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Title page

Prognostic Role of Neutrophil-Lymphocyte Ratio in Renal Cell Carcinoma: A Meta-Analysis

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Abstract

Objective: Increasing evidence suggests that cancer-associated inflammation is associated with poor prognosis in cancer patients. The role of neutrophil-lymphocyte ratio (NLR) as a predictor in renal cell carcinoma (RCC) remains controversial. We conducted the meta-analysis to determine the association between NLR and clinical outcome of RCC patients.

Methods and materials: Studies were identified from PubMed and EMBASE databases in March 2014. Meta-analysis was performed to generate combined hazard ratios (HRs) with 95% confidence intervals (95% CIs) for overall survival (OS) and recurrence/progress-free survival (RFS/PFS).

Results: Fifteen cohorts containing 3357 patients were included. Our analysis results indicated that elevated NLR predicted poorer OS (HR: 1.82, 95%CI: 1.51-2.19) and RFS/PFS (HR: 2.18, 95% CI: 1.75-2.71) in RCC patients. These findings were robust when stratified by study region, sample size, therapeutic intervention, types of RCC and study quality. However, it significantly differed by assessment of the cut-off value defining "elevated NLR" in RFS/PFS (p = 0.004). The heterogeneity in our meta-analysis was mild to moderate.

Conclusions: Elevated NLR indicates poorer prognosis for patients with RCC. NLR should be monitored in RCC patients for rational risk stratification and adjusting the management accordingly.

Keywords: neutrophil-lymphocyte ratio, renal cell carcinoma, prognosis, meta-analysis

Strengths and limitations of this study

Our study is the first systematic meta-analysis evaluating the relationship between elevated NLR

and prognosis in RCC patients. Our analysis provides substantial evidence that elevated NLR is significantly associated with poorer outcomes of RCC patients. However, there were some limitations in our study. The enrolled studies were retrospective cohort studies, publication bias inevitably existed. We conducted "trim and fill" analysis to show our conclusion was robust. There was some heterogeneity in the included patient populations, so we confirmed the prognostic role of NLR in patients with different disease stage, therapeutic intervention and types of RCC by subgroup analysis. We only searched limited databases, which might weaken the estimating power of the pooled estimate.

Introduction

Renal cell carcinoma (RCC) accounts for 2–3% of all malignant diseases in adults. It's the seventh most common cancer in men and the ninth in women worldwide[1,2]. The incidence of this cancer varies geographically and has increased over past decades owing to changes in life style and environment[1]. Despite a rapid development in surgical resection, immunotherapy and targeted therapy in RCC management, the long-term outcome is still not promising mainly due to common local recurrence, distal metastasis and limited drug response[3]. Hence, it is important to identify significant biomarkers, which can help clinicians to stratify patients in terms of prognosis and possibility of metastatic recurrence together with tumor staging system, *i.e.* the TNM staging system and Robson's staging system, and then set the most appropriate therapeutic strategy.

It is well recognized that the heterogeneity in clinical outcomes is determined by both oncological characteristics of tumor itself and host's response to the progressing malignancy[4]. The complicated mechanisms by which cancer and inflammation intersect have been gradually

revealed. Inflammation impacts every single step of tumorigenesis, from tumor initiation to promotion and metastatic progression[5]. Recently, several serum biomarkers and haematological indices representative of inflammatory response, notably C-reactive protein (CRP), neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR), have been demonstrated to be closely related to poor prognosis of RCC patients[6,7].

Generally speaking, lymphopenia well reflects impaired cell-mediated immunity, while neutrophilia represents a response to systematic inflammation[5]. So the NLR, defined as neutrophil counts divided by lymphocyte counts, is particularly noteworthy. Emerging evidences have shown that NLR gained its prognostic value in patients with colorectal cancer[8] and hepatocellular carcinoma[9]. RCC patients with elevated level of pre-treatment NLR may be more likely to gain a poorer clinical outcome[10]. But the exact role of NLR in RCC patients is not consistent in different studies due to the variance in study design, sample size and other factors. Some concluded significant relationship between higher NLR and poorer prognosis, while others did not. Therefore, it is necessary to perform a meta-analysis to systematically and comprehensively understand the prognostic value of NLR in RCC patients.

In this study, we aimed to assess the prognostic significance of high NLR for overall survival (OS) and recurrence-free survival (RFS) / progress-free survival (PFS) in RCC patients by pooling outcomes from available data.

Material and Methods

Search strategy

A comprehensive literature search of PubMed and EMBASE databases (Up to March 2014) was

conducted to identify relevant studies. The search strategy included terms for: "NLR" (e.g., "neutrophil to lymphocyte ratio", "neutrophil lymphocyte ratio" and "neutrophil-lymphocyte ratio"), "RCC" (e.g., "renal cancer", "renal carcinoma", "kidney cancer", clear cell carcinoma", "non-clear cell carcinoma", and "renal papillary carcinoma") and "prognosis" (e.g., "recurrence", "survival" and "outcome"). Abstracts and information from conferences were collected independently. The reference list was also checked for additional articles. Only studies published in English were included.

Study inclusion criteria and definitions

Two independent authors (Hu KM and Lou LX) reviewed the retrieved studies and extracted data from each included study. Discrepancies were resolved by discussion. Studies included in our meta-analysis must meet the following criteria: (1) The diagnosis of RCC was based on the current clinical guidelines; (2) NLR was measured by serum-based methods before formal treatment; (3) Studies reported hazard ratios (HRs) and 95% confidence intervals (95% CIs) for pre-treatment NLR in OS and (or) RFS/PFS, or allowed for calculation from raw data contained in the article; (4) Only primary data or data superseded earlier work were included, and articles were superior to conference abstracts.

NLR was defined as the serum absolute neutrophil count divided by lymphocyte count in peripheral blood[11]. OS was defined as the interval between the medical treatment and the death or the last follow-up of patients. RFS (disease free survival / metastasis free survival, DFS/MFS) was measured from the date of curative treatment until the detection of tumor recurrence. PFS was calculated from the date of treatment until progressing of disease. If all the patients in the

individual study only received curative nephrectomy, the study was classified into nephrectomy only subgroup, and the studies in which patients were mainly treated by non-surgical intervention were classified into mixed therapies subgroup.

Data extraction

We extracted data including: (1) study information including name of first author, year of publication, study region, sample size, time of research; (2) patient characters including age, gender, follow-up period and treatment methods; (3) data about RCC including type, size, stage and distal metastasis; (4) NLR data and cut-off value of NLR; (5) survival data including OS and RFS/PFS.

Quality assessment of primary studies

Quality assessment of included studies was evaluated with the Newcastle-Ottawa quality assessment scale (NOS) range from 0 to 8 by two independent investigators (Hu KM and Lou LX). Studies with NOS score of $\geqslant$ 6 were assigned as high-quality ones. Studies from conference abstracts were defined as low-quality studies. Any inconsistencies were resolved by joint discussion.

Statistical analysis

HR abstracted in each study greater than one favored that elevated NLR indicated a poor prognosis. Multivariate analysis for HR was superior to univariate analysis unless adjustment variables in multivariable analysis significantly interacting with NLR level. As heterogeneity was

detected among primary studies, meta-analysis was pooled using the random effects models with DerSimonian Laird method[12]. Between-study heterogeneity was assessed using Cochran Q test and I^2 statistic. $P < .10$ was considered statistically significant for Cochran Q test, $I^2 > 50\%$ indicating substantial heterogeneity between studies. Potential sources of heterogeneity were then investigated using subgroup analyses and meta-regression. All statistical tests were two-sided and the significance level was set at 0.05. The possibility of publication bias was assessed using the Begg test and visual insection of a funnel plot[13]. We also performed the Duval and Tweedie nonparametric "trim and fill" procedure to further assess the possible effect of publication bias in our meta-analysis[14]. All statistical manipulations were undertaken using the program STATA version 12.0 (Stata Corporation, College Station, TX).

Results

Study characteristics

The initial search algorithm retrieved a total of 403 studies. After the title and abstract reviewed, only 30 records were identified regarding the association of NLR and RCC (Figure 1). After full-text review, a total of 14 retrospective studies[10,15-27] (15 cohorts) with 3357 RCCs were included in our meta-analysis. The study by Hatakeyama et al[27] reported the HR and 95% CI of two different cohorts separately. If the patients were overlapping or partially overlapping in several studies, only the study with the most complete data was included.

The basic features of the 14 studies were summarized in Table 1. Median quality score of the involved studies was 6 (range: 4-8). Eight studies were from western countries, including the USA, Italy, Belgium, Austria, Canada, and Australia. The rest studies were from Turkey and Japan.

Seven of these cohorts enrolled more than 200 patients and eight had less than 200 patients. Radical and partial nephrectomy as only initial treatment for non-metastatic RCC was reported in four studies. Others were treated with mixed therapies, including nephrectomy, immunotherapy, targeted therapy and others. NLR was calculated using the white blood cell differentiated counts in all studies. In the study by Certin et al.[20], some of the adjustment variables used in multivariate analysis was significantly associated with NLR value, so HR and 95% CI from univariate analysis for both PFS and OS were used in our meta-analysis.

NLR and OS in RCC

There were 13 cohorts presenting the data of pre-treatment NLR and OS in RCC patients. Elevated NLR was significantly associated with shorter OS (HR = 1.82; 95% CI: 1.51-2.19; $p < 0.001$; Figure 2), but there was evidence of moderate heterogeneity between studies ($I^2 = 52.8\%$; $p = 0.013$).

NLR and RFS/PFS in RCC

There were 10 cohorts presenting the data of pre-treatment NLR and RFS/PFS in RCC patients. A significant relationship between elevated pre-treatment NLR and shorter RFS/PFS (HR = 2.18; 95% CI: 1.75-2.71; $p < 0.001$; Figure 3) with non-significant heterogeneity ($I^2 = 25.0\%$; $p = 0.214$) was detected according to our pooled estimates.

Subgroup analysis and meta-regression

To explore the heterogeneity, subgroup analysis and meta-regression were performed by study

region (eastern *vs.* western countries), sample size (≥200 *vs.* <200), cut-off value defining "elevated NLR" (>3 *vs.* ≤3), therapeutic intervention (nephrectomy only *vs.* mixed therapies), type of RCC (clear cell RCC *vs.* non-clear cell RCC/NA; If the majority of patients were clear cell RCC in one study, the study was assigned to clear cell RCC subgroup; NA: not mentioned) and NOS score (≥6 *vs.* <6). Subgroup analysis did not alter the prognostic role of NLR in OS or RFS/PFS substantially (Table 2), except for stratified analysis[28] by cut-off of NLR in PFS/RFS. Meta-regression showed consistent results with subgroup analysis.

Sensitivity analyses

Each single cohort included in our meta-analysis was deleted every time to investigate the influence of individual data set on the pooled HR. Results of sensitivity analyses indicated the robustness of our findings (data not shown).

Publication bias

Visual inspection of the Begg funnel plot revealed asymmetry (p = 0.001 in OS and p = 0.003 in RFS/PFS) (Figure 4A), which raised the possibility of publication bias. Because of this, we undertook sensitivity analysis using the trim and fill method, which conservatively imputes hypothetical negative unpublished studies to mirror the positive studies that cause funnel plot asymmetry. The imputed studies produced a symmetrical funnel plot (Figure 4B). The pooled analysis incorporating the hypothetical studies continued to show a statistically significant association between elevated NLR and prognosis of RCC patients (HR: 1.54, 95% CI: 1.25-1.88; p<0.001 in OS and HR: 1.85, 95% CI, 1.45-2.36; p<0.001 in RFS/PFS).

Discussion

The TNM staging and Robson's staging system cannot estimate the outcomes of RCC patients precisely or guide the clinical practice appropriately, lots of patients in the same stage turned out to be quite different in prognosis. Therefore, introduction of new laboratory index as a supplementary item to current RCC risk stratification system which mainly focuses on the biological characteristics of tumor itself is really urgent for personalizing the optimal treatment strategy.

As hematological tests are routinely conducted in RCC patients before medical intervention, NLR acts as a simple, robust and convenient parameter of the inflammatory response. To our knowledge, the present study is the first meta-analysis systemically and comprehensively determining the exact relationship between elevated NLR and clinical outcomes of RCC patients. We found that increased NLR has an unfavorable effect on both OS and RFS/PFS in RCC patients. As there was heterogeneity existing among included studies, we also conducted subgroup analyses based on study region, sample size, cut-off value of NLR, therapeutic intervention, type of RCC and NOS score. No significant change was found according to subgroups. From the results above, NLR is a promising prognostic biomarker to help make better clinical decision on RCC treatment and outcomes.

We tried to figure out the source of heterogeneity observed among included studies by meta-regression and interaction revisited between subgroup estimates analyses. Though meta-regression did not find any possible reasons for heterogeneity in our meta-analysis for OS, sample size (p = 0.132) and NOS score (p = 0.083) according to results of interaction revisited

between subgroup estimates may partially explain the inter-study heterogeneity. In the same way, we found NLR cut-off value ($p = 0.004$) and tumor type ($p = 0.151$) were responsible for the mild heterogeneity in RFS/PFS. It is inevitable that studies with smaller sample size or lower NOS score are more likely to gain statistic heterogeneity. Authors of included studies defined the cut-off value of NLR, which best discriminated between good and poor survival, on the basis of different methods. And pooled analysis of studies with cut-off value no more than 3 indicated a superior prognostic role in RCC patients than studies with cut-off value higher than 3. We suppose the variance of NLR between high and low risk groups is larger when cut-off value is small, which may more veritably reflect the role of NLR in outcome of RCC patients.

Although the funnel plot analysis showed some asymmetry in our meta-analysis suggesting the possibility of publication bias, the trim and fill sensitivity analysis did not change the general result, suggesting that the association of higher NLR value and poorer prognosis of RCC patients is not an artifact of unpublished negative studies.

In our analysis, subgroup defined as Nephrectomy only also represented patients group with clinically localized disease, while patients with metastatic disease were stratified to the mixed therapies subgroup. According to our results, elevated NLR was associated with both increased risk of future recurrence in localized disease and accelerated disease progression as well as shortened overall survival in advanced disease. Therefore, we should take a more active attitude in RCC patient treatment, for example, consolidation and maintenance therapy, cytoreductive nephrectomy, especially in patients with elevated NLR before treatment.

Owing to limited data from available studies, we did not conduct pooled analysis on the correlation between elevated NLR and the clinicopathological parameters of RCC. As reported in

several studies[20,22,25], high NLR was closely correlated with a more malignant tumor characteristics, as well as changed blood and biologic indexes. Taken all these into consideration, there may be a significant association between NLR and pathologic features and other known risk factors of RCC, but more clinical studies focusing on these relationships are still needed to help us better understand how NLR influences prognosis of RCC patients.

There are other laboratory markers of systemic inflammation reaction besides NLR, such as C-reactive protein[29] and modified Glasgow prognostic score[30,31], playing a prognostic role in RCC patients. What's more, gene polymorphisms[32] and biological markers[33,34] are also suggested to be predictors of prognosis in RCC patients. However, factoring in cost-effective analysis and accessibility, NLR stands out for its low economic costs and widely availableness even in primary hospitals. The results of our meta-analysis encourage routinely monitoring of NLR to predict recurrence, progress and survival outcomes in RCC patients, irrespective of the detailed therapeutic intervention, stage and type of tumor and geographic region.

NLR is an inflammation marker. High NLR represents systemic and local inflammatory response to tumor, which provids a favorable microenvironment for tumor invasion and metastasis[5]. As traditional chemotherapy and immunotherapy are with limited benefit in metastatic RCC, treatment remains quite a challenge for clinicians. Now targeted therapy on vascular endothelial growth factor (VEGF) is generally recognized as first choice for metastatic patients[35]. A major difficulty in developing anti-VEGF therapies is tumor intrinsic refractoriness and the emergence of treatment-induced resistance. Tumor-associated macrophages (TAMs) are identified to mediate refractoriness to anti-VEGF treatment recently[36]. TAMs promote systemic neutrophilia via secreting cytokines such as IL-6[37], so high NLR is associated with high infiltration of TAMs[38].

On the other hand, tumor can produce immunosuppressive cytokines and reduce cytotoxic T lymphocyte infiltration[39]. Thus NLR not only reflects system immune status but also tumor microenvironment which favors tumor invasion and suppresses the host immune surveillance. Hence, NLR acts as an effective prognostic predictor for VEGF-targeted therapy in metastatic patients.

In conclusion, the present meta-analysis demonstrates that elevated NLR is closely associated with poorer prognostic outcome of RCC patients in different stages. NLR is a widely available, robust and convenient predictor. It helps to figure out patients with high risk and not sensitive to targeted therapy, for whom clinician are urged to adjust the management accordingly. Further research on the best therapeutic schedule fitted with patients of high NLR is needed in the near future.

Contributorship Statement

Study concept and design: All authors. Acquisition, analysis, or interpretation of data: Hu, Lou. Drafting of the manuscript: Hu. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Hu, Lou. Obtained funding: Zhang. Administrative, technical, ormaterial support: Zhang. Study supervision: Zhang, Ye.

Competing Interests

There were no competing interests.

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Data Sharing

There was no additional unpublished data.

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Tables

Table 1. Main characteristics of included studies in the meta-analysis.

Study cohort	Year	Study region	Research time	Follow-up (month)	Treatment	No. (M/F)	Age (years)	Tumor type	No. of distal metastasis
Martino et al[13]	2013	USA	1995-2012	Mean:49; IQR: (15-71)	Radical and partial nephrectomy	202/79	Mean: 63; IQR: (54-72)	nonclear cell RCC	0
Ohno et al[14]	2012	Japan	1990-2008	Mean±SD: (75±54)	Radical and partial nephrectomy	186/64	Mean±SD:(61±12)	clear cell RCC	0
Ohno et al[10]	2014	Japan	1990-2008	Mean (range): 20.6(1-114)	Cytoreductive nephrectomy: Yes 48; No 25	61/12	Cytoreductive nephrectomy [Median (range)]: Yes: 63(38-79); No: 65(34-88)	mRCC	73
Dirican et al[16]	2013	Turkey	2006-2011	Median:13.43; Range: (1.97-40.91)	Nephrectomy, INF-α,sunitinib	17/6	Median (range): 59(43-76)	clear cell RCC:18; Non-clear cell RCC: 5	23
Keizman et al[17]	2014	USA, Israel	2004-2013	NA	Sunitinib	186/92	Median: 63	mRCC	278
Santoni et al[18]	2013	Italy	2005-2013	Median:46.9; 95% CI: (39.9-53.9)	Past nephrectomy: 91; second-line everolimus: 65; third-line everolimus: 32	70/27	Median:64; 95% CI: (44-82)	mRCC	97
Cetin et al[19]	2013	Turkey	2008-2011	Median:15; Range: (1-53)	First line therapy with IFN-α; second line therapy with VEGF targeted TKIs	76/24	Median (range): 58(24-80)	mRCC: clear cell 73; non-clear cell 24; unknown 3	100: liver 17; bone 24; lung 65
Forget et al[20]	2013	Belgium	1993-2005	Median:74.5; IQR: (31-112)	Radical nephrectomy	71/156	Mean±SD: (63±12)	Clear cell 166; tubulo-papillary 29;	0

								chromophobe 4; others 28	
Pichler et al[21]	2013	Austria	2000-2010	Mean (range): 44(0-130)	Curative radical or partial nephrectomy	Total: 678	Mean±SD: (63.7±11.9)	clear cell RCC	0
Kobayashi et al[22]	2013	Japan	2008-2012	Median:12; Range: (1.1-48.9)	Radical nephrectomy, cytokine therapy and sorafenib, sunitinib or mTORi	44/14;	Median (range): 64(53-81)	mRCC	26
Templeton et al[23]	2014	Canada	NA	NA	Targeted therapy	Total: 859	NA	RCC	NA
Fox et al[24]	2013	Australia	2002-2005	NA	As in EGF20001	268/94	Median (range): 62(19-84)	mRCC	362
Huang et al[25]	2011	USA	2004-2011	Median: 35	Sunitinib	Total: 109	NA	mRCC	109
Hatakeyama et al[26]	2013	Japan	1995-2013	Surgery： 26；immunotherapy or IFN-α： 5	Radical nephrectomy with thrombectomy, immunotherapy or IFN-α	55/30;	Mean±SD: (62±12)	RCC with tumor thrombus	14

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Study cohort	NLR value	Cut-off	No. of elevated NLR	Survival analysis	HR	Adjustment variables	NOS score
Martino et al[13]	Median (IQR): 2.6(1.9-3.6)	3.6	NA	RFS (DFS)	R(M)	Age, gender, ECOG performance score, pT stage, TNM group, grade, MVI, subtype, ANC, ALC	7
Ohno et al[14]	Mean±SD: 2.62 ± 1.44	2.7	84	RFS	R(M)	Age, presentation, nephrectomy, tumor size, pT, grade, MVI, eastern Cooperative Oncology Group, neutriphil, lymphocytes	8
Ohno et al[10]	Mean±SD: 3.98 ± 2.27	4	NA	OS	R(M)	Age, presentation mode, T stage, ECOG PS, Charlson comorbidity index, hemoglobin, LDH, corrected calcium, CRP, neutrophils, Lymphocytes	5

Dirican et al[16]	NA	3	NA	OS,PFS	E(U)	/	4
Keizman et al[17]	NA	3	NA	OS,PFS	R(M)	unclear	5
Santoni et al[18]	Median: 2.2	3	38	OS,PFS	R(M)	Gender, age, Motzer prognostic group, PFS on first-line therapy, neutrophilia	6
Cetin et al[19]	Median: 3.04	3.04	50	OS,PFS	R(U)	Age, tumor history, sex, hemoglobin level, red cell distribution width, albumin level, alkaline phosphatase level, PFS, site and number of metastatic orgen, MSKCC score, dose reduction, second-line mTOR inhibitors	5
Forget et al[20]	Median (IQR): 3.01(1.97-4.49)	5	52	OS,RFS	R(U)	Age, sex, node status, histological grade, stage	8
Pichler et al[21]	Mean±SD: 3.51 ± 2.49	3.3	398	OS,RFS(MFS),CSS	R(M)	Age, gender, T stage, tumor grade, presence of tumor necrosis	7
Kobayashi et al[22]	Mean±SD: sorafenib: 4.25±3.01; sunitinib: 4.50±3.43; mTORi: 4.26±2.87	4.41	sorafenib: 8; sunitinib: 23; mTORi: 16	OS,PFS	R(M) in OS, E(U) in PFS	Karnofsky PS, metastasis at presentation, number of metastasis, prior nephrectomy, prior cytokine therapy, initial targeted agent, Heng's risk classification, pre-treatment level of hemoglobin, platelet count, albumin, CRP, corrected calcium	5
Templeton et al[23]	Mean: 4.98; Median(95%CI): 3.51(1.42-14.0)	2.5	622	OS	R(M), E(U)	6 international metastatic renal cell carcinoma database consortium(IMDC)	/
Fox et al[24]	NA	3	188	OS	R(M)	MSKCC and systemic inflammation markers	7
Huang et al[25]	NA	3	57	OS,PFS	R(U)	/	/
Hatakeyama et al[26]	Mean±SD: 3.1 ± 1.5	NA	NA	OS	R(U,M)	Age, ECOG-performance status, gender, thrombus level, distant metastasis, underwent surgy, hemoglobin, serum albumin, eGFR,	5

						choline esterase, serum sodium, correlated calcium, LDH, CRP, Charison comorbidity index, molecular targeted agents	

NLR: neutrophil-lymphocyte ratio; mRCC: metastatic renal cell carcinoma; OS: overall survival; RFS: recurrence free survival; DFS: disease free survival; PFS: progress free survival; MFS: metastasis free survival; IQR: interquartile range; HR: hazard ratio, obtained by reporting in text (R), or estimating (E). "M" means the HR comes from multivariate analysis, "U" means the HR comes from univariate analysis; NA: not available; NOS: Newcastle-Ottawa Quality Scale.

Table 2. Summary of subgroup analyses results.

Analysis		N		Random-effects model		Heterogeneity		Interaction revisited		Meta-regression
			References	HR(95%CI)	p	I^2	p	Ratio of Hazard ratio (RHR) (95%CI)	p	p
Overal survival (OS)		12(13)	10,17-27	1.82(1.51-2.19)	＜0.001	52.80%				
Subgroup 1: Study region	Western countries	7	10,17,19,20,22-24	1.73(1.39-2.14)	＜0.001	39.80%	0.126			
	Eastern countries	5(6)	18,21,25-27	2.06(1.41-3.02)	＜0.001	67.70%	0.013	0.84(0.54-1.30)	0.434	0.680
Subgroup 2: Sample size	≥200	5	18,21,22,24,25	1.60(1.30-1.96)	＜0.001	34.60%	0.190			
	＜200	7(8)	10,17,19,20,23,26,27	2.16(1.55-3.01)	＜0.001	62.80%	0.013	0.74(0.50-1.09)	0.132	0.305
Subgroup 3: Cut-off value	＞3	5	10,20-23	2.04(1.47-2.82)	＜0.001	28.20%	0.234			

	≤3	6	17-19,24-26	2.07(1.51-2.83)	<0.001	63.60%	0.017	0.99(0.63-1.55)	0.950	0.959
Subgroup 4: Therapeutic intervention	Nephrectomy only	2	21,22	1.52(1.06-2.17)	0.022	0	0.615			
	Mixed therapies	10(11)	10,17-20,23-27	1.92(1.54-2.38)	<0.001	60.10%	0.005	0.79(0.52-1.20)	0.275	0.424
Subgroup 5: NOS score	≥6	4	19,21,22,25	1.51(1.24-1.84)	<0.001	0	0.594			
	<6	8(9)	10,17,18,20,23,24,26,27	2.06(1.51-2.70)	<0.001	65.10%	0.003	0.73(0.51-1.04)	0.083	0.313
Subgroup 6: Tumor type	Non-clear cell RCC/NA	7(8)	10,18,19,23,24,26,27	1.87(1.45-2.42)	<0.001	58.20%	0.065			
	Clear cell RCC	5	17,20-22,25	1.82(1.32-2.50)	<0.001	53.70%	0.067	1.03(0.68-1.55)	0.891	0.859
Progress free survival (PFS)/Recurrence free survival (RFS)		10	14,15,17-23,26	2.18(1.75-2.71)	<0.001	25%				
Subgroup 1: Study region	Western countries	6	14,18,19,21,22,26	2.20(1.64-2.96)	<0.001	35.70%	0.169			
	Eastern countries	4	15,17,20,23	2.23(1.51-3.28)	<0.001	28.60%	0.241	0.99(0.61-1.61)	0.957	0.958
Subgroup 2: Sample	≥200	5	14,15,18,21,22	2.25(1.56-3.24)	<0.001	51.30%	0.084			

size										
	＜200	5	17,19,20,23,26	2.15(1.62-2.85)	＜0.001	0	0.444	1.05(0.66-1.66)	0.847	0.950
Subgroup 3: Cut-off value	＞3	5	14,20-23	1.74(1.39-2.17)	＜0.001	0	0.675			
	≤3	5	15,17-19,26	3.08(2.24-4.24)	＜0.001	0	0.867	0.56(0.38-0.83)	0.004	0.020
Subgroup 4: Therapeutic intervention	Nephrectomy only	4	14,15,21,22	2.00(1.40-2.85)	＜0.001	39.90%	0.172			
	Mixed therapies	6	17-20,21,26	2.36(1.79-3.12)	＜0.001	11.40%	0.342	0.85(0.54-1.33)	0.472	0.404
Subgroup 5: NOS score	≥6	5	14,15,19,21,22	2.08(1.53-2.84)	＜0.001	33.60%	0.197			
	＜6	5	17,18,20,23,26	2.35(1.67-3.32)	＜0.001	26.50%	0.245	0.89(0.56-1.41)	0.605	0.622
Subgroup 6: Tumor type	Non-clear cell RCC/NA	5	14,18,19,23,26	2.62(1.94-3.53)	＜0.001	0	0.644			
	Clear cell RCC	5	15,17,20-22	1.92(1.42-2.59)	＜0.001	34.10%	0.194	1.36(0.89-2.09)	0.151	0.112

N: number of studies (cohorts); HR: hazard ratio; 95%CI: 95% confidence interval; Subgroup analyses for OS and RFS/PFS were performed by study region (eastern vs. western countries), sample size (≥200 vs. ＜200), cut-off value (＞3 vs. ≤3), therapeutic intervention (nephrectomy only vs. mixed therapies), type of RCC (Clear cell RCC vs. Non-clear cell RCC/NA) and NOS score (≥6 vs. ＜6). Interaction revisited of estimates between subgroups and meta-regression were also applied to figure out heterogeneity among studies.

Figure Legends

Figure 1. Flow chart of study selection process.

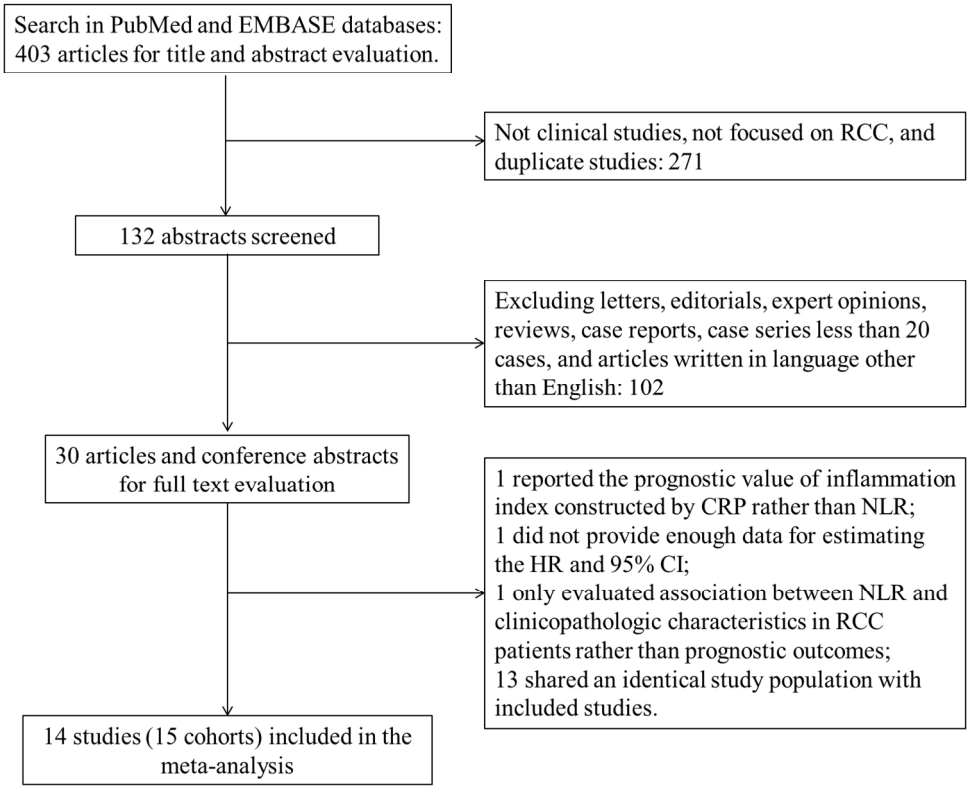
RCC: renal cell carcinoma; CRP: C-reactive protein; NLR: neutrophil-lymphocyte ratio; HR: hazard ratio; CI: confidence interval.

Figure 2. Meta-analysis of the association between elevated NLR and OS of RCC. Results are presented as individual and pooled hazard ratio (HR) and 95% confidence interval (CI).

Figure 3. Meta-analysis of the association between elevated NLR and RFS/PFS of RCC. Results are presented as individual and pooled hazard ratio (HR) and 95% confidence interval (CI).

Figure 4. Funnel plots without and with trim and fill.

The pseudo 95% confidence interval (CI) is computed as part of the analysis that produces the funnel plot, and corresponds to the expected 95% CI for a given standard error (SE). HR indicates hazard ratio.



Flow chart of study selection process.
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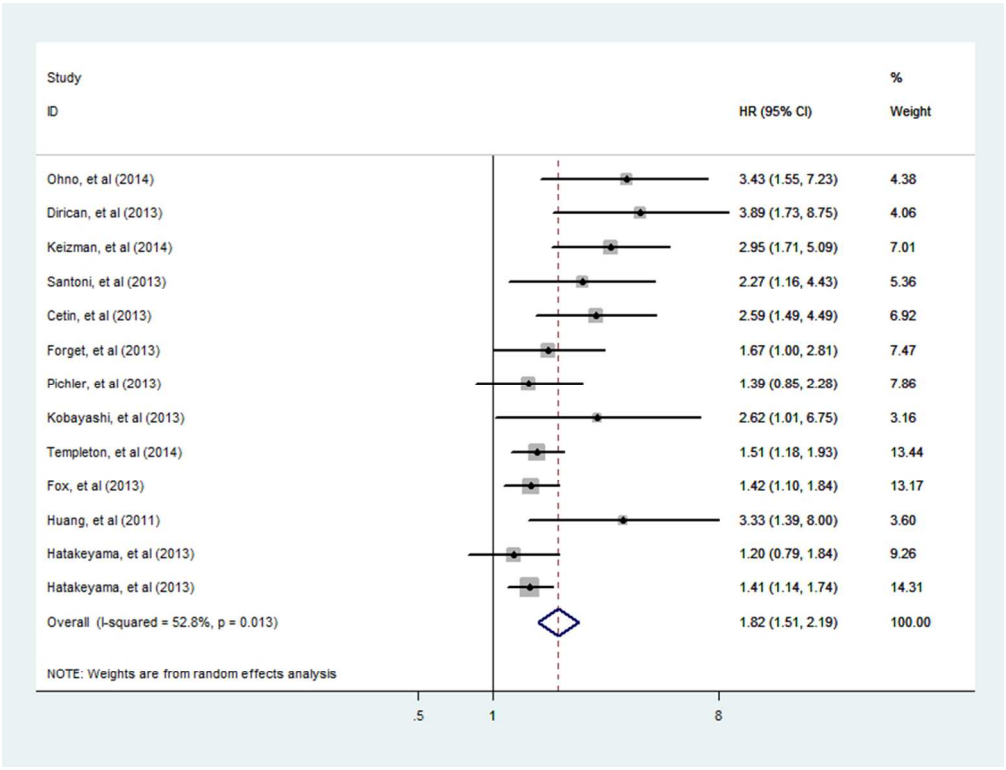

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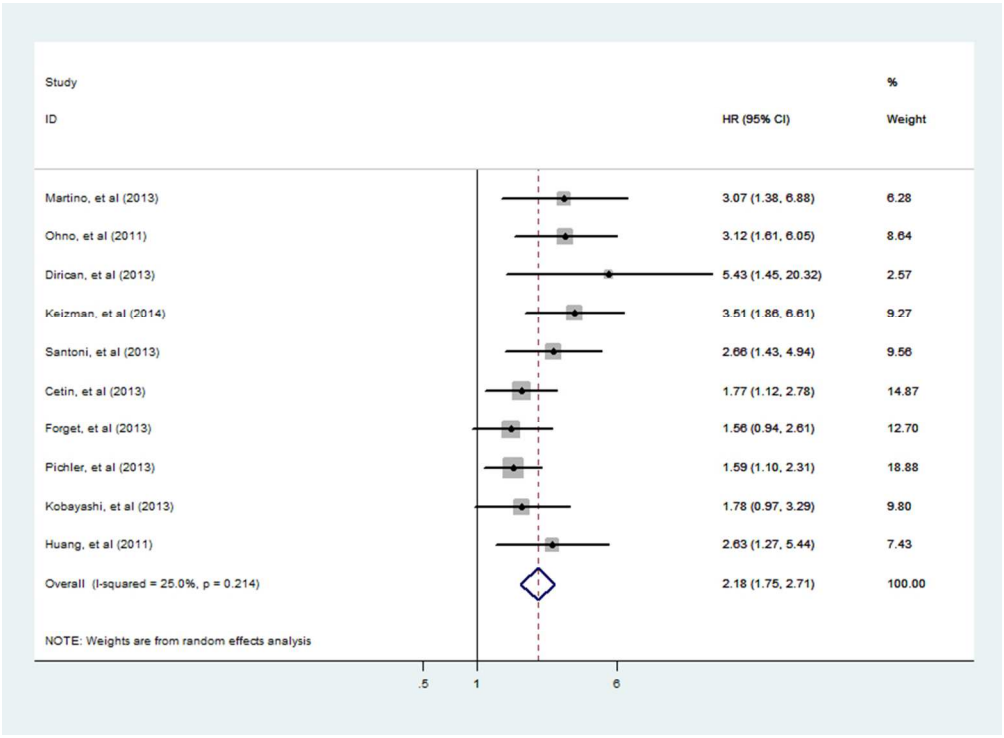

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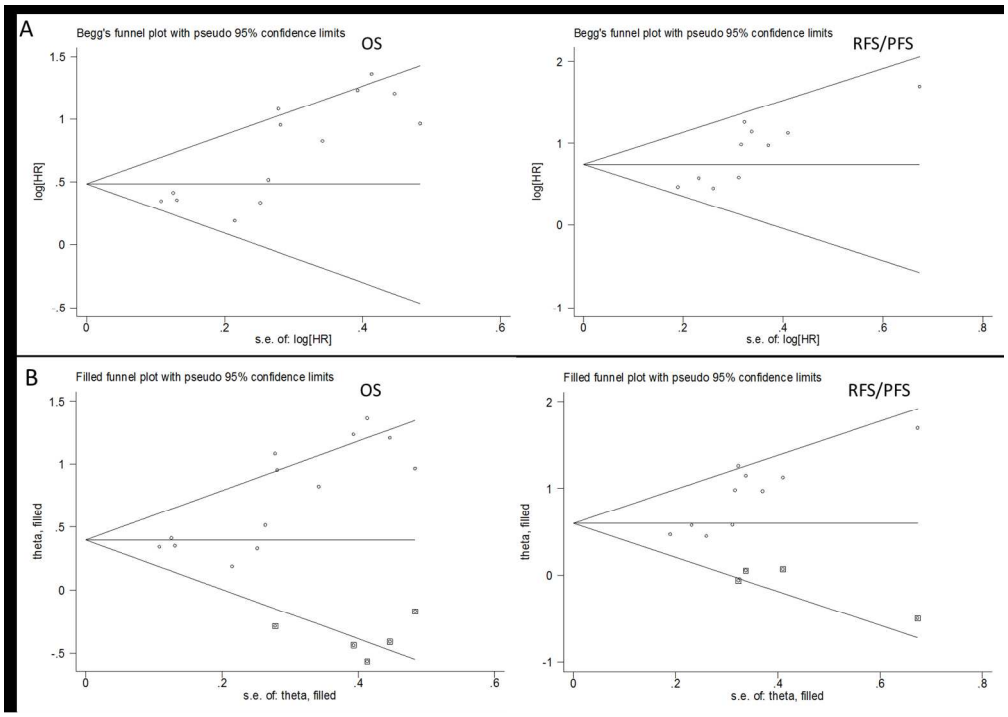

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Title page

Prognostic Role of Neutrophil-Lymphocyte Ratio in Renal Cell Carcinoma: A Meta-Analysis

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Abstract

Objective: Increasing evidence suggests that cancer-associated inflammation is associated with poor prognosis in cancer patients. The role of neutrophil-lymphocyte ratio (NLR) as a predictor in renal cell carcinoma (RCC) remains controversial. We conducted the meta-analysis to determine the association between NLR and clinical outcome of RCC patients.

Methods and materials: Studies were identified from PubMed and EMBASE databases in March 2014. Meta-analysis was performed to generate combined hazard ratios (HRs) with 95% confidence intervals (95% CIs) for overall survival (OS) and recurrence/progress-free survival (RFS/PFS).

Results: Fifteen cohorts containing 3357 patients were included. Our analysis results indicated that elevated NLR predicted poorer OS (HR: 1.82, 95%CI: 1.51-2.19) and RFS/PFS (HR: 2.18, 95% CI: 1.75-2.71) in RCC patients. These findings were robust when stratified by study region, sample size, therapeutic intervention, types of RCC and study quality. However, it significantly differed by assessment of the cut-off value defining "elevated NLR" in RFS/PFS (p = 0.004). The heterogeneity in our meta-analysis was mild to moderate.

Conclusions: Elevated NLR indicates poorer prognosis for patients with RCC. NLR should be monitored in RCC patients for rational risk stratification and adjusting the management accordingly.

Keywords: neutrophil-lymphocyte ratio, renal cell carcinoma, prognosis, meta-analysis

Strengths and limitations of this study

Our study is the first systematic meta-analysis evaluating the relationship between elevated NLR

and prognosis in RCC patients. Our analysis provides substantial evidence that elevated NLR is significantly associated with poorer outcomes of RCC patients. However, there were some limitations in our study. The enrolled studies were retrospective cohort studies, publication bias inevitably existed. We conducted "trim and fill" analysis to show our conclusion was robust. There was some heterogeneity in the included patient populations, so we confirmed the prognostic role of NLR in patients with different disease stage, therapeutic intervention and types of RCC by subgroup analysis. We only searched limited databases (PubMed and EMBASE), which might weaken the estimating power of the pooled estimate.

Introduction

Renal cell carcinoma (RCC) accounts for 2–3% of all malignant diseases in adults. It's the seventh most common cancer in men and the ninth in women worldwide[1,2]. The incidence of this cancer varies geographically and has increased over past decades owing to changes in life style and environment[1]. Despite a rapid development in surgical resection, immunotherapy and targeted therapy in RCC management, the long-term outcome is still not promising mainly due to common local recurrence, distal metastasis and limited drug response[3]. Hence, it is important to identify significant biomarkers, which can help clinicians to stratify patients in terms of prognosis and possibility of metastatic recurrence together with tumor staging system, *i.e.* the TNM staging system and Robson's staging system, and then set the most appropriate therapeutic strategy.

It is well recognized that the heterogeneity in clinical outcomes is determined by both oncological characteristics of tumor itself and host's response to the progressing malignancy[4]. The complicated mechanisms by which cancer and inflammation intersect have been gradually

revealed. Inflammation impacts every single step of tumorigenesis, from tumor initiation to promotion and metastatic progression[5]. Recently, several serum biomarkers and haematological indices representative of inflammatory response, notably C-reactive protein (CRP), fibrinogen, lymphocyte-monocyte ratio (LMR), neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR), have been demonstrated to be closely related to poor prognosis of RCC patients[6-9].

Generally speaking, lymphopenia well reflects impaired cell-mediated immunity, while neutrophilia represents a response to systematic inflammation[5]. So the NLR, defined as neutrophil counts divided by lymphocyte counts, is particularly noteworthy. Emerging evidences have shown that NLR gained its prognostic value in patients with colorectal cancer[10] and hepatocellular carcinoma[11]. RCC patients with elevated level of pre-treatment NLR may be more likely to gain a poorer clinical outcome[12]. But the exact role of NLR in RCC patients is not consistent in different studies due to the variance in study design, sample size and other factors. Some concluded significant relationship between higher NLR and poorer prognosis, while others did not. Therefore, it is necessary to perform a meta-analysis to systematically and comprehensively understand the prognostic value of NLR in RCC patients.

In this study, we aimed to assess the prognostic significance of high NLR for overall survival (OS) and recurrence-free survival (RFS) / progress-free survival (PFS) in RCC patients by pooling outcomes from available data.

Material and Methods

Search strategy

A comprehensive literature search of PubMed and EMBASE databases (Up to March 2014) was

conducted to identify relevant studies. The search strategy included terms for: "NLR" (e.g., "neutrophil to lymphocyte ratio", "neutrophil lymphocyte ratio" and "neutrophil-lymphocyte ratio"), "RCC" (e.g., "renal cancer", "renal carcinoma", "kidney cancer", clear cell carcinoma", "non-clear cell carcinoma", and "renal papillary carcinoma") and "prognosis" (e.g., "recurrence", "survival" and "outcome"). Abstracts and information from conferences were collected independently. The reference list was also checked for additional articles. Only studies published in English were included.

Study inclusion criteria and definitions

Two independent authors (Hu KM and Lou LX) reviewed the retrieved studies and extracted data from each included study. Discrepancies were resolved by discussion. Studies included in our meta-analysis must meet the following criteria: (1) The diagnosis of RCC was based on the current clinical guidelines; (2) NLR was measured by serum-based methods before formal treatment; (3) Studies reported hazard ratios (HRs) and 95% confidence intervals (95% CIs) for pre-treatment NLR in OS and (or) RFS/PFS, or allowed for calculation from raw data contained in the article; (4) Only primary data or data superseded earlier work were included, and articles were superior to conference abstracts.

NLR was defined as the serum absolute neutrophil count divided by lymphocyte count in peripheral blood[13]. OS was defined as the interval between the medical treatment and the death or the last follow-up of patients. RFS (disease free survival / metastasis free survival, DFS/MFS) was measured from the date of curative treatment until the detection of tumor recurrence. PFS was calculated from the date of treatment until progressing of disease. If all the patients in the

individual study only received curative nephrectomy, the study was classified into nephrectomy only subgroup, and the studies in which patients were mainly treated by non-surgical intervention were classified into mixed therapies subgroup.

Data extraction

We extracted data including: (1) study information including name of first author, year of publication, study region, sample size, time of research; (2) patient characters including age, gender, follow-up period and treatment methods; (3) data about RCC including type, size, stage and distal metastasis; (4) NLR data and cut-off value of NLR; (5) survival data including OS and RFS/PFS.

Quality assessment of primary studies

Quality assessment of included studies was evaluated with the Newcastle-Ottawa quality assessment scale (NOS) range from 0 to 8 by two independent investigators (Hu KM and Lou LX). Studies with NOS score of $\geq$ 6 were assigned as high-quality ones. Studies from conference abstracts were defined as low-quality studies. Any inconsistencies were resolved by joint discussion.

Statistical analysis

HR abstracted in each study greater than one favored that elevated NLR indicated a poor prognosis. Multivariate analysis for HR was superior to univariate analysis unless adjustment variables in multivariable analysis significantly interacting with NLR level. As heterogeneity was

detected among primary studies, meta-analysis was pooled using the random effects models with DerSimonian Laird method[14]. Between-study heterogeneity was assessed using Cochran Q test and I^2 statistic. $P < .10$ was considered statistically significant for Cochran Q test, $I^2 > 50\%$ indicating substantial heterogeneity between studies. Potential sources of heterogeneity were then investigated using subgroup analyses and meta-regression. All statistical tests were two-sided and the significance level was set at 0.05. The possibility of publication bias was assessed using the Begg test and visual insection of a funnel plot[15]. We also performed the Duval and Tweedie nonparametric "trim and fill" procedure to further assess the possible effect of publication bias in our meta-analysis[16]. All statistical manipulations were undertaken using the program STATA version 12.0 (Stata Corporation, College Station, TX).

Results

Study characteristics

The initial search algorithm retrieved a total of 403 studies. After the title and abstract reviewed, only 30 records were identified regarding the association of NLR and RCC (Figure 1). After full-text review, a total of 14 retrospective studies[12,17-29] (15 cohorts) with 3357 RCCs were included in our meta-analysis. The study by Hatakeyama et al[29] reported the HR and 95% CI of two different cohorts separately. If the patients were overlapping or partially overlapping in several studies, only the study with the most complete data was included.

The basic features of the 14 studies were summarized in Table 1. Median quality score of the involved studies was 6 (range: 4-8). Eight studies were from western countries, including the USA, Italy, Belgium, Austria, Canada, and Australia. The rest studies were from Turkey and Japan.

Seven of these cohorts enrolled more than 200 patients and eight had less than 200 patients. Radical and partial nephrectomy as only initial treatment for non-metastatic RCC was reported in four studies. Others were treated with mixed therapies, including nephrectomy, immunotherapy, targeted therapy and others. NLR was calculated using the white blood cell differentiated counts in all studies. In the study by Cetin et al.[22], some of the adjustment variables used in multivariate analysis was significantly associated with NLR value, so HR and 95% CI from univariate analysis for both PFS and OS were used in our meta-analysis.

NLR and OS in RCC

There were 13 cohorts presenting the data of pre-treatment NLR and OS in RCC patients. Elevated NLR was significantly associated with shorter OS (HR = 1.82; 95% CI: 1.51-2.19; $p < 0.001$; Figure 2), but there was evidence of moderate heterogeneity between studies ($I^2 = 52.8\%$; $p = 0.013$).

NLR and RFS/PFS in RCC

There were 10 cohorts presenting the data of pre-treatment NLR and RFS/PFS in RCC patients. A significant relationship between elevated pre-treatment NLR and shorter RFS/PFS (HR = 2.18; 95% CI: 1.75-2.71; $p < 0.001$; Figure 3) with non-significant heterogeneity ($I^2 = 25.0\%$; $p = 0.214$) was detected according to our pooled estimates.

Subgroup analysis and meta-regression

To explore the heterogeneity, subgroup analysis and meta-regression were performed by study

region (eastern *vs.* western countries), sample size (≥200 *vs.* <200), cut-off value defining "elevated NLR" (>3 *vs.* ≤3), therapeutic intervention (nephrectomy only *vs.* mixed therapies), type of RCC (clear cell RCC *vs.* non-clear cell RCC/NA; If the majority of patients were clear cell RCC in one study, the study was assigned to clear cell RCC subgroup; NA: not mentioned) and NOS score (≥6 *vs.* <6). Subgroup analysis did not alter the prognostic role of NLR in OS or RFS/PFS substantially (Table 2), except for stratified analysis[30] by cut-off of NLR in PFS/RFS. Meta-regression showed consistent results with subgroup analysis.

Sensitivity analyses

Each single cohort included in our meta-analysis was deleted every time to investigate the influence of individual data set on the pooled HR. Results of sensitivity analyses indicated the robustness of our findings (data not shown).

Publication bias

Visual inspection of the Begg funnel plot revealed asymmetry (p = 0.001 in OS and p = 0.003 in RFS/PFS) (Figure 4A), which raised the possibility of publication bias. Because of this, we undertook sensitivity analysis using the trim and fill method, which conservatively imputes hypothetical negative unpublished studies to mirror the positive studies that cause funnel plot asymmetry. The imputed studies produced a symmetrical funnel plot (Figure 4B). The pooled analysis incorporating the hypothetical studies continued to show a statistically significant association between elevated NLR and prognosis of RCC patients (HR: 1.54, 95% CI: 1.25-1.88; p<0.001 in OS and HR: 1.85, 95% CI, 1.45-2.36; p<0.001 in RFS/PFS).

Discussion

The TNM staging and Robson's staging system cannot estimate the outcomes of RCC patients precisely or guide the clinical practice appropriately, lots of patients in the same stage turned out to be quite different in prognosis. Therefore, introduction of new laboratory index as a supplementary item to current RCC risk stratification system which mainly focuses on the biological characteristics of tumor itself is really urgent for personalizing the optimal treatment strategy.

As hematological tests are routinely conducted in RCC patients before medical intervention, NLR acts as a simple, robust and convenient parameter of the inflammatory response. To our knowledge, the present study is the first meta-analysis systemically and comprehensively determining the exact relationship between elevated NLR and clinical outcomes of RCC patients. We found that increased NLR has an unfavorable effect on both OS and RFS/PFS in RCC patients. As there was heterogeneity existing among included studies, we also conducted subgroup analyses based on study region, sample size, cut-off value of NLR, therapeutic intervention, type of RCC and NOS score. No significant change was found according to subgroups. From the results above, NLR is a promising prognostic biomarker to help make better clinical decision on RCC treatment and outcomes.

We tried to figure out the source of heterogeneity observed among included studies by meta-regression and interaction revisited between subgroup estimates analyses. Though meta-regression did not find any possible reasons for heterogeneity in our meta-analysis for OS, sample size (p = 0.132) and NOS score (p = 0.083) according to results of interaction revisited

between subgroup estimates may partially explain the inter-study heterogeneity. In the same way, we found NLR cut-off value ($p = 0.004$) and tumor type ($p = 0.151$) were responsible for the mild heterogeneity in RFS/PFS. It is inevitable that studies with smaller sample size or lower NOS score are more likely to gain statistic heterogeneity. Authors of included studies defined the cut-off value of NLR, which best discriminated between good and poor survival, on the basis of different methods. Pooled analysis of studies with cut-off value no more than 3 indicated a superior prognostic role in RCC patients than studies with cut-off value higher than 3. We suppose that some patients with poor outcomes were wrongly classified into the low risk group if the cut-off is too large, which leads to an underestimate of the role of NLR in outcomes of RCC patients. Although NLR is a sensitive prognostic indicator in retrospective researches, prospective clinical trials are still warranted to evaluate the exact value of NLR in predicting the prognosis of RCC patients.

Although the funnel plot analysis showed some asymmetry in our meta-analysis suggesting the possibility of publication bias, the trim and fill sensitivity analysis did not change the general result, suggesting that the association of higher NLR value and poorer prognosis of RCC patients is not an artifact of unpublished negative studies.

In our analysis, subgroup defined as Nephrectomy only also represented patients group with clinically localized disease, while patients with metastatic disease were stratified to the mixed therapies subgroup. According to our results, elevated NLR was associated with both increased risk of future recurrence in localized disease and accelerated disease progression as well as shortened overall survival in advanced disease. Therefore, we should take a more active attitude in RCC patient treatment, for example, consolidation and maintenance therapy, cytoreductive

nephrectomy, especially in patients with elevated NLR before treatment.

Owing to limited data from available studies, we did not conduct pooled analysis on the correlation between elevated NLR and the clinicopathological parameters of RCC. As reported in several studies[22,24,27], high NLR was closely correlated with a more malignant tumor characteristics, as well as changed blood and biologic indexes. Taken all these into consideration, there may be a significant association between NLR and pathologic features and other known risk factors of RCC, but more clinical studies focusing on these relationships are still needed to help us better understand how NLR influences prognosis of RCC patients.

There are other laboratory markers of systemic inflammation reaction besides NLR, such as C-reactive protein[31] and modified Glasgow prognostic score[32,33], playing a prognostic role in RCC patients. What's more, gene polymorphisms[34] and biological markers[35,36] are also suggested to be predictors of prognosis in RCC patients. However, factoring in cost-effective analysis and accessibility, NLR stands out for its low economic costs and widely availableness even in primary hospitals. The results of our meta-analysis encourage routinely monitoring of NLR to predict recurrence, progress and survival outcomes in RCC patients, irrespective of the detailed therapeutic intervention, stage and type of tumor and geographic region.

NLR is an inflammation marker. High NLR represents systemic and local inflammatory response to tumor, which provids a favorable microenvironment for tumor invasion and metastasis[5]. As traditional chemotherapy and immunotherapy are with limited benefit in metastatic RCC, treatment remains quite a challenge for clinicians. Now targeted therapy on vascular endothelial growth factor (VEGF) is generally recognized as first choice for metastatic patients[37]. A major difficulty in developing anti-VEGF therapies is tumor intrinsic refractoriness and the

emergence of treatment-induced resistance. Tumor-associated macrophages (TAMs) are identified to mediate refractoriness to anti-VEGF treatment recently[38]. TAMs promote systemic neutrophilia via secreting cytokines such as IL-6[39], so high NLR is associated with high infiltration of TAMs[40]. On the other hand, tumor can produce immunosuppressive cytokines and reduce cytotoxic T lymphocyte infiltration[41]. Thus NLR not only reflects system immune status but also tumor microenvironment which favors tumor invasion and suppresses the host immune surveillance. Hence, NLR acts as an effective prognostic predictor for VEGF-targeted therapy in metastatic patients.

In conclusion, the present meta-analysis demonstrates that elevated NLR is closely associated with poorer prognostic outcome of RCC patients in different stages. NLR is a widely available, robust and convenient predictor. It helps to figure out patients with high risk and not sensitive to targeted therapy, for whom clinician are urged to adjust the management accordingly. Further research on the best therapeutic schedule fitted with patients of high NLR is needed in the near future.

Contributorship Statement

Study concept and design: All authors. Acquisition, analysis, or interpretation of data: Hu, Lou. Drafting of the manuscript: Hu. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Hu, Lou. Obtained funding: Zhang. Administrative, technical, ormaterial support: Zhang. Study supervision: Zhang, Ye.

Competing Interests

There were no competing interests.

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Data Sharing

There was no additional unpublished data.

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Tables

Table 1. Main characteristics of included studies in the meta-analysis.

Study cohort	Year	Study region	Research time	Follow-up (month)	Treatment	No. (M/F)	Age (years)	Tumor type	No. of distal metastasis
Martino *et al*[17]	2013	USA	1995-2012	Mean:49; IQR: (15-71)	Radical and partial nephrectomy	202/79	Mean: 63; IQR: (54-72)	nonclear cell RCC	0
Ohno *et al*[18]	2012	Japan	1990-2008	Mean±SD: (75±54)	Radical and partial nephrectomy	186/64	Mean±SD:(61±12)	clear cell RCC	0
Ohno *et al*[12]	2014	Japan	1990-2008	Mean (range): 20.6(1-114)	Cytoreductive nephrectomy: Yes 48; No 25	61/12	Cytoreductive nephrectomy [Median (range)]: Yes: 63(38-79); No: 65(34-88)	mRCC	73
Dirican *et al*[19]	2013	Turkey	2006-2011	Median:13.43; Range: (1.97-40.91)	Nephrectomy, INF-α,sunitinib	17/6	Median (range): 59(43-76)	clear cell RCC:18; Non-clear cell RCC: 5	23
Keizman *et al*[20]	2014	USA, Israel	2004-2013	NA	Sunitinib	186/92	Median: 63	mRCC	278
Santoni *et al*[21]	2013	Italy	2005-2013	Median:46.9; 95% CI: (39.9-53.9)	Past nephrectomy: 91; second-line everolimus: 65; third-line everolimus: 32	70/27	Median:64; 95% CI: (44-82)	mRCC	97
Cetin *et al*[22]	2013	Turkey	2008-2011	Median:15; Range: (1-53)	First line therapy with IFN-α; second line therapy with VEGF targeted TKIs	76/24	Median (range): 58(24-80)	mRCC: clear cell 73; non-clear cell 24; unknown 3	100: liver 17; bone 24; lung 65
Forget *et al*[23]	2013	Belgium	1993-2005	Median:74.5; IQR: (31-112)	Radical nephrectomy	71/156	Mean±SD: (63±12)	Clear cell 166; tubulo-papillary 29;	0

								chromophobe 4; others 28	
Pichler *et al*[24]	2013	Austria	2000-2010	Mean (range): 44(0-130)	Curative radical or partial nephrectomy	Total: 678	Mean±SD: (63.7±11.9)	clear cell RCC	0
Kobayashi *et al*[25]	2013	Japan	2008-2012	Median:12; Range: (1.1-48.9)	Radical nephrectomy, cytokine therapy and sorafenib, sunitinib or mTORi	44/14;	Median (range): 64(53-81)	mRCC	26
Templeton *et al*[26]	2014	Canada	NA	NA	Targeted therapy	Total: 859	NA	RCC	NA
Fox *et al*[27]	2013	Australia	2002-2005	NA	As in EGF20001	268/94	Median (range): 62(19-84)	mRCC	362
Huang *et al*[28]	2011	USA	2004-2011	Median: 35	Sunitinib	Total: 109	NA	mRCC	109
Hatakeyama *et al*[29]	2013	Japan	1995-2013	Surgery： 26；immunotherapy or IFN-α： 5	Radical nephrectomy with thrombectomy, immunotherapy or IFN-α	55/30;	Mean±SD: (62±12)	RCC with tumor thrombus	14

Study cohort	NLR value	Cut-off	No. of elevated NLR	Survival analysis	HR	Adjustment variables	NOS score
Martino *et al*[17]	Median (IQR): 2.6(1.9-3.6)	3.6	NA	RFS (DFS)	R(M)	Age, gender, ECOG performance score, pT stage, TNM group, grade, MVI, subtype, ANC, ALC	7
Ohno *et al*[18]	Mean±SD: 2.62 ± 1.44	2.7	84	RFS	R(M)	Age, presentation, nephrectomy, tumor size, pT, grade, MVI, eastern Cooperative Oncology Group, neutriphil, lymphocytes	8
Ohno *et al*[12]	Mean±SD: 3.98 ± 2.27	4	NA	OS	R(M)	Age, presentation mode, T stage, ECOG PS, Charlson comorbidity index, hemoglobin, LDH, corrected calcium, CRP, neutrophils, Lymphocytes	5

Dirican *et al*[19]	NA	3	NA	OS,PFS	E(U)	/	4
Keizman *et al*[20]	NA	3	NA	OS,PFS	R(M)	unclear	5
Santoni *et al*[21]	Median: 2.2	3	38	OS,PFS	R(M)	Gender, age, Motzer prognostic group, PFS on first-line therapy, neutrophilia	6
Cetin *et al*[22]	Median: 3.04	3.04	50	OS,PFS	R(U)	Age, tumor history, sex, hemoglobin level, red cell distribution width, albumin level, alkaline phosphatase level, PFS, site and number of metastatic organ, MSKCC score, dose reduction, second-line mTOR inhibitors	5
Forget *et al*[23]	Median (IQR): 3.01(1.97-4.49)	5	52	OS,RFS	R(U)	Age, sex, node status, histological grade, stage	8
Pichler *et al*[24]	Mean±SD: 3.51 ± 2.49	3.3	398	OS,RFS(MFS),CSS	R(M)	Age, gender, T stage, tumor grade, presence of tumor necrosis	7
Kobayashi *et al*[25]	Mean±SD: sorafenib: 4.25±3.01; sunitinib: 4.50±3.43; mTORi: 4.26±2.87	4.41	sorafenib: 8; sunitinib: 23; mTORi: 16	OS,PFS	R(M) in OS, E(U) in PFS	Karnofsky PS, metastasis at presentation, number of metastasis, prior nephrectomy, prior cytokine therapy, initial targeted agent, Heng's risk classification, pre-treatment level of hemoglobin, platelet count, albumin, CRP, corrected calcium	5
Templeton *et al*[26]	Mean: 4.98; Median(95%CI): 3.51(1.42-14.0)	2.5	622	OS	R(M), E(U)	6 international metastatic renal cell carcinoma database consortium(IMDC)	/
Fox *et al*[27]	NA	3	188	OS	R(M)	MSKCC and systemic inflammation markers	7
Huang *et al*[28]	NA	3	57	OS,PFS	R(U)	/	/
Hatakeyama *et al*[29]	Mean±SD: 3.1 ± 1.5	NA	NA	OS	R(U,M)	Age, ECOG-performance status, gender, thrombus level, distant metastasis, underwent surgy, hemoglobin, serum albumin, eGFR,	5

						choline esterase, serum sodium, correlated calcium, LDH, CRP, Charison comorbidity index, molecular targeted agents	

NLR: neutrophil-lymphocyte ratio; mRCC: metastatic renal cell carcinoma; OS: overall survival; RFS: recurrence free survival; DFS: disease free survival; PFS: progress free survival; MFS: metastasis free survival; IQR: interquartile range; HR: hazard ratio, obtained by reporting in text (R), or estimating (E). "M" means the HR comes from multivariate analysis, "U" means the HR comes from univariate analysis; NA: not available; NOS: Newcastle-Ottawa Quality Scale.

Table 2. Summary of subgroup analyses results.

Analysis		N		Random-effects model		Heterogeneity		Interaction revisited		Meta-regression
			References	HR(95%CI)	p	I^2	p	Ratio of Hazard ratio (RHR) (95%CI)	p	p
Overal survival (OS)		12(13)	12,19-29	1.82(1.51-2.19)	<0.001	52.80%				
Subgroup 1: Study region	Western countries	7	20,21,23,24,26-28	1.73(1.39-2.14)	<0.001	39.80%	0.126			
	Eastern countries	5(6)	12,19,22,25,29	2.06(1.41-3.02)	<0.001	67.70%	0.013	0.84(0.54-1.30)	0.434	0.680
Subgroup 2: Sample size	≥200	5	20,23,24,26,27	1.60(1.30-1.96)	<0.001	34.60%	0.190			
	<200	7(8)	12,19,21,22,25,28,29	2.16(1.55-3.01)	<0.001	62.80%	0.013	0.74(0.50-1.09)	0.132	0.305
Subgroup 3: Cut-off value	>3	5	12,22-25	2.04(1.47-2.82)	<0.001	28.20%	0.234			

	≤3	6	19-21,26-28	2.07(1.51-2.83)	<0.001	63.60%	0.017	0.99(0.63-1.55)	0.950	0.959
Subgroup 4: Therapeutic intervention	Nephrectomy only	2	23,24	1.52(1.06-2.17)	0.022	0	0.615			
	Mixed therapies	10(11)	12,19-22,25-29	1.92(1.54-2.38)	<0.001	60.10%	0.005	0.79(0.52-1.20)	0.275	0.424
Subgroup 5: NOS score	≥6	4	21,23,24,27	1.51(1.24-1.84)	<0.001	0	0.594			
	<6	8(9)	12,19,20,22,25,28,29	2.06(1.51-2.70)	<0.001	65.10%	0.003	0.73(0.51-1.04)	0.083	0.313
Subgroup 6: Tumor type	Non-clear cell RCC/NA	7(8)	12,20,21,25,26,28,29	1.87(1.45-2.42)	<0.001	58.20%	0.065			
	Clear cell RCC	5	19,22-24,27	1.82(1.32-2.50)	<0.001	53.70%	0.067	1.03(0.68-1.55)	0.891	0.859
Progress free survival (PFS)/Recurrence free survival (RFS)		10	17,18,19-25,28	2.18(1.75-2.71)	<0.001	25%				
Subgroup 1: Study region	Western countries	6	17,20,21,23,24,28	2.20(1.64-2.96)	<0.001	35.70%	0.169			
	Eastern countries	4	18,19,22,25	2.23(1.51-3.28)	<0.001	28.60%	0.241	0.99(0.61-1.61)	0.957	0.958
Subgroup 2: Sample	≥200	5	17,18,20,23,24	2.25(1.56-3.24)	<0.001	51.30%	0.084			

size										
	<200	5	19,21,22,25,28	2.15(1.62-2.85)	<0.001	0	0.444	1.05(0.66-1.66)	0.847	0.950
Subgroup 3: Cut-off value	>3	5	17,22-25	1.74(1.39-2.17)	<0.001	0	0.675			
	≤3	5	18,19-21,28	3.08(2.24-4.24)	<0.001	0	0.867	0.56(0.38-0.83)	0.004	0.020
Subgroup 4: Therapeutic intervention	Nephrectomy only	4	17,18,23,24	2.00(1.40-2.85)	<0.001	39.90%	0.172			
	Mixed therapies	6	19-22,25,28	2.36(1.79-3.12)	<0.001	11.40%	0.342	0.85(0.54-1.33)	0.472	0.404
Subgroup 5: NOS score	≥6	5	17,18,21,23,24	2.08(1.53-2.84)	<0.001	33.60%	0.197			
	<6	5	19,20,22,15,28	2.35(1.67-3.32)	<0.001	26.50%	0.245	0.89(0.56-1.41)	0.605	0.622
Subgroup 6: Tumor type	Non-clear cell RCC/NA	5	17,20,21,25,28	2.62(1.94-3.53)	<0.001	0	0.644			
	Clear cell RCC	5	18,19,22-24	1.92(1.42-2.59)	<0.001	34.10%	0.194	1.36(0.89-2.09)	0.151	0.112

N: number of studies (cohorts); HR: hazard ratio; 95%CI: 95% confidence interval; Subgroup analyses for OS and RFS/PFS were performed by study region (eastern vs. western countries), sample size (≥200 vs. <200), cut-off value (>3 vs. ≤3), therapeutic intervention (nephrectomy only vs. mixed therapies), type of RCC (Clear cell RCC vs. Non-clear cell RCC/NA) and NOS score (≥6 vs. <6). Interaction revisited of estimates between subgroups and meta-regression were also applied to figure out heterogeneity among studies.

Figure Legends

Figure 1. Flow chart of study selection process.

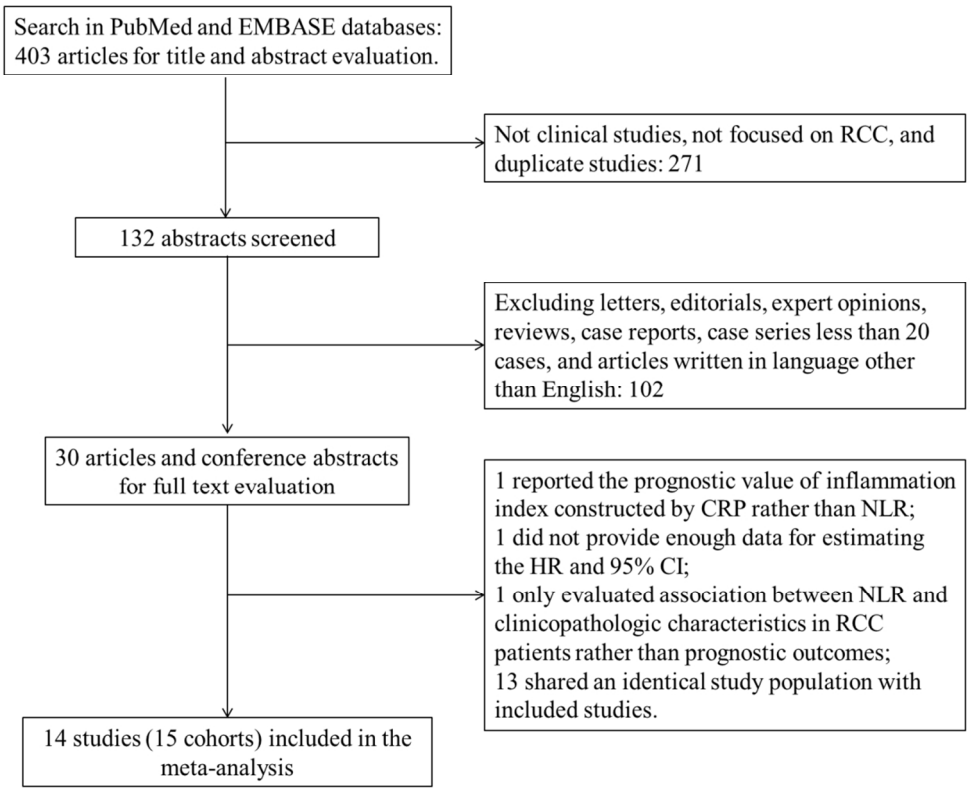
RCC: renal cell carcinoma; CRP: C-reactive protein; NLR: neutrophil-lymphocyte ratio; HR: hazard ratio; CI: confidence interval.

Figure 2. Meta-analysis of the association between elevated NLR and OS of RCC. Results are presented as individual and pooled hazard ratio (HR) and 95% confidence interval (CI).

Figure 3. Meta-analysis of the association between elevated NLR and RFS/PFS of RCC. Results are presented as individual and pooled hazard ratio (HR) and 95% confidence interval (CI).

Figure 4. Funnel plots without and with trim and fill.

The pseudo 95% confidence interval (CI) is computed as part of the analysis that produces the funnel plot, and corresponds to the expected 95% CI for a given standard error (SE). HR indicates hazard ratio.



Flow chart of study selection process.
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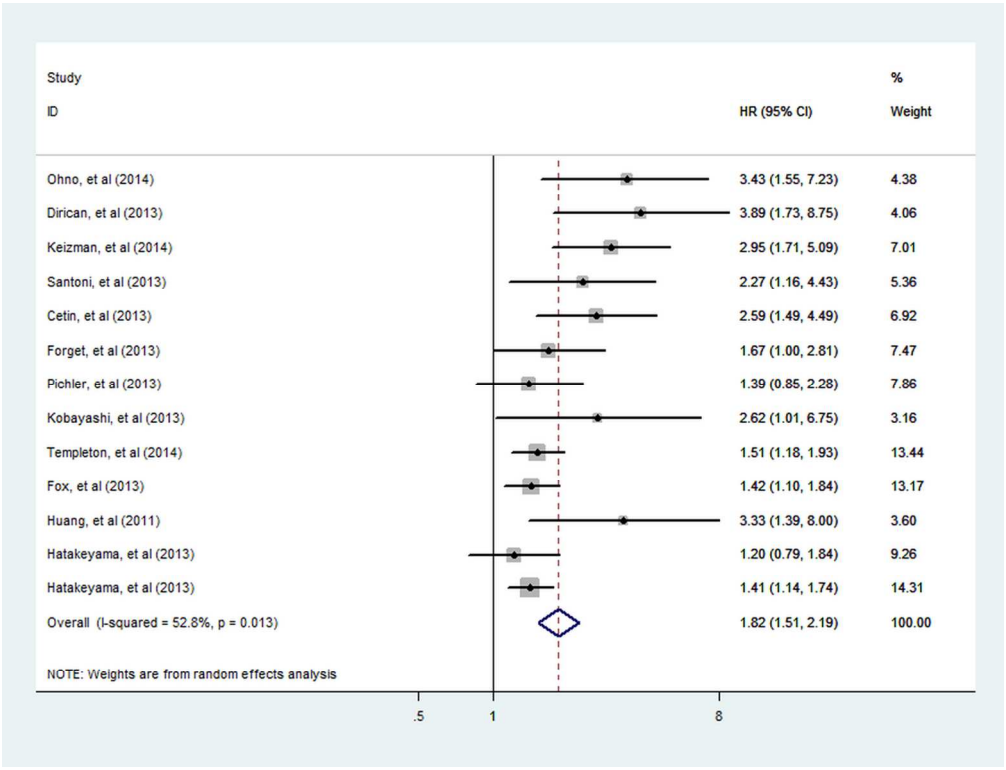

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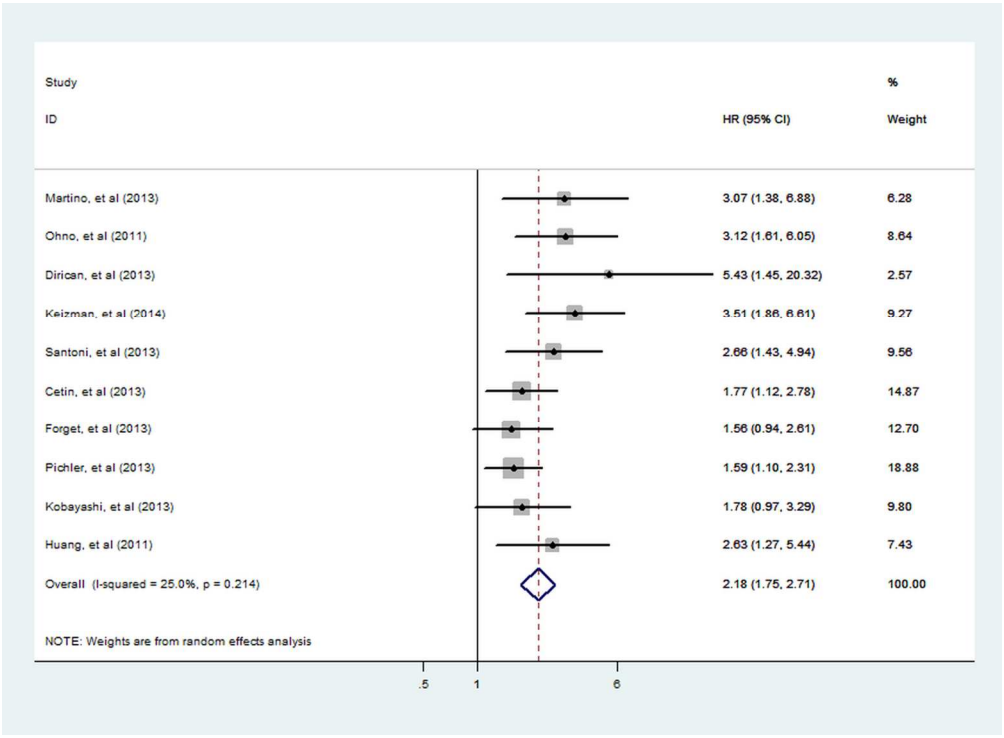

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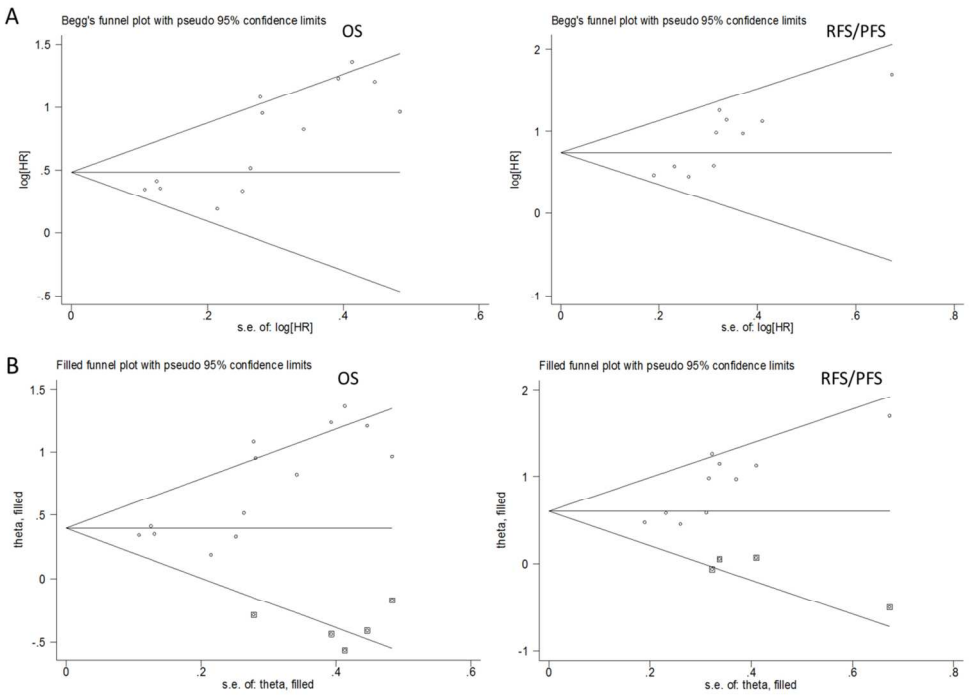

127x90mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	#1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	#2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	#3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	#4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	#4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	#5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	#4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	#4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	#5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	#5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	#5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	#5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	#5
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	#6

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	#6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	#6
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	#7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	#7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	#8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	#8
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	#8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	#9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	#9
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	#10-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	#2-3
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	#13
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	#14

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