	6.71
Lehmann et al[18] (2004)		6.11 (1.54–24.19)	1.77
Yildiz et al[19] (2004)		4.16 (0.48–36.39)	0.76
Kim et al[20] (2005)		1.49 (1.23–1.81)	16.23
Kramer et al[21] (2005)		1.15 (0.67–1.98)	7.64
Phuoc et al[23] (2007)		2.22 (0.69–7.12)	2.37
Tollefson et al[24] (2007)		1.69 (1.27–2.26)	13.51
Parker et al[26] (2009)		1.95 (1.42–2.67)	12.73
Zubac et al[27] (2009)		2.37 (1.23–4.58)	5.92
Toma et al[29] (2011)		2.34 (0.92–5.97)	3.46
Weber et al[30] (2013)		3.80 (1.10–12.90)	2.16
Gayed et al[31] (2014)		3.51 (0.67–18.35)	1.26
Teng et al[32] (2014)		5.05 (1.71–14.97)	2.69
Overall (I^2=41.0%, P=0.040)		2.01 (1.66–2.44)	100.00

Note: weights are from random effects analysis

0.5 1 5 35
Ratio

Figure 2 Forest plot HR for the correlation between Ki-67/MIB-1 expression and OS (**A**) or CSS (**B**) in RCC patients.
Abbreviations: CSS, cancer-specific survival; HR, hazard ratio; CI, confidence interval; RCC, renal cell carcinoma; OS, overall survival.
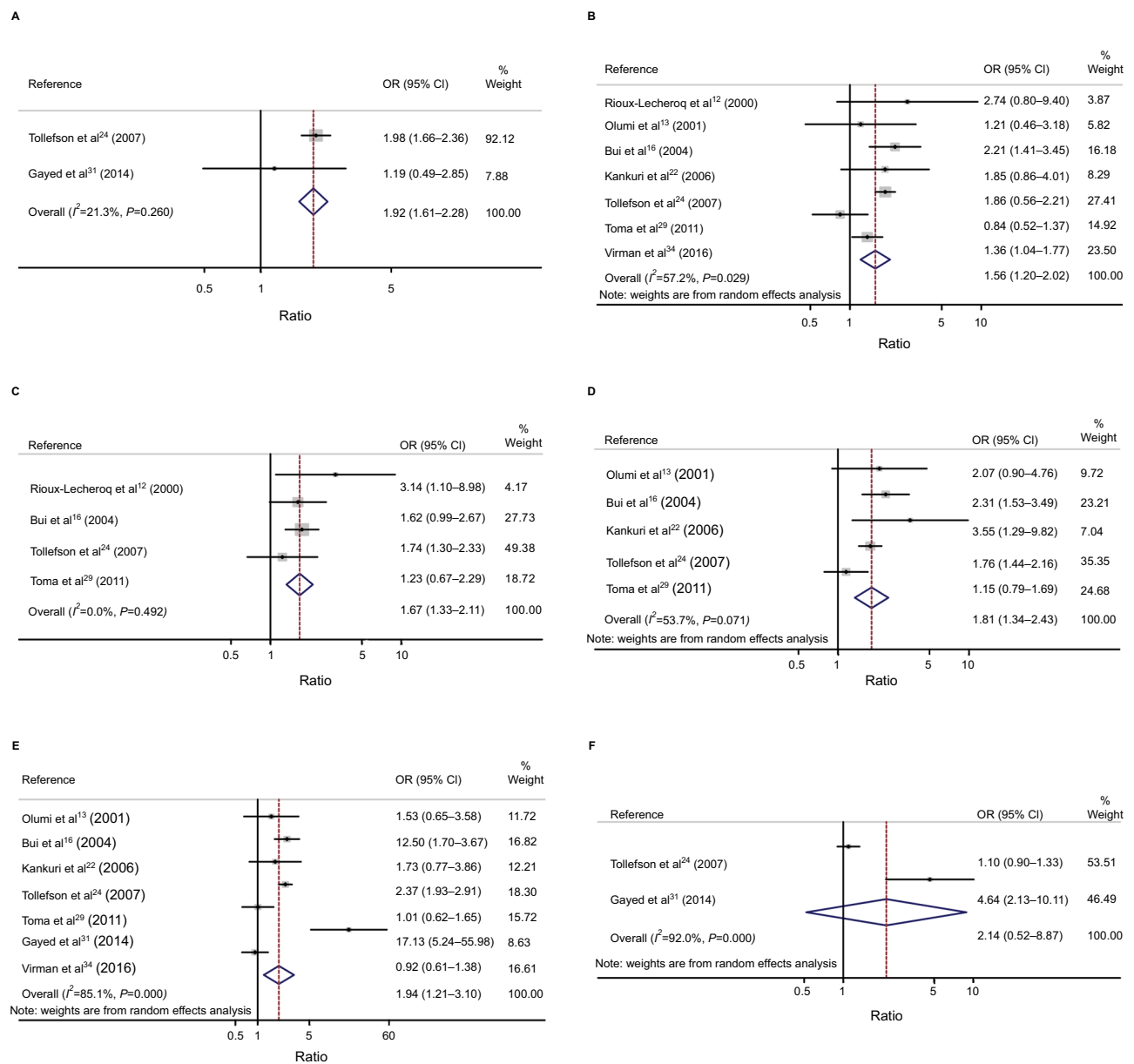
Figure 3 Association between Ki-67/MIB-1 expression and (**A**) TNM stage; (**B**) primary tumor stage; (**C**) lymph node metastasis; (**D**) distant metastasis; (**E**) grade; and (**F**) gender.
Abbreviations: OR, odds ratio; CI, confidence interval.

plot test (OS: P=1.000, CSS: P=0.149; Figure 4) and the Egger's (OS: P=0.494, CSS: P=0.010) test verified that there was no publication bias within the included cohorts. The funnel plots for clinical features also indicated no obvious publication bias (Figure 4, Table 3).

Sensitivity analysis

Sensitivity analysis was performed to examine the stability of the current meta-analysis. The selected studies were sequentially omitted to investigate whether any single study could have an influence on the pooled OS or CSS. As shown

in Figure 5, the stable overall HR was found to be not dominantly influenced by each individual study.

Discussion

MIB-1, a nuclear protein, is famous as a marker of cell proliferation and tumor growth. Since Gerdes et al[42] first suggested that Ki-67 labeling index predicted poor prognosis in non-Hodgkin's lymphomas, a number of studies have examined the usefulness of Ki-67 expression in various tumor types. In recent years, several reports suggested that high Ki-67 expression can serve as a promising biomarker

Table 3 Meta-analysis of Ki-67 expression and clinicopathological features in renal cell carcinoma

Group	No of studies	Chi-squared	P$_{heterogeneity}$	I^2 (%)	Pooled OR (95% CI)				Begg's test P-value	Egger's test P-value
					Fixed effect	P-value	Random effect	P-value		
Tumor stage (pT3/pT4 vs pT1/Pt2)	7	14.01	0.029	57.2	1.66 (1.46–1.89)	<0.001	1.56 (1.21–2.02)	0.001	0.764	0.286
N (N1–2 vs N0)	4	2.41	0.492	0	1.67 (1.33–2.12)	<0.001	1.68 (1.34–2.12)	<0.001	1.000	0.913
M (M1 vs M0)	5	8.63	0.071	53.7	1.83 (1.54–2.16)	<0.001	1.81 (1.34–2.43)	<0.001	1.000	0.975
TNM (III/IV vs I/II)	2	1.27	0.26	21.3	1.92 (1.61–2.28)	<0.001	1.84 (1.30–2.61)	0.001	1.000	–
Grade (3/4 vs 1/2)	7	40.18	<0.001	85.1	2.17 (1.87–2.51)	<0.001	1.94 (1.21–3.10)	0.006	1.000	0.472
Gender (male vs female)	2	12.56	<0.001	92	1.24 (1.03–1.50)	0.024	2.14 (0.52–8.87)	0.292	1.000	–

Abbreviations: OR, odds ratio; CI, confidence interval; N, lymph node involvement; M, distant metastasis; TNM, TNM stage.
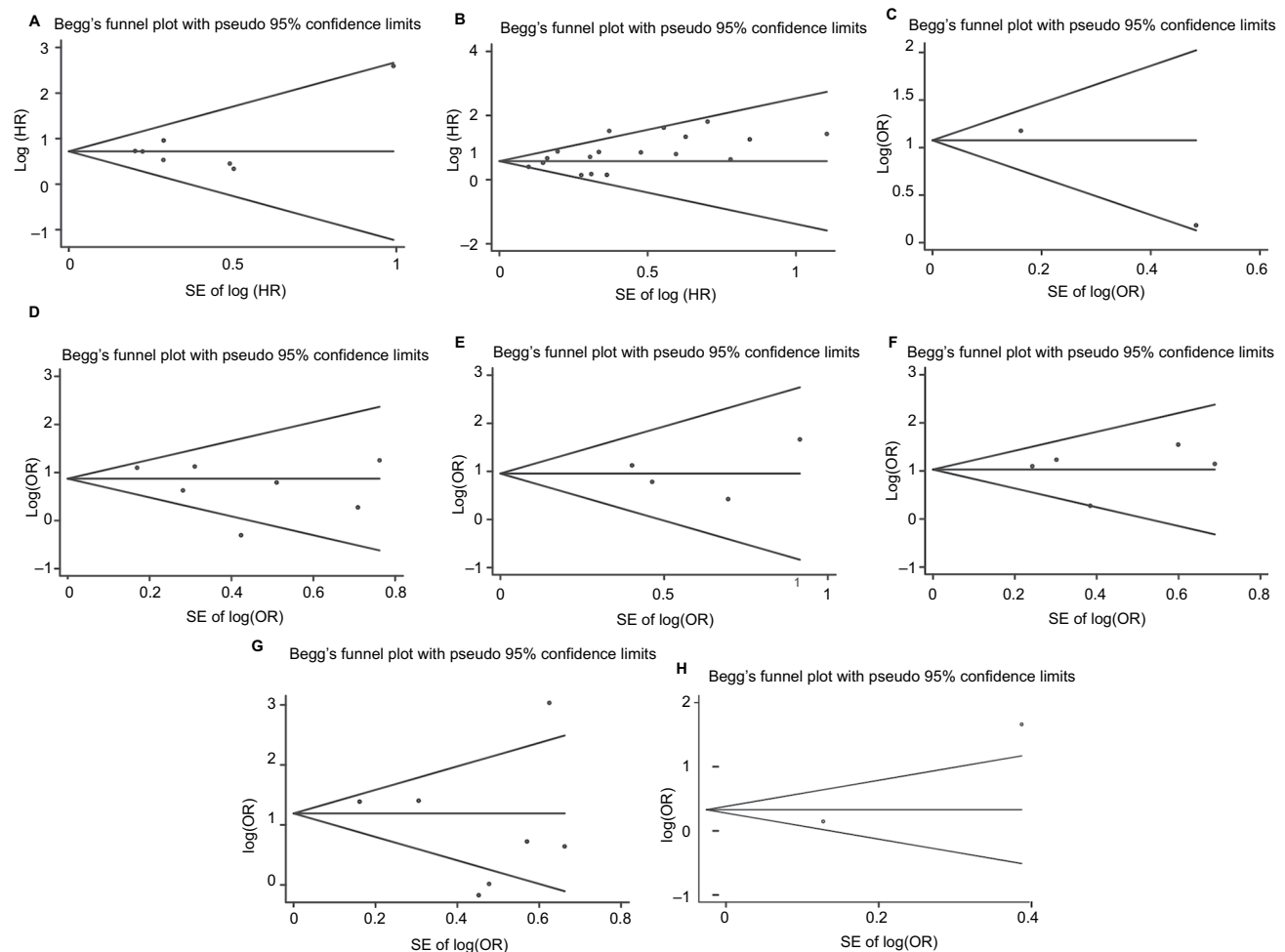
Figure 4 Funnel plots evaluating possible publication bias for (**A**) OS; (**B**) CSS; (**C**) TNM stage; (**D**) primary tumor stage; (**E**) lymph node involvement; (**F**) distant metastasis; (**G**) grade; (**H**) gender.
Abbreviations: CSS, cancer-specific survival; OR, odds ratio; OS, overall survival.

for prognostication in various tumors.[7–11] Many studies have also reported the prognostic value of Ki-67 expression in RCC, but the results were still conflicting.[12–34,41] Therefore, we performed this meta-analysis to explore the association between Ki-67/MIB-1 expression and prognostic value and clinicopathological features in patients with RCC.

Our analysis mainly reports the prognostic role of Ki-67/MIB-1 expression in RCC. Studies from different countries are included in the meta-analysis. Fixed effects model and random effects model were used for the meta-analysis. In this study, we focused on validating Ki-67/MIB-1 expression and evaluated the prognostic values of Ki-67/MIB-1 expression in
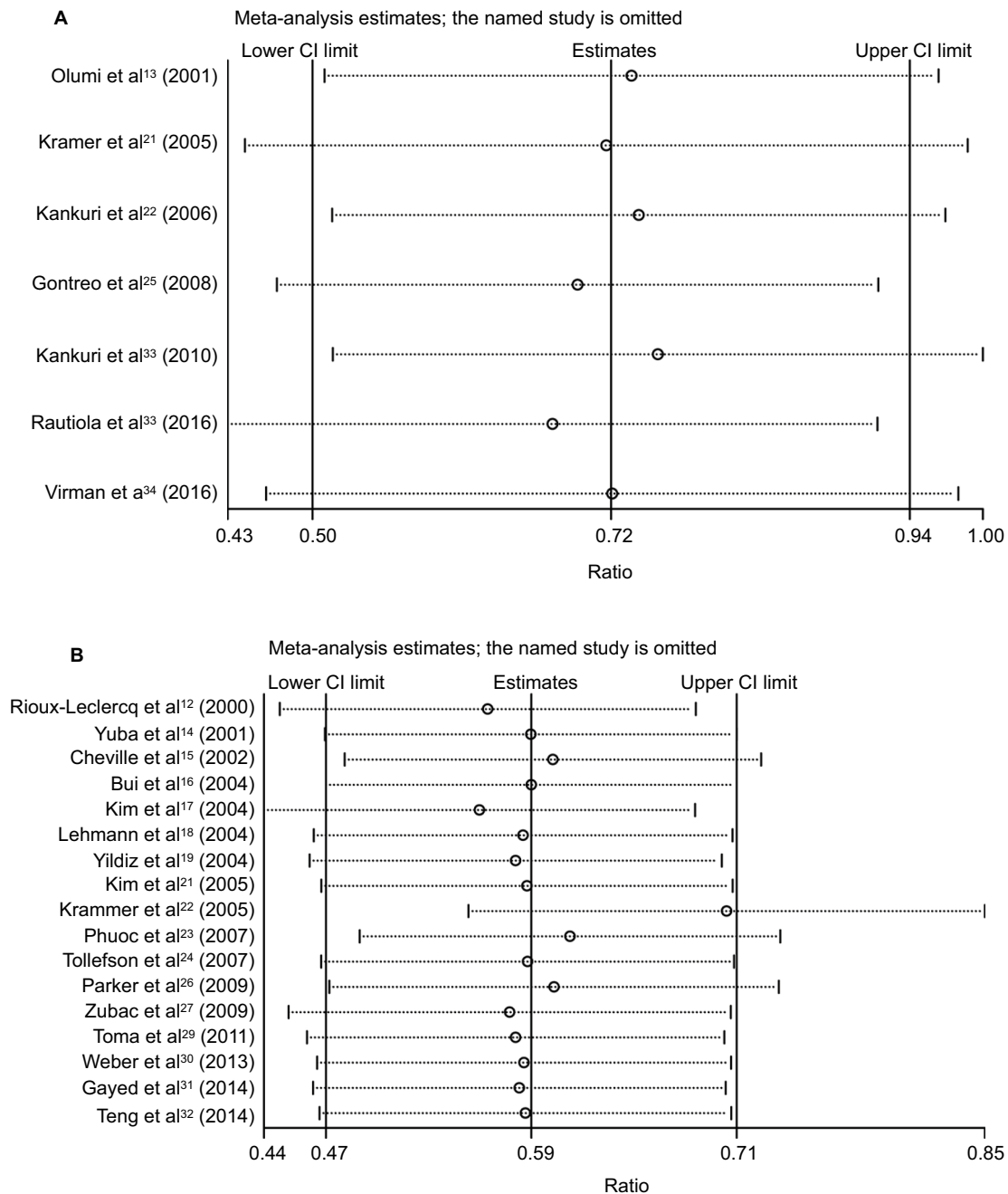
A

Meta-analysis estimates; the named study is omitted

Olumi et al[13] (2001)

Kramer et al[21] (2005)

Kankuri et al[22] (2006)

Gontreo et al[25] (2008)

Kankuri et al[33] (2010)

Rautiola et al[33] (2016)

Virman et a[34] (2016)

Ratio

B

Meta-analysis estimates; the named study is omitted

Rioux-Leclercq et al[12] (2000)
Yuba et al[14] (2001)
Cheville et al[15] (2002)
Bui et al[16] (2004)
Kim et al[17] (2004)
Lehmann et al[18] (2004)
Yildiz et al[19] (2004)
Kim et al[21] (2005)
Krammer et al[22] (2005)
Phuoc et al[23] (2007)
Tollefson et al[24] (2007)
Parker et al[26] (2009)
Zubac et al[27] (2009)
Toma et al[29] (2011)
Weber et al[30] (2013)
Gayed et al[31] (2014)
Teng et al[32] (2014)

Ratio

Figure 5 Effect of individual studies on the pooled HRs for Ki-67/MIB-1, OS, and CSS of patients.
Abbreviations: CI, confidence interval; CSS, cancer-specific survival; HRs, hazard ratios; OS, overall survival.

RCC. Based on results from 24 studies with 4579 participants, we concluded that Ki-67/MIB-1 expression predicted poor prognostic value for patients with RCC. RCC patients with Ki-67/MIB-1 expression exhibited poor OS and CSS. Subgroup analysis results revealed that the pooled HRs obtained from Kaplan–Meier curves and those directly extracted from studies both demonstrated that Ki-67/MIB-1 expression was significantly associated with poor OS and CSS. Our results showed that Ki-67/MIB-1 expression was an unfavorable predictor for prognosis in RCC, which were in accordance with conclusions from other solid cancer types, such as

breast cancer,[7] prostate cancer,[8] cervical cancer,[9] gliomas,[10] and hepatocellular carcinoma.[11] In addition, Ki-67/MIB-1 expression was also associated with clinical factors in RCC; Ki-67/MIB-1 expression had positive relationship with higher tumor stage and grade, as well as lymph node involvement and distant metastases, which suggested that Ki-67/MIB-1 had potential to be used as a dichotomous biomarker.

The relationship between Ki-67/MIB-1 expression and clinicopathological features was also evaluated. The result suggested that RCC patients with Ki-67/MIB-1 expression were significantly associated with primary tumor stage,

regional lymph node involvement, distant metastases, nuclear grade, and TNM stage. High Ki-67/MIB-1 expression was likely to have a higher primary tumor stage, TNM stage, positive regional lymph node involvement and distant metastasis, and a higher nuclear grade.

There are several limitations in this study that should be acknowledged. First, all included studies in this meta-analysis measured Ki-67/MIB-1 expression via IHC, but the cut-off criteria to determine the positive or negative expression of Ki-67/MIB-1 were inconsistent in different studies, which may potentially contribute to heterogeneity. Therefore, a more unified standard should be defined in the future. Second, the number of patients included in the most eligible studies was relatively small. Therefore, large-scale studies are needed to conceive more reliable results. Third, relatively few studies were extracted in some subgroup analyses, which might render premature results. Finally, research with positive results is potentially more likely to be submitted and published than work with negative results, which could cause publication bias, although this bias was not detected in the present analysis.[43]

Conclusion

Our meta-analysis suggests that Ki-67/MIB-1 expression predicted a poor OS and CSS in patients with RCC. The results also indicate that Ki-67/MIB-1 expression was associated with more aggressive clinical features in patients with RCC. Hence, the detection of Ki-67/MIB-1 in clinic will be beneficial to the treatment and prognostic evaluation for RCC patients. More prospective and large-scale studies are needed to clarify our results.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. *Lancet.* 2009;373(9669):1119–1132.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65(2):87–108.
3. Fitzmaurice C, Dicker D, Pain A, et al. The Global Burden of Cancer 2013. *JAMA Oncol.* 2015;1(4):505–527.
4. Janzen NK, Kim HL, Figlin RA, Belldegrun AS. Surveillance after radical or partial nephrectomy for localized renal cell carcinoma and management of recurrent disease. *Urol Clin North Am.* 2003;30(4):843–852.
5. Meskawi M, Sun M, Trinh QD, et al. A review of integrated staging systems for renal cell carcinoma. *Eur Urol.* 2012;62(2):303–314.
6. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol.* 2000;182(3):311–322.
7. Liedtke C, Packeisen J, Hess KR, et al. Systematic analysis of in vitro chemosensitivity and mib-1 expression in molecular breast cancer subtypes. *Eur J Cancer.* 2012;48(13):2066–2074.
8. Luczynska E, Gasinska A, Wilk W. Expression of Ki-67 (MIB-1) and GLUT-1 proteins in non-advanced prostatic cancer. *Pol J Pathol.* 2012;63(4):272–277.
9. Pan D, Wei K, Ling Y, Su S, Zhu M, Chen G. The prognostic role of Ki-67/MIB-1 in cervical cancer: a systematic review with meta-analysis. *Med Sci Monit.* 2015;21:882–889.
10. Skjulsvik AJ, Mork JN, Torp MO, Torp SH. Ki-67/MIB-1 immunostaining in a cohort of human gliomas. *Int J Clin Exp Pathol.* 2014;7(12):8905–8910.
11. Shi W, Hu J, Zhu S, et al. Expression of MTA2 and Ki-67 in hepatocellular carcinoma and their correlation with prognosis. *Int J Clin Exp Pathol.* 2015;8(10):13083–13089.
12. Rioux-Leclercq N, Turlin B, Bansard J, et al. Value of immunohistochemical Ki-67 and p53 determinations as predictive factors of outcome in renal cell carcinoma. *Urology.* 2000;55(4):501–505.
13. Olumi AF, Weidner N, Presti JC. p53 immunoreactivity correlates with Ki-67 and bcl-2 expression in renal cell carcinoma. *Urol Oncol.* 2001;6(2):63–67.
14. Yuba H, Okamura K, Ono Y, Ohshima S. Growth fractions of human renal cell carcinoma defined by monoclonal antibody Ki-67. Predictive values for prognosis. *Inte J Urol.* 2001;8(11):609–614.
15. Cheville JC, Zincke H, Lohse CM, et al. pT1 clear cell renal cell carcinoma: a study of the association between MIB-1 proliferative activity and pathologic features and cancer specific survival. *Cancer.* 2002;94(8):2180–2184.
16. Bui MH, Visapaa H, Seligson D, et al. Prognostic value of carbonic anhydrase IX and KI67 as predictors of survival for renal clear cell carcinoma. *J Urol.* 2004;171(6 Pt 1):2461–2466.
17. Kim HL, Seligson D, Liu X, et al. Using protein expressions to predict survival in clear cell renal carcinoma. *Clin Cancer Res.* 2004;10(16):5464–5471.
18. Lehmann J, Retz M, Nurnberg N, et al. The superior prognostic value of humoral factors compared with molecular proliferation markers in renal cell carcinoma. *Cancer.* 2004;101(7):1552–1562.
19. Yildiz E, Gokce G, Kilicarslan H, Ayan S, Goze OF, Gultekin EY. Prognostic value of the expression of Ki-67, CD44 and vascular endothelial growth factor, and microvessel invasion, in renal cell carcinoma. *BJU Int.* 2004;93(7):1087–1093.
20. Kim HL, Seligson D, Liu X, et al. Using tumor markers to predict the survival of patients with metastatic renal cell carcinoma. *J Urol.* 2005;173(5):1496–1501.
21. Kramer BA, Gao X, Davis M, Hall M, Holzbeierlein J, Tawfik O. Prognostic significance of ploidy, MIB-1 proliferation marker, and p53 in renal cell carcinoma. *J Am Coll Surg.* 2005;201(4):565–570.
22. Kankuri M, Soderstrom KO, Pelliniemi TT, Vahlberg T, Pyrhonen S, Salminen E. The association of immunoreactive p53 and Ki-67 with T-stage, grade, occurrence of metastases and survival in renal cell carcinoma. *Anticancer Res.* 2006;26(5B):3825–3833.
23. Phuoc NB, Ehara H, Gotoh T, et al. Immunohistochemical analysis with multiple antibodies in search of prognostic markers for clear cell renal cell carcinoma. *Urology.* 2007;69(5):843–848.
24. Tollefson MK, Thompson RH, Sheinin Y, et al. Ki-67 and coagulative tumor necrosis are independent predictors of poor outcome for patients with clear cell renal cell carcinoma and not surrogates for each other. *Cancer.* 2007;110(4):783–790.
25. Gontero P, Ceratti G, Guglielmetti S, et al. Prognostic factors in a prospective series of papillary renal cell carcinoma. *BJU Int.* 2008;102(6):697–702.
26. Parker AS, Leibovich BC, Lohse CM, et al. Development and evaluation of BioScore: a biomarker panel to enhance prognostic algorithms for clear cell renal cell carcinoma. *Cancer.* 2009;115(10):2092–2103.

27. Zubac DP, Bostad L, Kihl B, Seidal T, Wentzel-Larsen T, Haukaas SA. The expression of thrombospondin-1 and p53 in clear cell renal cell carcinoma: its relationship to angiogenesis, cell proliferation and cancer specific survival. *J Urol.* 2009;182(5):2144–2149.

28. Kankuri-Tammilehto MK, Soderstrom KO, Pelliniemi TT, Vahlberg T, Pyrhonen SO, Salminen EK. Prognostic evaluation of COX-2 expression in renal cell carcinoma. *Anticancer Res.* 2010;30(7):3023–3030.

29. Toma MI, Weber T, Meinhardt M, et al. Expression of the Forkhead transcription factor FOXP1 is associated with tumor grade and Ki67 expression in clear cell renal cell carcinoma. *Cancer Invest.* 2011;29(2):123–129.

30. Weber T, Meinhardt M, Zastrow S, Wienke A, Fuessel S, Wirth MP. Immunohistochemical analysis of prognostic protein markers for primary localized clear cell renal cell carcinoma. *Cancer Invest.* 2013;31(1):51–59.

31. Gayed BA, Youssef RF, Bagrodia 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):668–673.

32. Teng J, Gao Y, Chen M, et al. Prognostic value of clinical and pathological factors for surgically treated localized clear cell renal cell carcinoma. *Chin Med J.* 2014;127(9):1640–1644.

33. Rautiola J, Lampinen A, Mirtti T, et al. Association of angiopoietin-2 and Ki-67 expression with vascular density and sunitinib response in metastatic renal cell Carcinoma. *PLoS One.* 2016;11(4):e0153745.

34. Virman JP, Bono P, Luukkaala TH, Sunela KL, Kujala PM, Kellokumpu-Lehtinen PL. Combined angiogenesis and proliferation markers' expressions as long-term prognostic factors in renal cell cancer. *Clin Genitourin Cancer.* 2016;14(4):e283–e289.

35. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med.* 2009;6(7):e1000100.

36. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* 2010;25(9):603–605.

37. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials.* 2007;8:16.

38. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* 2000;283(15):2008–2012.

39. Handoll HH. Systematic reviews on rehabilitation interventions. *Arch Phys Med Rehabil.* 2006;87(6):875.

40. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* 2003;327(7414):557–560.

41. Visapaa H, Bui M, Huang Y, et al. Correlation of Ki-67 and gelsolin expression to clinical outcome in renal clear cell carcinoma. *Urology.* 2003;61(4):845–850.

42. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer.* 1983;31(1):13–20.

43. Sutton AJ, Song F, Gilbody SM, Abrams KR. Modelling publication bias in meta-analysis: a review. *Stat Methods Med Res.* 2000;9(5):421–445.