BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035031
Article Type:	Original research
Date Submitted by the Author:	17-Oct-2019
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Keywords:	Immunology < BASIC SCIENCES, Epidemiology < ONCOLOGY, Respiratory tract tumours < ONCOLOGY

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Correlation of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis

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Running title: Neutrophil to lymphocyte ratio and lung cancer

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Article summary

Strengths and limitations of this study

1. Verifying the prognostic value of NLR in a large of lung cancer patients with immunotherapy

2. Different clinical characteristics could affect the prognostic value

3. High heterogeneity was present in this analysis

Patient and Public Involvement: No patient involved

Abstract:

Objectives: We conducted a meta-analysis to explore the relationship between pretreatment or posttreatment neutrophil to lymphocyte ratio (NLR) and overall survival (OS)/progress free survival (PFS) in lung cancer patients receiving immunotherapy.

Setting: Medical Center in Southwestern of China

Participants: Studies reporting the prognostic value of NLR in lung cancer patients receiving immunotherapy were enrolled.

Primary and secondary outcome measures: We searched PubMed, Cochrane Library, Embase and Web of Science to collect relevant studies conducted until July 2019. We carefully reviewed the full text of included publications and combined the hazard ratios (HRs) and 95% confidence intervals (CIs) to assess the correlation between the NLR and survival time in lung cancer patients receiving immunotherapy.

Results: Twenty-three studies with 2068 patients were enrolled. Among all patients, 1305 (64.0%) were males, and 643 (31.38%) were diagnosed with squamous carcinoma. In a pooled analysis of OS and PFS from all studies, an elevated NLR predicted poorer OS ((HR=1.62; 95% CI: 1.41-1.87; P< 0.001) and PFS (HR=1.47; 95% CI: 1.25-1.72; P< 0.0001). Subgroup analyses stratified showed that the

posttreatment NLR was not significantly related to OS and that patients in Asia were significantly associated with higher HRs than those in Europe and America. Furthermore, the proportion of squamous cell carcinoma and baseline level of NLR affect the prognosis value of NLR.

Conclusions: Our study found that an elevated NLR was associated with poorer OS and PFS in lung cancer patients receiving immunotherapy and that several clinical factors might have an impact on the predictive value of NLR in the survival of lung cancer patients. However, further studies are warranted to draw firm conclusions.

Trial registration: The registration number of PROSPERO: CRD42018104856.

Keywords: NLR; systemic inflammation; prognostic markers; lung cancer

Introduction:

Lung cancer is the most prevalent cancer and life-threatening malignancy worldwide.(1) The pathogenesis of lung cancer is complicated, and the primary treatments for lung cancer patients are surgery and chemotherapy. Unfortunately, most patients with lung cancer are diagnosed at advanced stages, and the benefits achieved from chemotherapy in advanced lung cancer patients are relatively small. Recently, many studies have revealed that tumor cells can evade the antitumor responses of T-cells by controlling the combined responses of programmed cell death protein 1 (PD-1) and programmed cell death ligand-1 (PD-L1).(2) Nivolumab, pembrolizumab, atezolizumab, durvalumab, ipilimumab and tremelimumab have successfully changed clinical experiences in lung cancer treatment.(3) Tumor mutational burden,(4) neoantigens(5) and classical monocytes in the peripheral blood(6) are effective predictive biomarkers for immune checkpoint therapy in lung cancer, especially PD-L1 expression on tumor cells.(7) Systemic inflammation in patients with cancer is considered to influence the growth and migration of tumors by some inflammatory factors.(8) Elevated levels of systemic inflammation, including Glasgow prognostic score (GPS), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), C-reactive protein to albumin ratio (CAR), has been

indicated to be associated with worse survival in solid tumors.(9-11) However, the data of prognosis from pretreatment NLR in lung cancer patients receiving immunotherapy trials are still scarce and inconsistent. Therefore, we collected the available publications and conducted a meta-analysis to explore the prognostic value of pretreatment NLR for OS and PFS in clinical trials of lung cancer patients receiving immunotherapy.

Materials and Methods

Search strategy

PRISMA guidelines for systematic review and meta-analysis were followed strictly in this article. An online search was conducted to identify relevant publications in PubMed, Cochrane Library, Web of Science and Embase databases. The following words were used: “Pulmonary Neoplasms”, “neutrophil lymphocyte ratio”, “immunotherapy”, “programmed death receptor-1”, and “immune checkpoint inhibitor” for studies on the associations between pretreatment NLR and survival time in patients with lung cancer published before July 2019. A full electronic search strategy is provided in the supplement materials (Supplement Table 1). Additional studies were selected for the full text to be reviewed by exploring the references cited in the selected articles and relevant reviews. The articles were limited to the English

language but no restrictions were used for the minimum number of patients. Two authors (J Jin and L Yang) independently reviewed the titles and abstracts of the retrieved articles to select the potentially relevant articles that to be more carefully assessed.

Eligibility criteria

The inclusion criteria were as follows: 1) retrospective or prospective studies published before July 2019; 2) all patients enrolled in studies were diagnosed with lung cancer by biopsy and received immunotherapy; 3) the value of NLR was calculated based on the level of neutrophils and lymphocytes; and 4) HRs and 95% CIs were provided and data necessary to calculate them were reported.

The exclusion criteria: 1) review, meeting abstract, letter, or full text unavailable in English; 2) nonhuman studies; and 3) research did not present the value of the NLR.

Data extraction

From each study, the name of the study, first author, year of publication, study design, number of patients, sex distribution, age, median follow-up time, histology, NLR cutoff value, NLR at baseline, line of therapy, drugs and HRs with 95% CIs for overall survival (OS) and progress free survival (PFS) were extracted by two authors (D Liu and L Yang). If univariate and multivariate analysis results were

simultaneously reported, only multivariate analysis results were extracted. Any disagreements between the authors were resolved by discussion and consensus. The most recent study was chosen when duplicate studies occurred.

Quality assessment

The primary studies were assessed by the NOS (Newcastle-Ottawa quality assessment Scale). The quality assessment was conducted by two independent researchers (J Jin and D Liu). The studies in which the mark was between 6 and 9 points were regarded as high-quality studies.

(http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)

Statistical analysis

The primary endpoints were OS and PFS of lung cancer patients receiving immunotherapy. PFS was defined as the time from the initial date of immunotherapy to the date of progression or death. OS was calculated from the date of inclusion to the time of death from any cause. HRs with 95% CIs were directly obtained from the articles or estimated from the Kaplan-Meier curves according to the methods reported by Tierney et al.(12) We calculated the pooled HRs of OS and PFS using random effects or fixed effects model. We performed the Q-test to assess between-study heterogeneity and calculated the I^2 statistic, which expresses the percentage of the

total observed variability due to study heterogeneity. The heterogeneity between studies was considered small if the I^2 statistic was less than 50% and the P value for Q-test was less than 0.05. We performed a subgroup analysis to detect the source of heterogeneity. In addition, we only considered subgroups that included more than two studies. Publication bias was assessed by Egger's and Begg's test, and a significant publication bias was defined as a $P<0.10$.(13) Trim and fill method was applied when significant publication bias was found to confirm the pooled results. Sensitivity analyses were carried out by excluding each study individually from the meta-analysis.(14) All statistical analyses were performed with R (Version: 3.5.2).

Result

The characteristics of the included studies

A total of 1102 studies were retrieved in this meta-analysis, and 26 of these studies were selected for full-text review. In total, 23 studies with 2068 patients fulfilled the inclusion criteria, with publication dates ranging from 2017 to 2018.(15-37) The flow diagram of this study is shown in Fig 1. The sample size was between 19 and 201 patients. Of these studies, 9 were conducted in Europe, (16, 17, 21, 24, 27, 30, 35, 36) 5 were conducted in America, (22, 28, 31, 33, 37) and the remaining studies were conducted in Asia. Among all patients included, 1305 (64.0%) were males, and 643

(31.38%) were diagnosed with squamous carcinoma. Twenty studies explored the association between NLR and OS; fifteen studies investigated the relationship between PFS and NLR. Additionally, 7 of 23 studies provided data about posttreatment NLR. (21, 23, 25, 28, 29, 32, 33) If the study provided data about posttreatment NLR and OS, we treated it as an independent research in the subsequent analysis. Six trials were performed as first-line therapy, (16, 19, 25, 28, 31, 36) and the other studies were second or additional lines of therapy. Almost all patients received nivolumab as an immunotherapy for PD-1 inhibitor. The cutoff value of NLR was not the same in all studies; the value of 5 was mostly used among all publications, and the median cutoff value for all enrolled publications was also 5. The NOS scores of the enrolled studies ranged from 6-9. Detailed information about these studies is presented in Table 1.

Relationship between NLR and OS in lung cancer patients receiving immunotherapy

A total of 1629 patients treated with immunotherapy from 20 studies provided the NLR value or the data to calculate the NLR and OS values. Five of these studies provided data about posttreatment NLR and OS. A total of 23 researches to combine the HR and 95% CI. In the pooled analysis of NLR and OS, we found that a higher

level of NLR was associated with a poorer OS with a high heterogeneity (HR=1.62; 95% CI: 1.41-1.87; P< 0.001) (I^2=81.7%, P< 0.0001) (Fig 2). To detect the source of heterogeneity, we conducted a subgroup analysis on some clinical factors that may influence the final results, such as study design, the time of detecting NLR, ethnicity, sex ratio, the proportion of squamous cell carcinoma (SCC%), the level of NLR at baseline, the treatment line, median follow-up time, sample size and the drug for immunotherapy (Fig 3). Interestingly, the association between the pretreatment NLR and OS showed a similar trend to the pooled result (HR=1.87; 95% CI: 1.46-2.39; P < 0.0001). However, the posttreatment NLR seemed to not be significantly related to the OS in lung cancer patients (HR=1.80; 95% CI: 0.81-4.00; P= 0.11). However, these results were still highly heterogeneous (pretreatment: I^2=79.80%, P< 0.0001; posttreatment: I^2=83.5%, P< 0.0001). Furthermore, the NLR was significantly unrelated to the OS in the studies in which the proportion of patients with squamous cell carcinoma or the proportion of patients whose NLR baseline levels exceeded the cutoff value was greater than 50% (Fig 3).The subgroup analysis stratified by ethnicity found that patients in Asia were significantly associated with a higher HR (HR=2.76; 95% CI: 1.88- 4.06) and smaller heterogeneity (I^2=45.7%, P=0.09) than those in Europe and America ($P_{interaction}$=0.030).

Relationship between NLR and PFS in lung cancer patients receiving immunotherapy

The data for NLR and PFS of 1612 patients treated with immunotherapy in 15 studies (20 researches) were extracted to obtain a pooled HR and 95% CI. Four of these studies provided the posttreatment NLR and its relationship with PFS. The random effects model revealed a significant association between an elevated NLR and PFS in lung cancer patients receiving immunotherapy (HR=1.47; 95% CI: 1.25-1.72; P< 0.0001) with high heterogeneity (I^2=72.5%, P< 0.0001) (Fig 4). To detect the potential source of heterogeneity in studies reporting PFS data, subgroup analysis stratified by the factors that affect the HR were proposed, as previously mentioned (Fig 5). Similar to the relationship between the NLR and OS, the NLR was significantly unrelated to the PFS in studies in which the proportion of patients with squamous cell carcinoma was greater than 50% ($P_{interaction}$=0.005). However, the pooled results for subgroups by other factors were not markedly changed with low-level heterogeneity.

Sensitivity analysis and Publication bias

We found high heterogeneity among studies enrolled in the analysis of the relationship among pretreatment NLR, OS and PFS. Therefore, we performed a

sensitivity analysis on all enrolled studies. The effect of each single study set on the combined HRs was evaluated by excluding each study individually from the meta-analysis. The results of the sensitivity analysis showed that the pooled HRs for OS and PFS were robust in our meta-analysis (Fig 6A and 6B). We conducted a subgroup analysis stratified by various factors to detect the source of heterogeneity. The Begg's test presented no evidence of obvious publication bias in both studies reporting the association between NLR and OS (P=0.673) and those reporting the association between NLR and PFS (P= 0.074), but the Egger's test showed significant publication bias in both (P<0.001 for both). Therefore, we performed a trim and fill analysis on studies reporting the relationship between NLR and OS/PFS. However, the result was unchanged after eliminating the influence of publication bias (OS: HR=1.40; 95% CI:1.22-1.60; P< 0.0001, PFS: HR=1.33; 95% CI:1.14-1.56; P= 0.0004, supplement fig 1).

Discussion:

The results of our meta-analysis revealed the prognostic effect of both pretreatment and posttreatment NLR on OS and PFS in lung cancer patients receiving immunotherapy. Twenty-three studies involving a total of 2049 lung cancer patients showed that an increased NLR was significantly associated with a poorer OS

(HR=1.62; 95% CI: 1.41-1.87; P< 0.001) and PFS (HR=1.47; 95% CI: 1.25-1.72; P< 0.0001). Interestingly, the posttreatment NLR did not seem to be significantly related to OS, and patients in Asia were significantly associated with higher HRs than those in Europe and America.

The immune checkpoint is a kind of mechanism that plays a protective role in the human immune system and acts like a brake to prevent inflammatory damage caused by excessive activation of T cells.(38) Human anti-PD-1 IgG4 mAb is now widely used and shows higher efficacy than standard therapies in lung cancer. (39) Despite a wide consensus on testing tumor tissues for PD-L1 expression, it is limited by its "unperfected dichotomy" across studies and molecules; patients with low levels of PD-L1 expression have responded at rates of up to 17%, and roughly half of patients are "not-responders" despite high tumor PD-L1 levels. Several factors could affect the response and survival of patients receiving immunotherapy.(39) In addition to tumor mutation loads and the expression of tumor antigens, the status of systemic inflammation also occupies an important position in lung cancer patients receiving immunotherapy. The tumor-associated cytokine and relevant signaling pathway could be reflected by the level of systemic inflammation, which has been proven to be associated with a worse survival in patients with solid tumors.(8) Biomarkers such as

NLR, PLR, GPS and mGPS have been used as prognostic factors in lung cancer.(9-11) In addition, the role of systemic inflammation in patients receiving immunotherapy is particularly important for their survival. Several studies have explored the effect of pretreatment NLR on lung cancer patients receiving immunotherapy.(31, 40-45) There are also two meta-analyses concerning pretreatment NLR and survival in patients with advanced cancer. (46) (47) In summary, NLR is a reliable prognostic factor for patients with various cancer types. Sacdalan, D. B. reported that an increased NLR resulted in a worse PFS among several kinds of cancers, such as melanoma, non-small-cell lung cancer and genitourinary cancer,(46) which was consistent with our results. However, only three publications about lung cancer were enrolled in that meta-analysis, and a nonsignificant association was discovered between pretreatment NLR and OS. In addition, two of the three studies included in the meta-analysis previously mentioned only provided abstracts, and we cannot obtain more details about those cohorts or study designs. Another meta-analysis conducted by Jiang, T also revealed a trend similar to our results, but the results of the subgroup analysis showed that posttreatment NLR was also significantly related to a poorer OS and PFS, and this result was different from ours. We enrolled more research articles in our study. In addition, we performed subgroup analyses stratified by more

clinical factors. Furthermore, our results showed that the ethnicity, NLR level at baseline and proportion of squamous cell carcinoma may affect the prognosis value of the NLR. However, due to the high heterogeneity, the results must be interpreted with caution. Neutrophils were the most abundant immune cell type identified in NSCLC patients and accounted for nearly 20% of all CD45+ cells in patients from America.(48) However, this result was not found in Asia or Europe. The systemic inflammatory response might be different among different ethnicities. Furthermore, we collected baseline patient information, including the proportion of squamous cell carcinoma, from all studies, and our results showed that the histology of lung cancer might have an impact on the prognosis value of NLR. However, the mechanism needs further exploration, and many cofounding factors could affect the systemic inflammatory response.

The current research had several limitations. First, high heterogeneity was present in this analysis although we conducted sensitivity analyses on all studies. The results were robust after eliminating every study from a combination of HRs. In addition, we performed subgroup analyses on some possible impact factors to detect the source of heterogeneity. Second, the Egger's test showed that obvious publication bias was present in this current study. The pooled results should be treated with caution

although a trim and fill analysis testing indicated credibility for this study. Additionally, considering the high heterogeneity after subgroup analysis, other factors might be responsible for the high heterogeneity in this meta-analysis.

Conclusion:

Generally, our meta-analysis focused on the clinical prognostic agreement of NLR for OS and PFS in lung cancer patients. Importantly, given the limitations mentioned above, these findings should be treated with caution in clinical practice. More prospective cohort studies are needed to test our results.

Contributorship statement:

(I) Conception and design: W Li, J Jin, Lan Y; II) Administrative support: J Jin, W Li; (III) Provision of study materials or patients: D Liu; (IV) Collection and assembly of data: J Jin, D Liu; (V) Data analysis and interpretation: D Liu, Lan Y; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Competing interests

The authors have no conflicts of interest to declare

Funding

None

Data sharing statement:

All data in the current study were available in published articles

Acknowledgments:

This work was supported by the Transformation Projects of Sci-Tech Achievements of Sichuan Province (2016CZYD0001), the Sci-Tech Support Program of Science and Technology Department of Sichuan Province (2016SZ0073), the National Major Sci-Tech Project (2017ZX10103004-012) and the National Key Development Plan for Precision Medicine Research (2017YFC0910004).

Compliance with Ethical Standards

Ethical approval: All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendment.

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Figure Legends

Figure 1 Flow chart of study selection

Figure 2 Forest plot of the association between NLR and OS in patients with lung cancer receiving immunotherapy

Figure 3 Subgroup analysis of the relationship NLR and OS in patients with lung cancer receiving immunotherapy

Abbreviation: ICI: Immune-Checkpoint Inhibitor; M/F: male/female; SCC%: Proportion of Squamous cell carcinoma;※: the data here shows the proportion of patients whose NLR baseline levels exceeded the cutoff value; N: Nivolumab; P:

Pembrolizumab; D: Durvalumab; E: Embrolizumab; A: Atezolizumab

Figure 4 Forest plot of the association between NLR and PFS in patients with lung cancer receiving immunotherapy

Figure 5 Subgroup analysis of the relationship NLR and PFS in patients with lung cancer receiving immunotherapy

Abbreviation: ICI: Immune-Checkpoint Inhibitor; M/F: male/female; SCC%: Proportion of Squamous cell carcinoma;※: 15 studies (20 researches) provided the data for pretreatment NLR and PFS, and 5 of them also provided posttreatment NLR and PFS; N: Nivolumab; P: Pembrolizumab; D: Durvalumab; E: Embrolizumab; A: Atezolizumab

Figure 6 Sensitivity analysis on OS (A) and PFS (B)

Table

Table 1 The basic characteristic of enrolled studies

Study	Year	Country	Ethnicity	Sample size	MFP	M/F	NLR at baseline
Russo A	2018	Italy	European	28	17	25/3	NM
Zer A	2018	America	American	88	5.3	43/45	NLR＞4:56.8%
Nakaya A	2018	Japan	Asian	101	8.9	77/24	NLR≥3:46.5%
Maymani H	2018	America	American	74	12.3	36/38	NLR＞6:20.3%
Mezquita L	2018	Europe	European	161	12	100/61	NLR＞3:39%
Diem S	2017	Europe	European	52	NM	29/23	5.0(2.7-8.3) *
Bagley SJ	2017	America	American	175	NM	80/95	NLR≥5:58%
Fukui T	2018	Japan	Asian	52	10.9	37/15	NLR≥5:34.6%
Park W	2018	America	American	159	11.5	82/77	4.3(0.5-24.1) *
Ren, F	2019	China	Asian	147	2.6	94/53	NLR＞2.5:59.9%

Pavan, A	2019	Italy	European	184	56.3	125/59	NLR≥3:57.5%
Passiglia, F	2019	Italy	European	45	9.1	32/13	NLR＞3.3:51.1%
Minami, S	2019	Japan	Asian	76	NM	49/27	NLR≥6:14.5%
Ichiki, Y.	2019	Japan	Asian	44	4.83	38/6	NM
Dusselier, M.	2019	France	European	59	NM	44/15	NLR＞5:62.7%
Takeda, T.	2018	Japan	Asian	30	NM	19/11	NLR＞5:30%
Svaton, M	2018	Czech Republic	European	120	NM	71/49	NLR＞3.8:50%
Suh, Koung Jin	2018	Korea	Asian	54	26.2	42/12	NLR＞5:14.8%
Shiroyama, Takayuki	2018	Japan	Asian	201	12.4	135/66	NLR＞4:39.3%
Kiriu, T	2018	Japan	Asian	19.00	NM	19	NLR＞5:31.6%
Khunger, M	2018	America	American	109	30	56/53	NLR≥5:50.5%
Inomata, M	2018	Japan	Asian	36	NM	27/9	NLR≥5:44.4%
Facchinetti, F	2018	Italy	European	54	12.6	45/9	NM

Study	SCC%	Treatment lines	Outcome	Study design	Cut-off	IO
Russo A	60.71%	at least second line therapy	OS/PFS	RO	3	N
Zer A	17.05%	at least second line therapy	OS/PFS/DCR	RO	4	NM
Nakaya A	36.63%	at least second line therapy	PFS/irAEs	RO	3	N
Maymani H	16.22%	including first line therapy	OS/PFS	RO	6	N/P/D
Mezquita L	28.57%	at least second line therapy	OS/PFS	RO	3	N/E/A/D
Diem S	34.62%	including first line therapy	OS/PFS	RO	5	N
Bagley SJ	24.00%	at least second line therapy	OS/PFS	RO	5	N
Fukui T	30.77%	at least second line therapy	OS/PFS/irAEs	PO	5	N
Park W	24.53%	including first line therapy	OS/PFS	RO	5	N
Ren, F	42.18%	at least second line therapy	OS/PFS	RO	2.5	N/P
Pavan, A	32.07%	including first line therapy	OS/PFS/irAEs	RO	3	N/P/A
Passiglia, F	44.44%	at least second line therapy	OS/TTP	RO	3.3	N
Minami, S	23.68%	at least second line therapy	OS/PFS	RO	6	N/P/A
Ichiki, Y.	65.91%	including first line therapy	OS/PFS/irAEs	RO	NM	N/P
Dusselier, M.	20.34%	at least second line therapy	OS	RO	5	N
Takeda, T.	30.00%	at least second line therapy	PFS	RO	5	N
Svaton, M	33.33%	at least second line therapy	OS/PFS	RO	3.8	N
Suh, Koung Jin	31.48%	including first line therapy	OS/PFS/irAEs	RO	5	N/P
Shiroyama, Takayuki	30.35%	at least second line therapy	PFS/RR	RO	4	N

Kiriu, T	31.58%	at least second line therapy	OS/PFS/TTF	RO	5	N
Khunger, M	23.85%	at least second line therapy	OS	RO	5	N
Inomata, M	44.44%	at least second line therapy	PFS	RO	5	N/P
Facchinetti, F	48.15%	at least second line therapy	OS/PFS/TTF/DP	PO	4	N

Abbreviation: NLR: neutrophil to lymphocyte ratio; NM: not mentioned; M/F: male/female; MFP: Median follow-up (month); SCC%: Proportion of Squamous cell carcinoma; IO: immunotherapy; N: Nivolumab; P: Pembrolizumab; D: Durvalumab; E: Embrolizumab; A: Atezolizumab; OS: overall survival; PFS: progress free survival; DCR: disease control rate; irAEs: immune-related adverse events; RR: response rate; TTF: treatment failure; RO: retrospective studies; PO: prospective studies; NOS: Ottawa quality assessment Scale; *: the study just provided the median number and the range of NLR at baseline.

Supporting information

S1File. PRISMA 2009 checklist.

S2 Search strategy. This file provides a full electronic search strategy for PubMed

S3 Figure1 Trim and fill analysis on the data of OS and PFS

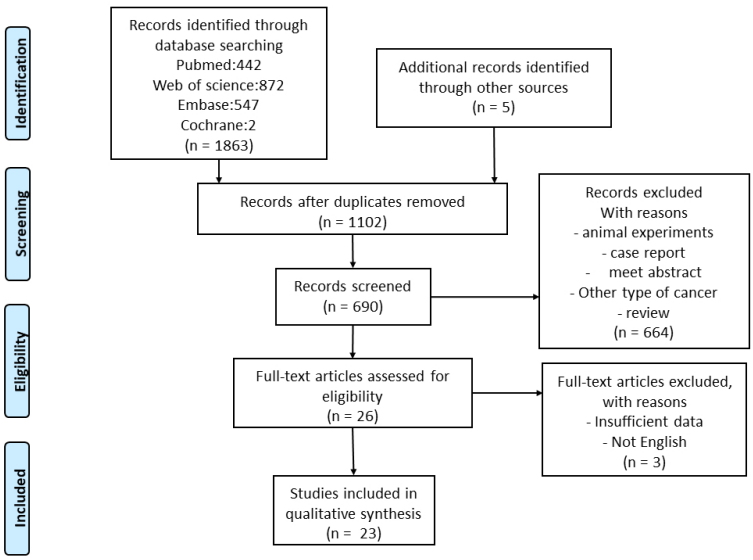

Figure 1 Flow chart of study selection

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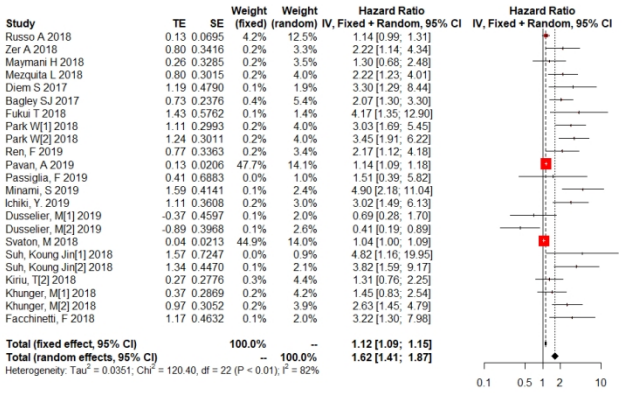

Figure 2 Forest plot of the association between NLR and OS in patients with lung cancer receiving immunotherapy

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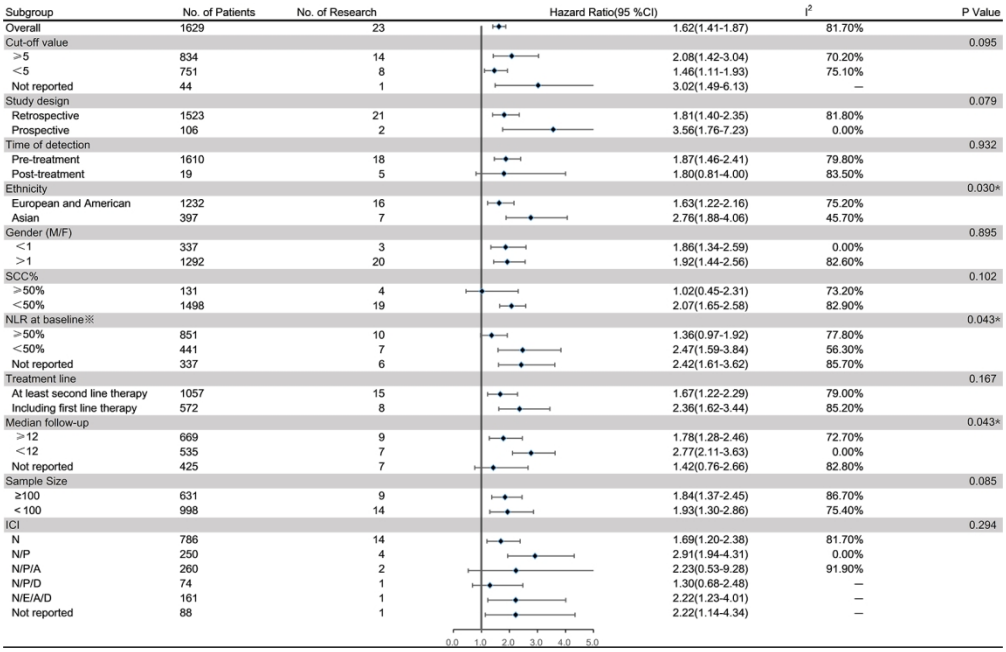


Subgroup analysis of the relationship NLR and OS in patients with lung cancer receiving immunotherapy
Abbreviation: ICI: Immune-Checkpoint Inhibitor; M/F: male/female; SCC%: Proportion of Squamous cell carcinoma;※: the data here shows the proportion of patients whose NLR baseline levels exceeded the cutoff value; N: Nivolumab; P: Pembrolizumab; D: Durvalumab; E: Embrolizumab; A: Atezolizumab

154x99mm (300 x 300 DPI)

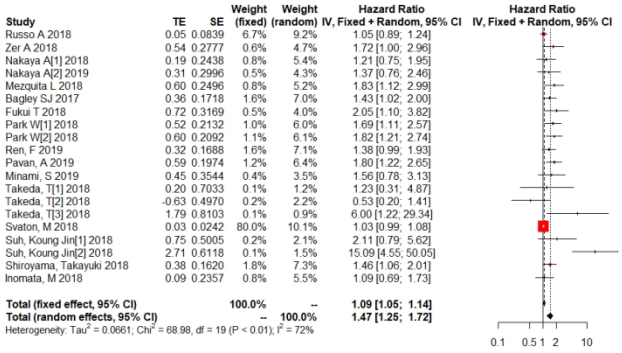
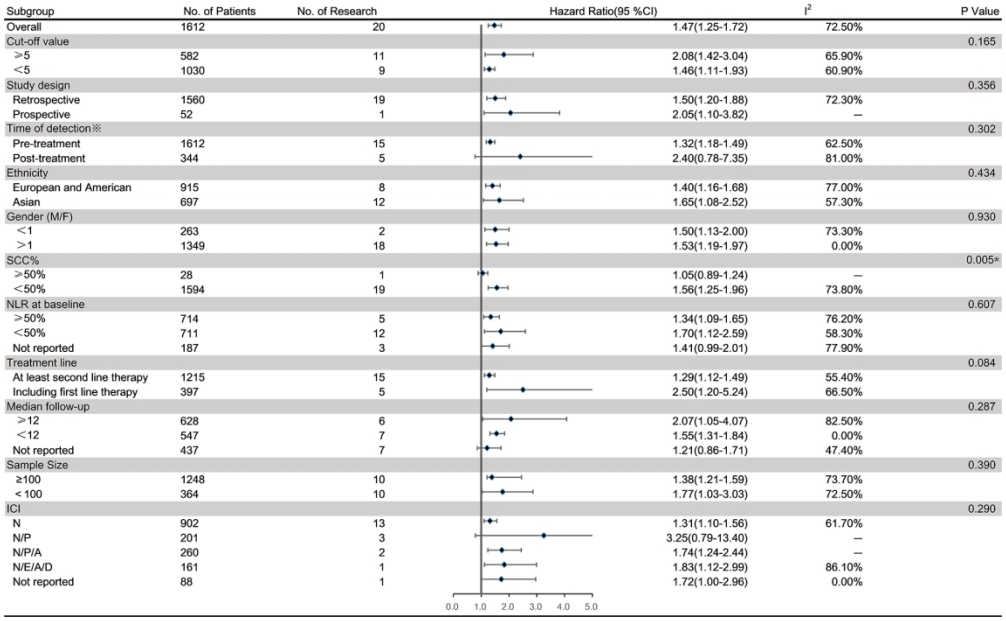


Figure 4 Forest plot of the association between NLR and PFS in patients with lung cancer receiving immunotherapy

396x246mm (96 x 96 DPI)



Subgroup analysis of the relationship NLR and PFS in patients with lung cancer receiving immunotherapy
Abbreviation: ICI: Immune-Checkpoint Inhibitor; M/F: male/female; SCC%: Proportion of Squamous cell carcinoma;※: 15 studies (20 researches) provided the data for pretreatment NLR and PFS, and 5 of them also provided posttreatment NLR and PFS; N: Nivolumab; P: Pembrolizumab; D: Durvalumab; E: Embrolizumab; A: Atezolizumab

162x99mm (300 x 300 DPI)

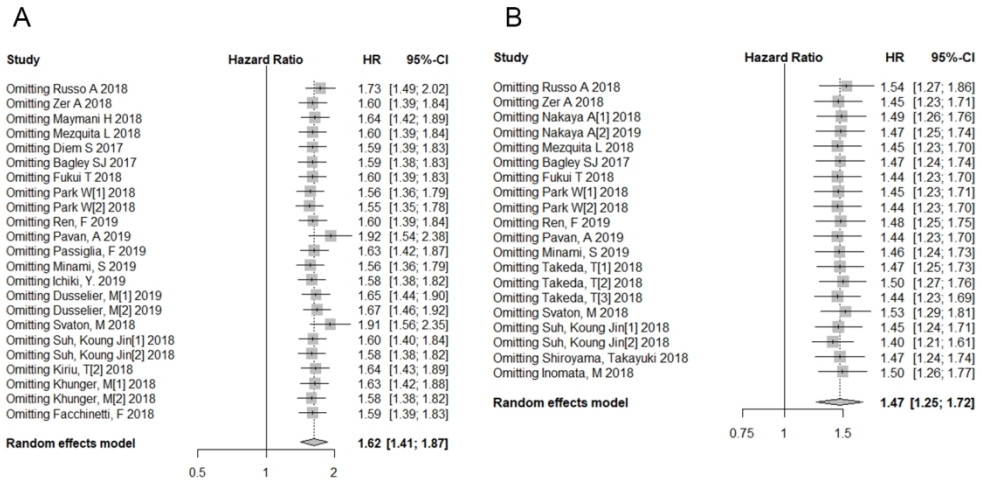


Figure 6 Sensitivity analysis on OS (A) and PFS (B)

143x74mm (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.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
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.	5-6
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.	6
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.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5-6 and supplements
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).	5-8
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.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
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.	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
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.	7-8

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).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
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.	8-9
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.	9-10
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).	11-12
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.	8-11
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11-12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12
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).	12-13
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).	15-16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
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.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Supplement Table1 Search strategy for meta-analysis of Correlation of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis (PubMed via NLM)

	Search terms: ***neutrophil to lymphocyte ratio and lung cancer patients with immunotherapy***
Population: persons with lung cancer receiving immunotherapy	
1	(((((Cancer of Lung) OR Pulmonary Neoplasms) OR Neoplasms, Lung) OR Lung Neoplasm) OR Neoplasm, Lung) OR Neoplasms, Pulmonary) OR Neoplasm, Pulmonary) OR Pulmonary Neoplasm) OR Lung Cancer) OR Cancer, Lung) OR Cancers, Lung) OR Lung Cancers) OR Pulmonary Cancer) OR Cancer, Pulmonary) OR Cancers, Pulmonary) OR Pulmonary Cancers) OR Cancer of the Lung) OR "Lung Neoplasms"[Mesh])))) AND (("Immunotherapy"[Mesh]) AND Immunotherapies)
Intervention (Expose): neutrophil to lymphocyte ratio	
2	((NLR) OR (neutrophil to lymphocyte ratio) OR neutrophil lymphocyte ratio))
Combined sets	
3	1 and 2
Limits	
4	3 AND English [Language]

[Mesh] = Term from the Medline controlled vocabulary, including terms found below this term in the Mesh hierarchy

BMJ Open

Association of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035031.R1
Article Type:	Original research
Date Submitted by the Author:	14-Feb-2020
Complete List of Authors:	Jin, Jing; Sichuan University West China Hospital Yang, Lan; Sichuan University West China Hospital Liu, Dan; Sichuan University West China Hospital Li, Weimin; Sichuan University West China Hospital,
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Respiratory medicine
Keywords:	Immunology < BASIC SCIENCES, Epidemiology < ONCOLOGY, Respiratory tract tumours < ONCOLOGY

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Association of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis

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Running title: Neutrophil to lymphocyte ratio and lung cancer

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Abstract:

Objectives: To explore the relationship between the pretreatment or posttreatment neutrophil to lymphocyte ratio (NLR) and overall survival (OS)/ progression-free survival (PFS) in lung cancer patients receiving immunotherapy.

Design: We searched several databases to collect relevant studies conducted until July 2019. We carefully reviewed the full text of the included publications and combined the hazard ratios (HRs) and 95% confidence intervals (CIs) to assess the association between the NLR and survival time in lung cancer patients receiving immunotherapy.

Data Sources: PubMed, the Cochrane Library, Embase and Web of Science

Eligibility Criteria: Studies reporting the prognostic value of the NLR in lung cancer patients receiving immunotherapy were enrolled.

Data extraction and synthesis: Basic information on the articles and patients(NLR cutoff value, NLR at baseline, and HRs with 95% CIs for OS and PFS) was extracted by two authors independently. The pooled HRs of OS and PFS were synthesized using the random effects or fixed effects model.

Results: Twenty-three studies with 2068 patients were enrolled. Among all patients, 1305 (64.0%) were males, and 643 (31.4%) were diagnosed with squamous cell carcinoma. In a pooled analysis of OS and PFS from all studies, an elevated NLR

predicted poor OS (HR=1.62; 95% CI: 1.41-1.87; P< 0.001) and PFS (HR=1.47; 95% CI: 1.25-1.72; P< 0.0001). Subgroup analyses stratified showed that the posttreatment NLR was not significantly related to OS and that patients in Asia had significantly higher HRs than those in Europe and America. Furthermore, the proportion of squamous cell carcinoma and baseline NLR could affect the prognostic value of the NLR.

Conclusions: Our study found that an elevated NLR was associated with poor OS and PFS in lung cancer patients receiving immunotherapy and that several clinical factors might have an impact on the predictive value of the NLR in the survival of lung cancer patients.

Strengths and limitations of this study

1. Verification of the prognostic value of the NLR in a large number of lung cancer patients who received immunotherapy

2. Different clinical characteristics could affect the prognostic value of the NLR

3. High heterogeneity was present in this analysis

Keywords: NLR; systemic inflammation; prognostic markers; lung cancer

Introduction:

Lung cancer is the most prevalent cancer and life-threatening malignancy worldwide.(1) The pathogenesis of lung cancer is complicated, and the primary treatments for lung cancer patients are surgery and chemotherapy. Unfortunately, most patients with lung cancer are diagnosed at advanced stages, and the benefits achieved from chemotherapy in advanced lung cancer patients are relatively small. Recently, many studies have revealed that tumor cells can evade the antitumor responses of T cells by controlling the combined responses of programmed cell death protein 1 (PD-1) and programmed cell death ligand-1 (PD-L1).(2) Nivolumab, pembrolizumab, atezolizumab, durvalumab, ipilimumab and tremelimumab have successfully changed clinical experiences in lung cancer treatment.(3) Tumor mutational burden,(4) neoantigens(5) and classical monocytes in the peripheral blood(6) and PD-L1 expression on tumor cells in particular, (7) are effective predictive biomarkers for immune checkpoint therapy in lung cancer. Systemic inflammation in cancer patients is believed to influence the growth and migration of tumors via certain inflammatory factors.(8) An elevated level of systemic inflammation, including Glasgow prognostic score (GPS), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and C-reactive protein to albumin

ratio (CAR), have been indicated to be associated with poor survival in patients with solid tumors.(9-11) However, data on the prognostic value of the pretreatment NLR in lung cancer patients receiving immunotherapy remain scarce and inconsistent. Therefore, we reviewed available publications and conducted a meta-analysis to explore the prognostic value of the pretreatment NLR for r overall survival (OS) and progression-free survival (PFS) in clinical trials on lung cancer patients receiving immunotherapy.

Materials and Methods

Patient and Public Involvement: No patient was involved

Search strategy

The PRISMA guidelines for a systematic review and meta-analysis were strictly followed in this article (registration number PROSPERO: CRD42018104856). An online search was conducted to identify relevant publications in the PubMed, Cochrane Library, Web of Science and Embase databases. The following words were used to search for studies on the associations between the pretreatment NLR and survival time in patients with lung cancer published before July 2019: “pulmonary neoplasms”, “neutrophil lymphocyte ratio”, “immunotherapy”, “programmed death receptor-1”, and “immune checkpoint inhibitor”. A full electronic search strategy is

provided in the supplementary information (Supplementary Table 1). Additional studies were selected for a full-text review were selected by exploring the references cited in the selected articles and relevant reviews. The articles were limited to the English language, but there were no restrictions on the minimum number of patients. Two authors (J Jin and L Yang) independently reviewed the titles and abstracts of the retrieved articles to select the potentially relevant articles for a careful assessment.

Eligibility criteria

The inclusion criteria were as follows: 1) retrospective or prospective studies published before July 2019; 2) all patients enrolled in the studies were diagnosed with lung cancer by biopsy and received immunotherapy; 3) the value of the NLR was calculated based on the level of neutrophils and lymphocytes; and 4) HRs and 95% CIs were provided and data necessary to calculate them were reported.

The exclusion criteria were as follows: 1) review, meeting abstract, letter, or full text unavailable in English; 2) nonhuman studies; and 3) research that did not provide the value of the NLR.

Data extraction

From each study, the name of the study, first author, year of publication, study design, number of patients, sex distribution, age, median follow-up time, histology, NLR

cutoff value, NLR at baseline, line of therapy, drugs and HRs with 95% CIs for OS and PFS were extracted by two authors (D Liu and L Yang). If univariate and multivariate analysis results were simultaneously reported, only the multivariate analysis results were extracted. Any disagreements between the authors were resolved by a discussion and consensus. The most recent study was chosen when duplicate studies occurred.

Quality assessment

The primary studies were assessed by the Newcastle-Ottawa quality assessment Scale (NOS). The quality assessment was conducted by two independent researchers (J Jin and D Liu). The studies in which the mark was between 6 and 9 points were regarded as high-quality studies.

(http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)

Statistical analysis

The primary endpoints were the OS and PFS of lung cancer patients receiving immunotherapy. PFS was defined as the time from the initial date of immunotherapy to the date of progression or death. OS was calculated from the date of inclusion to the time of death from any cause. HRs with 95% CIs were directly obtained from the articles or estimated from the Kaplan-Meier curves according to the methods reported

by Tierney et al.(12) We calculated the pooled HRs of OS and PFS using random effects or fixed effects model. We performed the Q-test to assess between-study heterogeneity and calculated the I^2 statistic, which expresses the percentage of the total observed variability due to study heterogeneity. The heterogeneity between studies was considered small if the I^2 statistic was less than 50% and the P value for the Q-test was less than 0.05. We performed a subgroup analysis to detect the source of heterogeneity. In addition, we considered only subgroups that included more than two studies. Publication bias was assessed by Egger's and Begg's test, and significant publication bias was defined as a $P<0.10$.(13) The trim and fill method was applied when significant publication bias was found to confirm the pooled results. Sensitivity analyses were carried out by excluding each study individually from the meta-analysis.(14) All statistical analyses were performed with R (Version: 3.5.2).

Result

The characteristics of the included studies

A total of 1102 studies were retrieved in this meta-analysis, and 279 studies were selected for full-text review. In total, 23 studies with 2068 patients fulfilled the inclusion and exclusion criteria, with publication dates ranging from 2017 to 2018.(15-37) The flow diagram of this study is shown in Figure 1. The sample size

was between 19 and 201. Of these studies, 9 were conducted in Europe, (16, 17, 21, 24, 27, 30, 35, 36) 5 were conducted in America, (22, 28, 31, 33, 37) and the remaining studies were conducted in Asia. Among all patients included, 1305 (64.0%) were males, and 643 (31.4%) were diagnosed with squamous cell carcinoma. Twenty studies explored the association between the NLR and OS; fifteen studies investigated the relationship between the NLR and PFS. Additionally, 7 of 23 studies provided data on the posttreatment NLR. (21, 23, 25, 28, 29, 32, 33) If the study provided data about posttreatment NLR and OS, we treated it as an independent study in the subsequent analysis. Six trials performed first-line therapy, (16, 19, 25, 28, 31, 36) and the other trails performed second or additional-lines of therapy. Most patients received nivolumab, a PD-1 inhibitor, as immunotherapy. The cutoff value of the NLR was not the same in all studies; a value of 5 was used frequently, and the median cutoff value for all enrolled publications was also 5. The NOS scores of the enrolled studies ranged from 6-9. Detailed information on these studies is presented in Table 1.

Relationship between the NLR and OS in lung cancer patients receiving immunotherapy

Twenty studies on a total of 1629 patients treated with immunotherapy provided the NLR value or data that could be used to calculate the NLR and OS. Five of these

studies provided data on the posttreatment NLR and OS. Data from a total of 23 studies were used to combine HRs and 95% CIs. In the pooled analysis of the NLR and OS, we found that a higher NLR was associated with poorer OS, with high heterogeneity (HR=1.62; 95% CI: 1.41-1.87; P< 0.001) (I^2=81.7%, P< 0.001) (Figure 2). To detect the source of heterogeneity, we conducted a subgroup analysis on certain clinical factors that may influence the final results, such as study design, the time at which the NLR was determined, ethnicity, sex ratio, the proportion of patients with squamous cell carcinoma (SCC%), the NLR at baseline, the treatment line, the median follow-up time, sample size and the drug given for immunotherapy (Figure 3). Interestingly, the association between the pretreatment NLR and OS showed a similar trend to the pooled result (HR=1.87; 95% CI: 1.46-2.39; P < 0.001). However, the posttreatment NLR was not significantly related to the OS in lung cancer patients (HR=1.80; 95% CI: 0.81-4.00; P= 0.111). However, these results were still highly heterogeneous (pretreatment: I^2=79.80%, P< 0.001; posttreatment: I^2=83.5%, P< 0.001). Furthermore, the NLR was significantly unrelated to the OS in studies in which the proportion of patients with squamous cell carcinoma or whose baseline NLR exceeded the cutoff value was greater than 50% (Figure 3).The subgroup analysis stratified by ethnicity found that patients in Asia had significantly higher HR

(HR=2.76; 95% CI: 1.88- 4.06) and less heterogeneity (I^2=45.7%, P=0.091) than those in Europe and America ($P_{interaction}$=0.030) (Figure 3).

Relationship between the NLR and PFS in lung cancer patients receiving immunotherapy

Data on the NLR and PFS of 1612 patients treated with immunotherapy in 20 studies were extracted to obtain the pooled HR and 95% CI. Four of these studies provided the posttreatment NLR and its relationship with PFS. The random effects model revealed a significant association between an elevated NLR and PFS in lung cancer patients receiving immunotherapy (HR=1.47; 95% CI: 1.25-1.72; P< 0.001) with high heterogeneity (I^2=72.5%, P< 0.001) (Figure 4). To detect the potential source of heterogeneity in studies reporting PFS data, a subgroup analysis stratified by the factors that affect the NLR was performed as previously described (Figure 5). Similar to the relationship between the NLR and OS, the NLR was significantly unrelated to the PFS in studies in which the proportion of patients with squamous cell carcinoma was greater than 50% ($P_{interaction}$=0.005). However, the pooled results for subgroups based on other factors were not markedly changed with a low level of heterogeneity.

Sensitivity analysis and Publication bias

We found high heterogeneity among studies in which the relationship between the

pretreatment NLR, OS and PFS was analyzed. Therefore, we performed a sensitivity analysis on all enrolled studies. The effect of each study set on the combined HRs was evaluated by excluding each study individually from the meta-analysis. The results of the sensitivity analysis showed that the pooled HRs for OS and PFS were robust in our meta-analysis (Figure 6A and 6B). We also conducted a subgroup analysis stratified by various factors to detect the source of heterogeneity. Begg's test presented no evidence of obvious publication bias in studies reporting the association between the NLR and OS (P=0.673) or in those reporting the association between the NLR and PFS (P= 0.074), but Egger's test showed significant publication bias in which both were reported (P<0.001 for both). Therefore, we performed a trim and fill analysis on studies reporting the relationship between the NLR and OS/PFS. However, the result was unchanged after eliminating the influence of publication bias (OS: HR=1.40; 95% CI:1.22-1.60; P< 0.001, PFS: HR=1.33; 95% CI:1.14-1.56; P< 0.001, Supplementary Figure 1).

Discussion:

The results of our meta-analysis revealed the prognostic effect of both the pretreatment and posttreatment NLR on OS and PFS in lung cancer patients receiving immunotherapy. Twenty-three studies involving a total of 2049 lung cancer patients

showed that an increased NLR was significantly associated with poor OS (HR=1.62; 95% CI: 1.41-1.87; P< 0.001) and PFS (HR=1.47; 95% CI: 1.25-1.72; P< 0.001). Interestingly, the posttreatment NLR was not significantly associated with OS, and patients in Asia had significantly higher HRs than those in Europe and America.

The immune checkpoint is a kind of mechanism that plays a protective role in the human immune system and acts as a brake to prevent inflammatory damage caused by the excessive activation of T cells.(38) Human anti-PD-1 IgG4 mAb is now widely used and shows higher efficacy than standard therapies in lung cancer therapies. (39) Despite a wide consensus on testing tumor tissues for PD-L1 expression, the human anti-PD-1 IgG4 mAb is limited by its “unperfected dichotomy” across studies and molecules; patients with low levels of PD-L1 expression have response rates of up to 17%, and roughly half of patients are “not-responders” despite having high tumor levels of PD-L1. Several factors could affect the response and survival of patients receiving immunotherapy.(39) In addition to tumor mutation loads and the expression of tumor antigens, the status of systemic inflammation also plays an important role in lung cancer patients receiving immunotherapy. Tumor-associated cytokines and the relevant signaling pathways could be reflected by the level of systemic inflammation, which has been proven to be associated with poor survival in patients with solid

tumors.(8) Biomarkers such as NLR, PLR, GPS and modified GPS(mGPS) have been used as prognostic factors in lung cancer.(9-11) In addition, the role of systemic inflammation in patients receiving immunotherapy is particularly important for their survival. Several studies have explored the effect of the pretreatment NLR on lung cancer patients receiving immunotherapy.(31, 40-45) There are also two meta-analyses concerning the pretreatment NLR and survival in patients with advanced cancer. (46) (47) In summary, the NLR is a reliable prognostic factor for patients with various cancer types.

Sacdalan D. B reported that a high NLR resulted in poor PFS in patients with several kinds of cancers, such as melanoma, non-small-cell lung cancer (NSCLC) and genitourinary cancer,(46) which was consistent with our results. However, only three publications on lung cancer were enrolled in the previous meta-analysis, and a nonsignificant association was discovered between the pretreatment NLR and OS was discovered. In addition, two of the three studies included in the meta-analysis previously mentioned only provided only abstracts, and we cloud not obtain more details about those cohorts or study designs. Another meta-analysis conducted by Jiang T also revealed a trend similar to ours, but the results of the subgroup analysis showed that posttreatment NLR was significantly associated with poor OS and PFS, which is

inconsistent with our result. We enrolled more research articles in our study. In addition, we performed subgroup analyses stratified by additional clinical factors. Furthermore, our results showed that the ethnicity, the NLR at baseline and the proportion of patients with squamous cell carcinoma may affect the prognostic value of the NLR. However, due to the high heterogeneity, the results must be interpreted with caution. We found that patients in Asia had a significant higher HR than those in Europe and America in the subgroup analysis of the relationship between the NLR and OS. Some studies showed that neutrophils were the most abundant immune cell type identified in NSCLC patients and accounted for nearly 20% of all CD45+ cells in patients from America.(48) However, this result was not found in patients from Asia or Europe. The systemic inflammatory response in different ethnicities might differ. Furthermore, we collected baseline patient information, including the proportion of patients with squamous cell carcinoma, from all studies, and our results showed that the histology of lung cancer might have an impact on the prognostic value of the NLR. Many factors including tumor mutation load and the expression of tumor antigens, affect patient response and survival. (39) Patients with lung adenocarcinoma have a high EGFR mutation rate and some studies revealed that patients with targetable oncogenes were associated with a poor response to immunotherapy. (49) This may account for the results of our article.

The current study had several limitations. First, high heterogeneity was present in this analysis although we conducted sensitivity analyses on all studies. The results were robust after eliminating each study from the analysis. In addition, we performed subgroup analyses on certain possible impact factors to detect the source of heterogeneity. Second, Egger's test showed that obvious publication bias in the current study. The pooled results should be treated with caution, although trim and fill analysis testing indicated credibility for this study. Additionally, considering the high heterogeneity after subgroup analysis, other factors might be responsible for the high heterogeneity in this meta-analysis.

Conclusion:

Generally, our meta-analysis focused on the clinical prognostic agreement of the NLR and OS and PFS in lung cancer patients. Importantly, given the limitations mentioned above, these findings should be treated with caution in clinical practice. More prospective cohort studies are needed to confirm our results.

Contributorship statement:

(I) Conception and design: W Li, J Jin and Lan Y; II) Administrative support: J Jin, W Li; (III) Provision of study materials or patients: D Liu; (IV) Collection and assembly of data: J Jin, D Liu; (V) Data analysis and interpretation: D Liu, Lan Y; (VI)

Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Competing interests

The authors have no conflicts of interest to declare

Funding

None

Data sharing statement:

All data in the current study are available in the published articles

Acknowledgments:

This work was supported by the Transformation Projects of Sci-Tech Achievements of Sichuan Province (2016CZYD0001), the Sci-Tech Support Program of Science and Technology Department of Sichuan Province (2016SZ0073), the National Major Sci-Tech Project (2017ZX10103004-012) and the National Key Development Plan for Precision Medicine Research (2017YFC0910004).

Compliance with Ethical Standards

Ethical approval: All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendment.

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Figure Legends

Figure 1 Flow chart of study selection

Figure 2 Forest plot of the association between the NLR and OS in patients with lung cancer receiving immunotherapy

Figure 3 Subgroup analysis of the relationship between the NLR and OS in patients with lung cancer receiving immunotherapy

Abbreviations: ICI: immune checkpoint inhibitor; M/F: male/female; SCC%: proportion of patients with squamous cell carcinoma;※: the data here show the proportion of patients whose baseline NLR exceeded the cutoff value; N: nivolumab; P: pembrolizumab; D: durvalumab; E: embrolizumab; A: atezolizumab

Figure 4 Forest plot of the association between the NLR and PFS in patients with lung cancer receiving immunotherapy

Figure 5 Subgroup analysis of the relationship between the NLR and PFS in patients with lung cancer receiving immunotherapy

Abbreviations: ICI: immune checkpoint inhibitor; M/F: male/female; SCC%: proportion of patients with squamous cell carcinoma;※: 20 studies provided the data on the pretreatment NLR and PFS, and 5 of them also provided the posttreatment NLR and PFS; N: nivolumab; P: pembrolizumab; D: durvalumab; E: embrolizumab; A: atezolizumab

Figure 6 Sensitivity analysis of OS (A) and PFS (B)

Table

Table 1 The basic characteristics of the enrolled studies

Study	Year	Country	Ethnicity	Sample size	MFP	M/F	NLR at baseline
Diem S	2017	Europe	European	52	NM	29/23	5.0(2.7-8.3) *
Bagley SJ	2017	America	American	175	NM	80/95	NLR≥5:58.0%
Russo A	2018	Italy	European	28	17	25/3	NM
Zer A	2018	America	American	88	5.3	43/45	NLR＞4:56.8%
Nakaya A	2018	Japan	Asian	101	8.9	77/24	NLR≥3:46.5%
Maymani H	2018	America	American	74	12.3	36/38	NLR＞6:20.3%
Mezquita L	2018	Europe	European	161	12	100/61	NLR＞3:39.0%
Fukui T	2018	Japan	Asian	52	10.9	37/15	NLR≥5:34.6%
Park W	2018	America	American	159	11.5	82/77	4.3(0.5-24.1) *
Takeda T	2018	Japan	Asian	30	NM	19/11	NLR＞5:30.0%
Svaton M	2018	Czech Republic	European	120	NM	71/49	NLR＞3.8:50.0%
Suh Koung Jin	2018	Korea	Asian	54	26.2	42/12	NLR＞5:14.8%
Shiroyama Takayuki	2018	Japan	Asian	201	12.4	135/66	NLR＞4:39.3%
Kiriu T	2018	Japan	Asian	19.00	NM	19	NLR＞5:31.6%
Khunger M	2018	America	American	109	30	56/53	NLR≥5:50.5%
Inomata M	2018	Japan	Asian	36	NM	27/9	NLR≥5:44.4%
Facchinetti F	2018	Italy	European	54	12.6	45/9	NM
Ren F	2019	China	Asian	147	2.6	94/53	NLR＞2.5:59.9%
Pavan A	2019	Italy	European	184	56.3	125/59	NLR≥3:57.5%
Passiglia F	2019	Italy	European	45	9.1	32/13	NLR＞3.3:51.1%
Minami S	2019	Japan	Asian	76	NM	49/27	NLR≥6:14.5%
Ichiki Y	2019	Japan	Asian	44	4.83	38/6	NM
Dusselier M	2019	France	European	59	NM	44/15	NLR＞5:62.7%

Study	SCC%	Treatment lines	Outcome	Study design	NOS	Cutoff	IO
Diem S	34.6%	including first line therapy	OS/PFS	RO	6	5	N
Bagley SJ	24.0%	at least second-line therapy	OS/PFS	RO	6	5	N
Russo A	60.7%	at least second-line therapy	OS/PFS	RO	7	3	N
Zer A	17.1%	at least second-line therapy	OS/PFS/DCR	RO	7	4	NM
Nakaya A	36.6%	at least second-line therapy	PFS/irAEs	RO	6	3	N
Maymani H	16.2%	including first line therapy	OS/PFS	RO	7	6	N/P/D
Mezquita L	28.6%	at least second line therapy	OS/PFS	RO	9	3	N/E/A/D
Fukui T	30.8%	at least second-line therapy	OS/PFS/irAEs	PO	7	5	N

Park W	24.5%	including first line therapy	OS/PFS	RO	7	5	N
Takeda T	30.0%	at least second-line therapy	PFS	RO	6	5	N
Svaton M	33.3%	at least second-line therapy	OS/PFS	RO	7	3.8	N
Suh Koung Jin	31.5%	including first line therapy	OS/PFS/irAEs	RO	8	5	N/P
Shiroyama Takayuki	30.4%	at least second-line therapy	PFS/RR	RO	7	4	N
Kiriu T	31.5%	at least second-line therapy	OS/PFS/TTF	RO	7	5	N
Khunger M	23.9%	at least second-line therapy	OS	RO	6	5	N
Inomata M	44.4%	at least second-line therapy	PFS	RO	6	5	N/P
Facchinetti F	48.2%	at least second-line therapy	OS/PFS/TTF/DP	PO	8	4	N
Ren F	42.2%	at least second-line therapy	OS/PFS	RO	6	2.5	N/P
Pavan A	32.1%	including first line therapy	OS/PFS/irAEs	RO	8	3	N/P/A
Passiglia F	44.4%	at least second-line therapy	OS/TTP	RO	8	3.3	N
Minami S	23.7%	at least second-line therapy	OS/PFS	RO	9	6	N/P/A
Ichiki Y	65.9%	including first line therapy	OS/PFS/irAEs	RO	7	NM	N/P
Dusselier M	20.3%	at least second-line therapy	OS	RO	8	5	N

Abbreviations: NLR: neutrophil to lymphocyte ratio; NM: not mentioned; M/F: male/female; MFP: median follow-up (months); SCC%: proportion of patients with squamous cell carcinoma; IO: immunotherapy; N: nivolumab; P: pembrolizumab; D: durvalumab; E: embrolizumab; A: atezolizumab; OS: overall survival; PFS: progression-free survival; DCR: disease control rate; irAEs: immune-related adverse events; RR: response rate; TTF: time to treatment failure; RO: retrospective study; PO: prospective study; NOS: Newcastle-Ottawa quality assessment Scale; *: the study provided only the median NLR and range at baseline.

Supplementary information

S1: File. PRISMA 2009 checklist.

1 S2: Search strategy. This file provides the full electronic search strategy for PubMed

2 S3: Figure 1.Trim and fill analysis of OS and PFS

3 S4: Professional editing certificaiton

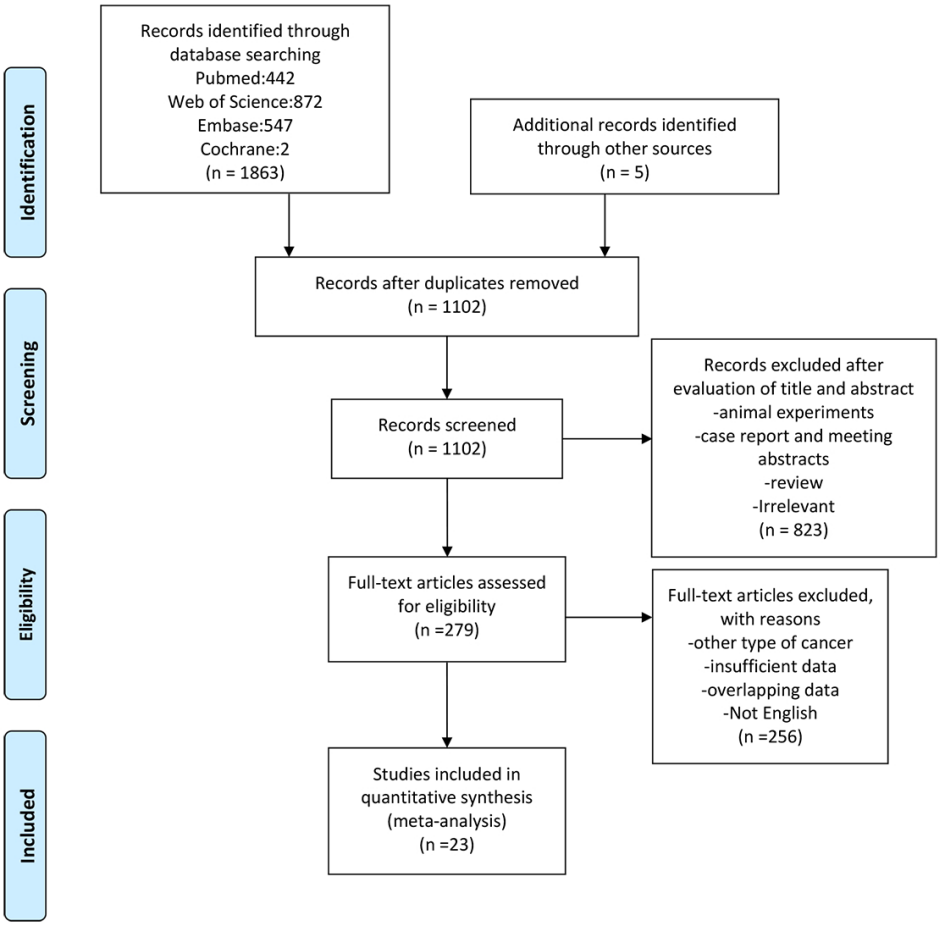


Figure 1 Flow chart of study selection

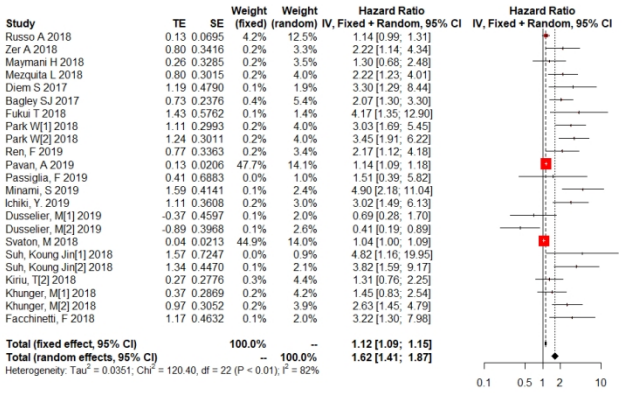


Figure 2 Forest plot of the association between NLR and OS in patients with lung cancer receiving immunotherapy

396x246mm (96 x 96 DPI)

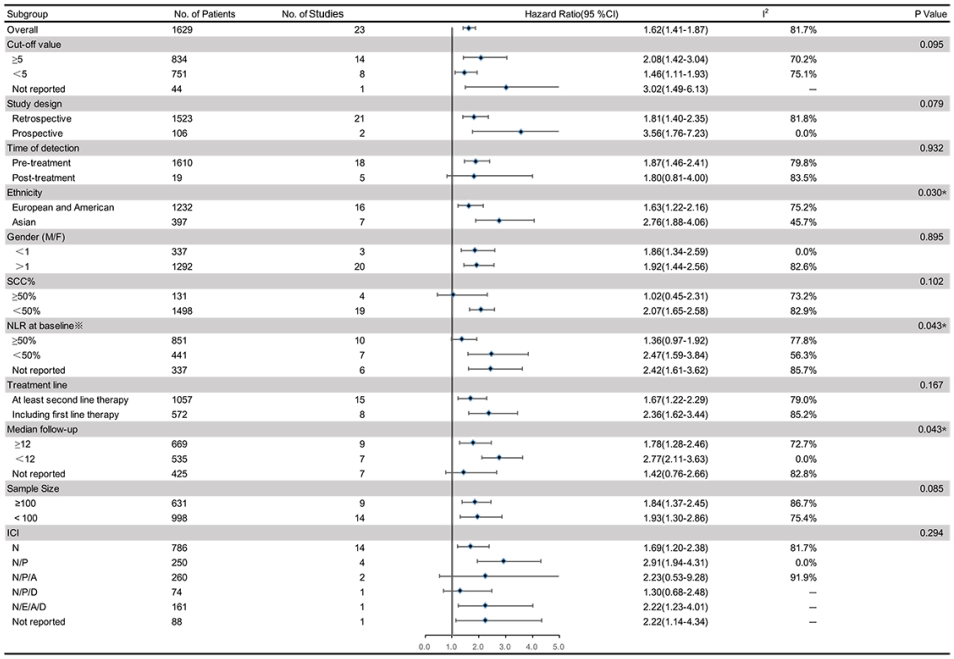


Figure 3 Subgroup analysis of the relationship between the NLR and OS in patients with lung cancer receiving immunotherapy

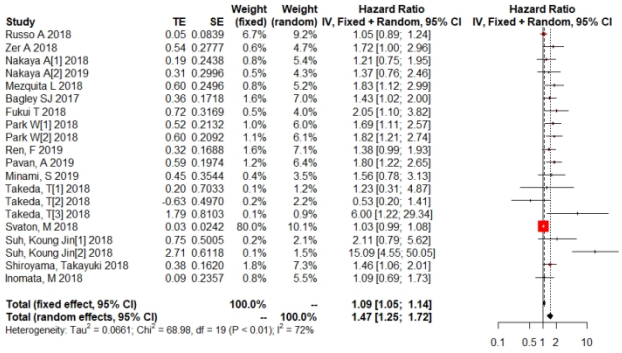


Figure 4 Forest plot of the association between NLR and PFS in patients with lung cancer receiving immunotherapy

396x246mm (96 x 96 DPI)

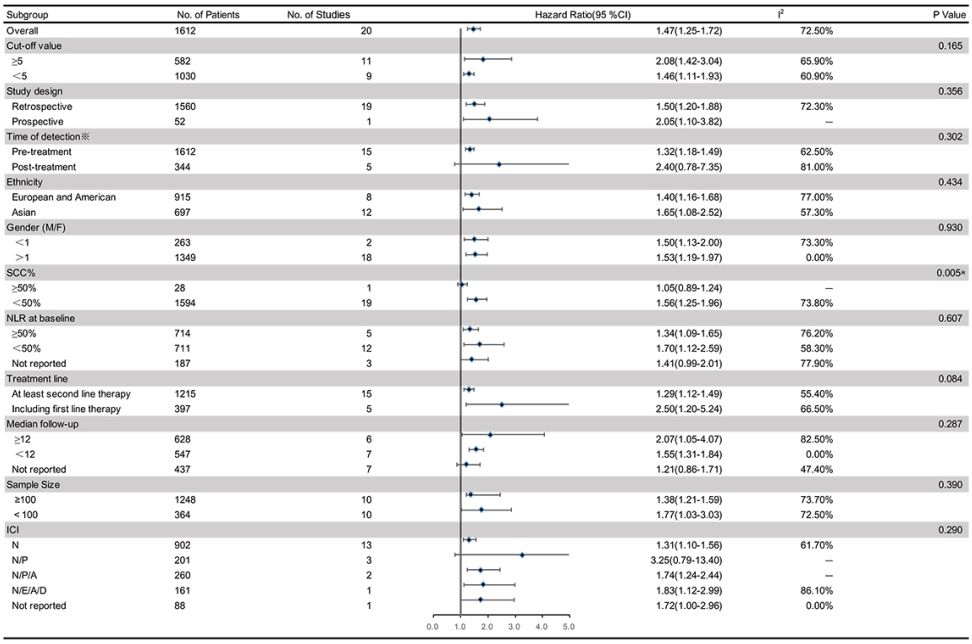


Figure 5 Subgroup analysis of the relationship between the NLR and PFS in patients with lung cancer receiving immunotherapy

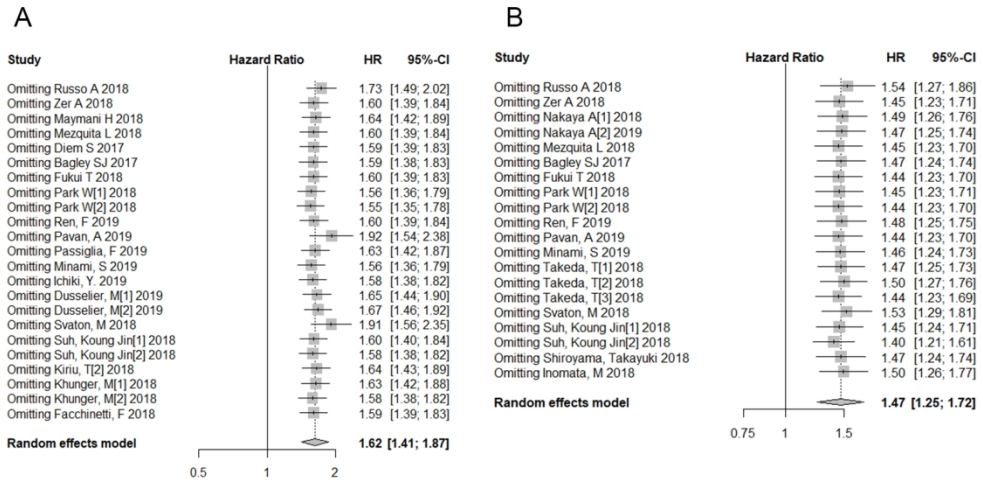


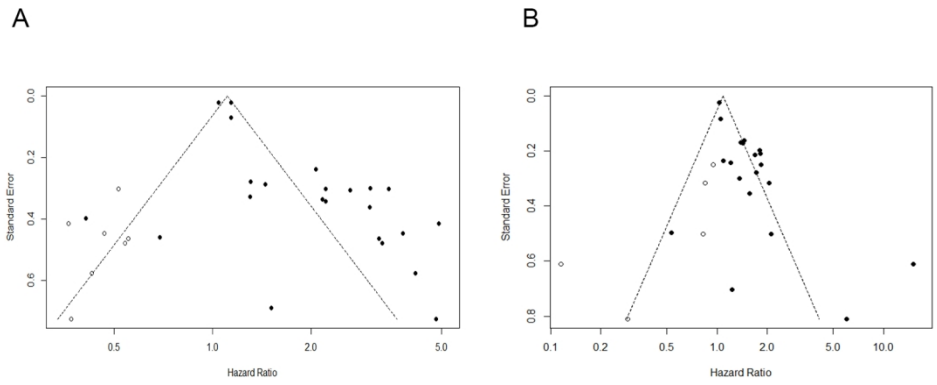
Figure 6 Sensitivity analysis on OS (A) and PFS (B)

143x74mm (300 x 300 DPI)

Supplement Table1 Search strategy for meta-analysis of Correlation of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis (PubMed via NLM)

	Search terms: ***neutrophil to lymphocyte ratio and lung cancer patients with immunotherapy***
Population: persons with lung cancer receiving immunotherapy	
1	(((((Cancer of Lung) OR Pulmonary Neoplasms) OR Neoplasms, Lung) OR Lung Neoplasm) OR Neoplasm, Lung) OR Neoplasms, Pulmonary) OR Neoplasm, Pulmonary) OR Pulmonary Neoplasm) OR Lung Cancer) OR Cancer, Lung) OR Cancers, Lung) OR Lung Cancers) OR Pulmonary Cancer) OR Cancer, Pulmonary) OR Cancers, Pulmonary) OR Pulmonary Cancers) OR Cancer of the Lung) OR "Lung Neoplasms"[Mesh])))) AND (("Immunotherapy"[Mesh]) AND Immunotherapies)
Intervention (Expose): neutrophil to lymphocyte ratio	
2	((NLR) OR (neutrophil to lymphocyte ratio) OR neutrophil lymphocyte ratio))
Combined sets	
3	1 and 2
Limits	
4	3 AND English [Language]

[Mesh] = Term from the Medline controlled vocabulary, including terms found below this term in the Mesh hierarchy



150x60mm (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.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
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.	5
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.	6
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.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5-6 and supplements
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).	5-8
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.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
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.	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
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.	7-8

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).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
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.	8-9
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.	8-9
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).	11-12
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.	8-11
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11-12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12
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).	12-13
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).	15-16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
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.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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BMJ Open

Association of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-035031.R2
Article Type:	Original research
Date Submitted by the Author:	14-Apr-2020
Complete List of Authors:	Jin, Jing; Sichuan University West China Hospital Yang, Lan; Sichuan University West China Hospital Liu, Dan; Sichuan University West China Hospital Li, Weimin; Sichuan University West China Hospital,
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Respiratory medicine
Keywords:	Immunology < BASIC SCIENCES, Epidemiology < ONCOLOGY, Respiratory tract tumours < ONCOLOGY

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Association of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis

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Running title: Neutrophil to lymphocyte ratio and lung cancer

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Article summary

Strengths and limitations of this study

1. Verification of the prognostic value of the NLR in a large number of lung cancer patients who received immunotherapy

2. Different clinical characteristics could affect the prognostic value of the NLR

3. High heterogeneity was present in this analysis

Abstract:

Objectives: To explore the relationship between the pretreatment or posttreatment neutrophil to lymphocyte ratio (NLR) and overall survival (OS)/ progression-free survival (PFS) in lung cancer patients receiving immunotherapy.

Design: We searched several databases to collect relevant studies conducted until July 2019. We carefully reviewed the full text of the included publications and combined the hazard ratios (HRs) and 95% confidence intervals (CIs) to assess the association between the NLR and survival time in lung cancer patients receiving immunotherapy.

Data Sources: PubMed, the Cochrane Library, Embase and Web of Science

Eligibility Criteria: Studies reporting the prognostic value of the NLR in lung cancer patients receiving immunotherapy were enrolled.

Data extraction and synthesis: Basic information on the articles and patients (NLR cutoff value, NLR at baseline, and HRs with 95% CIs for OS and PFS) was extracted by two authors independently. The pooled HRs of OS and PFS were synthesized using the random effects or fixed effects model.

Results: Twenty-three studies with 2068 patients were enrolled. Among all patients, 1305 (64.0%) were males, and 643 (31.4%) were diagnosed with squamous cell carcinoma. In a pooled analysis of OS and PFS from all studies, an elevated NLR

predicted poor OS (HR=1.62; 95% CI: 1.41-1.87; P< 0.001) and PFS (HR=1.47; 95% CI: 1.25-1.72; P< 0.0001). Subgroup analyses stratified showed that the posttreatment NLR was not significantly related to OS and that patients in Asia had significantly higher HRs than those in Europe and America. Furthermore, the proportion of squamous cell carcinoma and baseline NLR could affect the prognostic value of the NLR.

Conclusions: Our study found that an elevated NLR was associated with poor OS and PFS in lung cancer patients receiving immunotherapy and that several clinical factors might have an impact on the predictive value of the NLR in the survival of lung cancer patients.

Keywords: NLR; systemic inflammation; prognostic markers; lung cancer

Introduction:

Lung cancer is the most prevalent cancer and life-threatening malignancy worldwide.(1) The pathogenesis of lung cancer is complicated, and the primary treatments for lung cancer patients are surgery and chemotherapy. Unfortunately, most patients with lung cancer are diagnosed at advanced stages, and the benefits achieved from chemotherapy in advanced lung cancer patients are relatively small. Recently, many studies have revealed that tumor cells can evade the antitumor responses of T cells by controlling the combined responses of programmed cell death protein 1 (PD-1) and programmed cell death ligand-1 (PD-L1).(2) Nivolumab, pembrolizumab, atezolizumab, durvalumab, ipilimumab and tremelimumab have successfully changed clinical experiences in lung cancer treatment.(3) Tumor mutational burden,(4) neoantigens(5) and classical monocytes in the peripheral blood(6) and PD-L1 expression on tumor cells in particular, (7) are effective predictive biomarkers for immune checkpoint therapy in lung cancer. Systemic inflammation in cancer patients is believed to influence the growth and migration of tumors via certain inflammatory factors.(8) An elevated level of systemic inflammation, including Glasgow prognostic score (GPS), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), and C-reactive protein to albumin

ratio (CAR), have been indicated to be associated with poor survival in patients with solid tumors.(9-11) However, data on the prognostic value of the pretreatment NLR in lung cancer patients receiving immunotherapy remain scarce and inconsistent. Therefore, we reviewed available publications and conducted a meta-analysis to explore the prognostic value of the pretreatment NLR for r overall survival (OS) and progression-free survival (PFS) in clinical trials on lung cancer patients receiving immunotherapy.

Materials and Methods

Patient and Public Involvement: No patient was involved

Search strategy

The PRISMA guidelines for a systematic review and meta-analysis were strictly followed in this article (registration number PROSPERO: CRD42018104856). An online search was conducted to identify relevant publications in the PubMed, Cochrane Library, Web of Science and Embase databases. The following words were used to search for studies on the associations between the pretreatment NLR and survival time in patients with lung cancer published before July 2019: “pulmonary neoplasms”, “neutrophil lymphocyte ratio”, “immunotherapy”, “programmed death receptor-1”, and “immune checkpoint inhibitor”. A full electronic search strategy is

provided in the supplementary information (Supplementary Table 1). Additional studies were selected for a full-text review were selected by exploring the references cited in the selected articles and relevant reviews. The articles were limited to the English language, but there were no restrictions on the minimum number of patients. Two authors (J Jin and L Yang) independently reviewed the titles and abstracts of the retrieved articles to select the potentially relevant articles for a careful assessment.

Eligibility criteria

The inclusion criteria were as follows: 1) retrospective or prospective studies published before July 2019; 2) all patients enrolled in the studies were diagnosed with lung cancer by biopsy and received immunotherapy; 3) the value of the NLR was calculated based on the level of neutrophils and lymphocytes; and 4) HRs and 95% CIs were provided and data necessary to calculate them were reported.

The exclusion criteria were as follows: 1) review, meeting abstract, letter, or full text unavailable in English; 2) nonhuman studies; and 3) research that did not provide the value of the NLR.

Data extraction

From each study, the name of the study, first author, year of publication, study design, number of patients, sex distribution, age, median follow-up time, histology, NLR

cutoff value, NLR at baseline, line of therapy, drugs and HRs with 95% CIs for OS and PFS were extracted by two authors (D Liu and L Yang). If univariate and multivariate analysis results were simultaneously reported, only the multivariate analysis results were extracted. Any disagreements between the authors were resolved by a discussion and consensus. The most recent study was chosen when duplicate studies occurred.

Quality assessment

The primary studies were assessed by the Newcastle-Ottawa quality assessment Scale (NOS). The quality assessment was conducted by two independent researchers (J Jin and D Liu). The studies in which the mark was between 6 and 9 points were regarded as high-quality studies.

(http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp)

Statistical analysis

The primary endpoints were the OS and PFS of lung cancer patients receiving immunotherapy. PFS was defined as the time from the initial date of immunotherapy to the date of progression or death. OS was calculated from the date of inclusion to the time of death from any cause. HRs with 95% CIs were directly obtained from the articles or estimated from the Kaplan-Meier curves according to the methods reported

by Tierney et al.(12) We calculated the pooled HRs of OS and PFS using random effects or fixed effects model. We performed the Q-test to assess between-study heterogeneity and calculated the I^2 statistic, which expresses the percentage of the total observed variability due to study heterogeneity. The heterogeneity between studies was considered small if the I^2 statistic was less than 50% and the P value for the Q-test was less than 0.05. We performed a subgroup analysis to detect the source of heterogeneity. In addition, we considered only subgroups that included more than two studies. Publication bias was assessed by Egger's and Begg's test, and significant publication bias was defined as a $P<0.10$.(13) The trim and fill method was applied when significant publication bias was found to confirm the pooled results. Sensitivity analyses were carried out by excluding each study individually from the meta-analysis.(14) All statistical analyses were performed with R (Version: 3.5.2).

Result

The characteristics of the included studies

A total of 1102 studies were retrieved in this meta-analysis, and 279 studies were selected for full-text review. In total, 23 studies with 2068 patients fulfilled the inclusion and exclusion criteria, with publication dates ranging from 2017 to 2018.(15-37) The flow diagram of this study is shown in Figure 1. The sample size

was between 19 and 201. Of these studies, 9 were conducted in Europe, (16, 17, 21, 24, 27, 30, 35, 36) 5 were conducted in America, (22, 28, 31, 33, 37) and the remaining studies were conducted in Asia. Among all patients included, 1305 (64.0%) were males, and 643 (31.4%) were diagnosed with squamous cell carcinoma. Twenty studies explored the association between the NLR and OS; fifteen studies investigated the relationship between the NLR and PFS. Additionally, 7 of 23 studies provided data on the posttreatment NLR. (21, 23, 25, 28, 29, 32, 33) If the study provided data about posttreatment NLR and OS, we treated it as an independent study in the subsequent analysis. Six trials performed first-line therapy, (16, 19, 25, 28, 31, 36) and the other trails performed second or additional-lines of therapy. Most patients received nivolumab, a PD-1 inhibitor, as immunotherapy. The cutoff value of the NLR was not the same in all studies; a value of 5 was used frequently, and the median cutoff value for all enrolled publications was also 5. The NOS scores of the enrolled studies ranged from 6-9. Detailed information on these studies is presented in Table 1.

Relationship between the NLR and OS in lung cancer patients receiving immunotherapy

Twenty studies on a total of 1629 patients treated with immunotherapy provided the NLR value or data that could be used to calculate the NLR and OS. Five of these

studies provided data on the posttreatment NLR and OS. Data from a total of 23 studies were used to combine HRs and 95% CIs. In the pooled analysis of the NLR and OS, we found that a higher NLR was associated with poorer OS, with high heterogeneity (HR=1.62; 95% CI: 1.41-1.87; P< 0.001) (I^2=81.7%, P< 0.001) (Figure 2). To detect the source of heterogeneity, we conducted a subgroup analysis on certain clinical factors that may influence the final results, such as study design, the time at which the NLR was determined, ethnicity, sex ratio, the proportion of patients with squamous cell carcinoma (SCC%), the NLR at baseline, the treatment line, the median follow-up time, sample size and the drug given for immunotherapy (Figure 3). Interestingly, the association between the pretreatment NLR and OS showed a similar trend to the pooled result (HR=1.87; 95% CI: 1.46-2.39; P < 0.001). However, the posttreatment NLR was not significantly related to the OS in lung cancer patients (HR=1.80; 95% CI: 0.81-4.00; P= 0.111). However, these results were still highly heterogeneous (pretreatment: I^2=79.80%, P< 0.001; posttreatment: I^2=83.5%, P< 0.001). Furthermore, the NLR was significantly unrelated to the OS in studies in which the proportion of patients with squamous cell carcinoma or whose baseline NLR exceeded the cutoff value was greater than 50% (Figure 3).The subgroup analysis stratified by ethnicity found that patients in Asia had significantly higher HR

(HR=2.76; 95% CI: 1.88- 4.06) and less heterogeneity (I^2=45.7%, P=0.091) than those in Europe and America ($P_{interaction}$=0.030) (Figure 3).

Relationship between the NLR and PFS in lung cancer patients receiving immunotherapy

Data on the NLR and PFS of 1612 patients treated with immunotherapy in 20 studies were extracted to obtain the pooled HR and 95% CI. Four of these studies provided the posttreatment NLR and its relationship with PFS. The random effects model revealed a significant association between an elevated NLR and PFS in lung cancer patients receiving immunotherapy (HR=1.47; 95% CI: 1.25-1.72; P< 0.001) with high heterogeneity (I^2=72.5%, P< 0.001) (Figure 4). To detect the potential source of heterogeneity in studies reporting PFS data, a subgroup analysis stratified by the factors that affect the NLR was performed as previously described (Figure 5). Similar to the relationship between the NLR and OS, the NLR was significantly unrelated to the PFS in studies in which the proportion of patients with squamous cell carcinoma was greater than 50% ($P_{interaction}$=0.005). However, the pooled results for subgroups based on other factors were not markedly changed with a low level of heterogeneity.

Sensitivity analysis and Publication bias

We found high heterogeneity among studies in which the relationship between the

pretreatment NLR, OS and PFS was analyzed. Therefore, we performed a sensitivity analysis on all enrolled studies. The effect of each study set on the combined HRs was evaluated by excluding each study individually from the meta-analysis. The results of the sensitivity analysis showed that the pooled HRs for OS and PFS were robust in our meta-analysis (Figure 6A and 6B). We also conducted a subgroup analysis stratified by various factors to detect the source of heterogeneity. Begg's test presented no evidence of obvious publication bias in studies reporting the association between the NLR and OS (P=0.673) or in those reporting the association between the NLR and PFS (P= 0.074), but Egger's test showed significant publication bias in which both were reported (P<0.001 for both). Therefore, we performed a trim and fill analysis on studies reporting the relationship between the NLR and OS/PFS. However, the result was unchanged after eliminating the influence of publication bias (OS: HR=1.40; 95% CI:1.22-1.60; P< 0.001, PFS: HR=1.33; 95% CI:1.14-1.56; P< 0.001, Supplementary Figure 1).

Discussion:

The results of our meta-analysis revealed the prognostic effect of both the pretreatment and posttreatment NLR on OS and PFS in lung cancer patients receiving immunotherapy. Twenty-three studies showed that an increased NLR was

significantly associated with poor OS and PFS. Interestingly, the posttreatment NLR was not significantly associated with OS, and patients in Asia had significantly higher HRs than those in Europe and America.

The immune checkpoint is a kind of mechanism that plays a protective role in the human immune system and acts as a brake to prevent inflammatory damage caused by the excessive activation of T cells.(38) Human anti-PD-1 IgG4 mAb is now widely used and shows higher efficacy than standard therapies in lung cancer therapies. (39) Despite a wide consensus on testing tumor tissues for PD-L1 expression, the human anti-PD-1 IgG4 mAb is limited by its "unperfected dichotomy" across studies and molecules; patients with low levels of PD-L1 expression have response rates of up to 17%, and roughly half of patients are "not-responders" despite having high tumor levels of PD-L1. Several factors could affect the response and survival of patients receiving immunotherapy.(39) In addition to tumor mutation loads and the expression of tumor antigens, the status of systemic inflammation also plays an important role in lung cancer patients receiving immunotherapy. Tumor-associated cytokines and the relevant signaling pathways could be reflected by the level of systemic inflammation, which has been proven to be associated with poor survival in patients with solid tumors.(8) Biomarkers such as NLR, PLR, GPS and modified GPS(mGPS) have been

used as prognostic factors in lung cancer.(9-11) In addition, the role of systemic inflammation in patients receiving immunotherapy is particularly important for their survival. Several studies have explored the effect of the pretreatment NLR on lung cancer patients receiving immunotherapy.(31, 40-45) There are also two meta-analyses concerning the pretreatment NLR and survival in patients with advanced cancer. (46) (47) In summary, the NLR is a reliable prognostic factor for patients with various cancer types.

Sacdalan D. B reported that a high NLR resulted in poor PFS in patients with several kinds of cancers, such as melanoma, non-small-cell lung cancer (NSCLC) and genitourinary cancer,(46) which was consistent with our results. However, only three publications on lung cancer were enrolled in the previous meta-analysis, and a nonsignificant association was discovered between the pretreatment NLR and OS was discovered. In addition, two of the three studies included in the meta-analysis previously mentioned only provided only abstracts, and we cloud not obtain more details about those cohorts or study designs. Another meta-analysis conducted by Jiang T also revealed a trend similar to ours, but the results of the subgroup analysis showed that posttreatment NLR was significantly associated with poor OS and PFS, which is inconsistent with our result. Different with the study mentioned before, we enrolled

more research articles andperformed subgroup analyses stratified by additional clinical factors. Furthermore, our results showed that the ethnicity, the NLR at baseline and the proportion of patients with squamous cell carcinoma may affect the prognostic value of the NLR. However, due to the high heterogeneity, the results must be interpreted with caution. We also found that patients in Asia had a significant higher HR than those in Europe and America in the subgroup analysis of the relationship between the NLR and OS. Some studies showed that neutrophils were the most abundant immune cell type identified in NSCLC patients and accounted for nearly 20% of all CD45+ cells in patients from America.(48) However, this result was not found in patients from Asia or Europe. The systemic inflammatory response in different ethnicities might differ. Furthermore, we collected baseline patient information, including the proportion of patients with squamous cell carcinoma, from all studies, and our results showed that the histology of lung cancer might have an impact on the prognostic value of the NLR. Many factors including tumor mutation load and the expression of tumor antigens, affect patient response and survival. (39) Patients with lung adenocarcinoma have a high EGFR mutation rate and some studies revealed that patients with targetable oncogenes were associated with a poor response to immunotherapy. (49) This may account for the results of our article.

The current study had several limitations. First, high heterogeneity was present in this analysis although we conducted sensitivity analyses on all studies. The results were robust after eliminating each study from the analysis. In addition, we performed subgroup analyses on certain possible impact factors to detect the source of heterogeneity. Second, Egger's test showed that obvious publication bias in the current study. The pooled results should be treated with caution, although trim and fill analysis testing indicated credibility for this study. Additionally, considering the high heterogeneity after subgroup analysis, other factors might be responsible for the high heterogeneity in this meta-analysis.

Conclusion:

Generally, our meta-analysis focused on the clinical prognostic agreement of the NLR and OS and PFS in lung cancer patients. Importantly, given the limitations mentioned above, these findings should be treated with caution in clinical practice. More prospective cohort studies are needed to confirm our results.

Contributorship statement:

(I) Conception and design: W Li, J Jin and Lan Y; II) Administrative support: J Jin, W Li; (III) Provision of study materials or patients: D Liu; (IV) Collection and assembly of data: J Jin, D Liu; (V) Data analysis and interpretation: D Liu, Lan Y; (VI)

Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Competing interests

The authors have no conflicts of interest to declare

Funding

None

Data sharing statement:

All data in the current study are available in the published articles

Acknowledgments:

This work was supported by the Transformation Projects of Sci-Tech Achievements of Sichuan Province (2016CZYD0001), the Sci-Tech Support Program of Science and Technology Department of Sichuan Province (2016SZ0073), the National Major Sci-Tech Project (2017ZX10103004-012) and the National Key Development Plan for Precision Medicine Research (2017YFC0910004).

Compliance with Ethical Standards

Ethical approval: All procedures performed in the studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendment.

Reference:

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Figure Legends

Figure 1 Flow chart of study selection

Figure 2 Forest plot of the association between the NLR and OS in patients with lung cancer receiving immunotherapy

Figure 3 Subgroup analysis of the relationship between the NLR and OS in patients with lung cancer receiving immunotherapy

Abbreviations: ICI: immune checkpoint inhibitor; M/F: male/female; SCC%: proportion of patients with squamous cell carcinoma;※: the data here show the proportion of patients whose baseline NLR exceeded the cutoff value; N: nivolumab; P: pembrolizumab; D: durvalumab; E: embrolizumab; A: atezolizumab

Figure 4 Forest plot of the association between the NLR and PFS in patients with lung cancer receiving immunotherapy

Figure 5 Subgroup analysis of the relationship between the NLR and PFS in patients with lung cancer receiving immunotherapy

Abbreviations: ICI: immune checkpoint inhibitor; M/F: male/female; SCC%: proportion of patients with squamous cell carcinoma;※: 20 studies provided the data on the pretreatment NLR and PFS, and 5 of them also provided the posttreatment NLR and PFS; N: nivolumab; P: pembrolizumab; D: durvalumab; E: embrolizumab; A: atezolizumab

Figure 6 Sensitivity analysis of OS (A) and PFS (B)

Table

Table 1 The basic characteristics of the enrolled studies

Study	Year	Country	Ethnicity	Sample size	MFP	M/F	NLR at baseline
Diem S	2017	Europe	European	52	NM	29/23	5.0(2.7-8.3) *
Bagley SJ	2017	America	American	175	NM	80/95	NLR≥5:58.0%
Russo A	2018	Italy	European	28	17	25/3	NM
Zer A	2018	America	American	88	5.3	43/45	NLR＞4:56.8%
Nakaya A	2018	Japan	Asian	101	8.9	77/24	NLR≥3:46.5%
Maymani H	2018	America	American	74	12.3	36/38	NLR＞6:20.3%
Mezquita L	2018	Europe	European	161	12	100/61	NLR＞3:39.0%
Fukui T	2018	Japan	Asian	52	10.9	37/15	NLR≥5:34.6%
Park W	2018	America	American	159	11.5	82/77	4.3(0.5-24.1) *
Takeda T	2018	Japan	Asian	30	NM	19/11	NLR＞5:30.0%
Svaton M	2018	Czech Republic	European	120	NM	71/49	NLR＞3.8:50.0%
Suh Koung Jin	2018	Korea	Asian	54	26.2	42/12	NLR＞5:14.8%
Shiroyama Takayuki	2018	Japan	Asian	201	12.4	135/66	NLR＞4:39.3%
Kiriu T	2018	Japan	Asian	19.00	NM	19	NLR＞5:31.6%
Khunger M	2018	America	American	109	30	56/53	NLR≥5:50.5%
Inomata M	2018	Japan	Asian	36	NM	27/9	NLR≥5:44.4%
Facchinetti F	2018	Italy	European	54	12.6	45/9	NM
Ren F	2019	China	Asian	147	2.6	94/53	NLR＞2.5:59.9%
Pavan A	2019	Italy	European	184	56.3	125/59	NLR≥3:57.5%
Passiglia F	2019	Italy	European	45	9.1	32/13	NLR＞3.3:51.1%
Minami S	2019	Japan	Asian	76	NM	49/27	NLR≥6:14.5%
Ichiki Y	2019	Japan	Asian	44	4.83	38/6	NM
Dusselier M	2019	France	European	59	NM	44/15	NLR＞5:62.7%

Study	SCC%	Treatment lines	Outcome	Study design	NOS	Cutoff	IO
Diem S	34.6%	including first line therapy	OS/PFS	RO	6	5	N
Bagley SJ	24.0%	at least second-line therapy	OS/PFS	RO	6	5	N
Russo A	60.7%	at least second-line therapy	OS/PFS	RO	7	3	N
Zer A	17.1%	at least second-line therapy	OS/PFS/DCR	RO	7	4	NM
Nakaya A	36.6%	at least second-line therapy	PFS/irAEs	RO	6	3	N
Maymani H	16.2%	including first line therapy	OS/PFS	RO	7	6	N/P/D
Mezquita L	28.6%	at least second line therapy	OS/PFS	RO	9	3	N/E/A/D
Fukui T	30.8%	at least second-line therapy	OS/PFS/irAEs	PO	7	5	N

Park W	24.5%	including first line therapy	OS/PFS	RO	7	5	N
Takeda T	30.0%	at least second-line therapy	PFS	RO	6	5	N
Svaton M	33.3%	at least second-line therapy	OS/PFS	RO	7	3.8	N
Suh Koung Jin	31.5%	including first line therapy	OS/PFS/irAEs	RO	8	5	N/P
Shiroyama Takayuki	30.4%	at least second-line therapy	PFS/RR	RO	7	4	N
Kiriu T	31.5%	at least second-line therapy	OS/PFS/TTF	RO	7	5	N
Khunger M	23.9%	at least second-line therapy	OS	RO	6	5	N
Inomata M	44.4%	at least second-line therapy	PFS	RO	6	5	N/P
Facchinetti F	48.2%	at least second-line therapy	OS/PFS/TTF/DP	PO	8	4	N
Ren F	42.2%	at least second-line therapy	OS/PFS	RO	6	2.5	N/P
Pavan A	32.1%	including first line therapy	OS/PFS/irAEs	RO	8	3	N/P/A
Passiglia F	44.4%	at least second-line therapy	OS/TTP	RO	8	3.3	N
Minami S	23.7%	at least second-line therapy	OS/PFS	RO	9	6	N/P/A
Ichiki Y	65.9%	including first line therapy	OS/PFS/irAEs	RO	7	NM	N/P
Dusselier M	20.3%	at least second-line therapy	OS	RO	8	5	N

Abbreviations: NLR: neutrophil to lymphocyte ratio; NM: not mentioned; M/F: male/female; MFP: median follow-up (months); SCC%: proportion of patients with squamous cell carcinoma; IO: immunotherapy; N: nivolumab; P: pembrolizumab; D: durvalumab; E: embrolizumab; A: atezolizumab; OS: overall survival; PFS: progression-free survival; DCR: disease control rate; irAEs: immune-related adverse events; RR: response rate; TTF: time to treatment failure; RO: retrospective study; PO: prospective study; NOS: Newcastle-Ottawa quality assessment Scale; *: the study provided only the median NLR and range at baseline.

Supplementary information

S1: Search strategy. This file provides the full electronic search strategy for PubMed

S2: Figure 1.Trim and fill analysis of OS and PFS

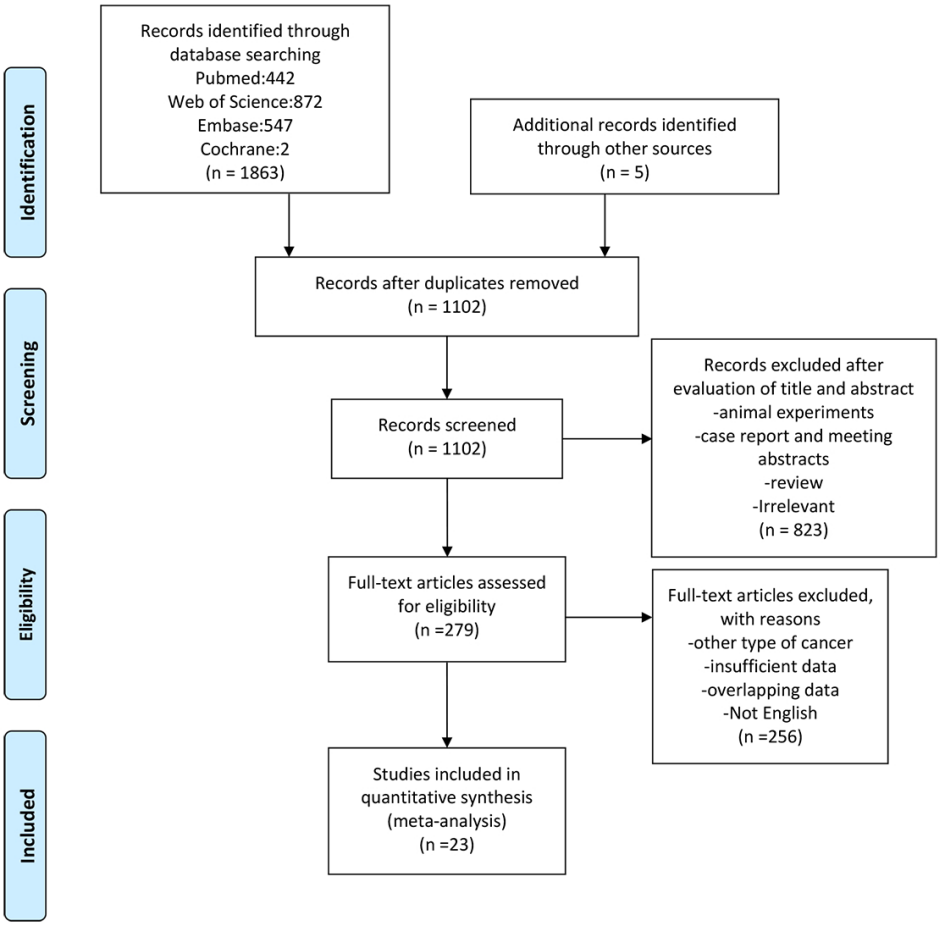


Figure 1 Flow chart of study selection

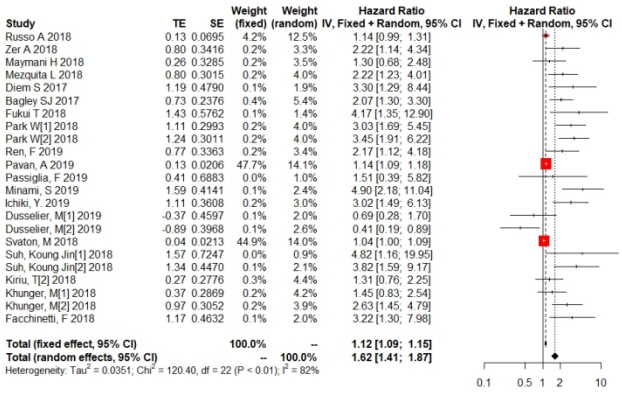


Figure 2 Forest plot of the association between NLR and OS in patients with lung cancer receiving immunotherapy

396x246mm (96 x 96 DPI)

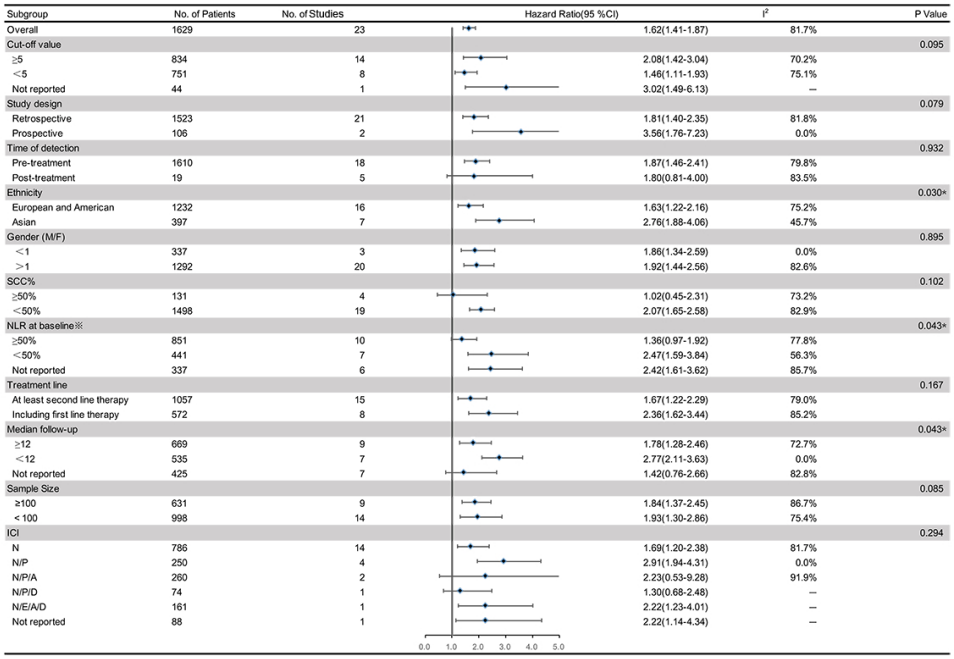


Figure 3 Subgroup analysis of the relationship between the NLR and OS in patients with lung cancer receiving immunotherapy

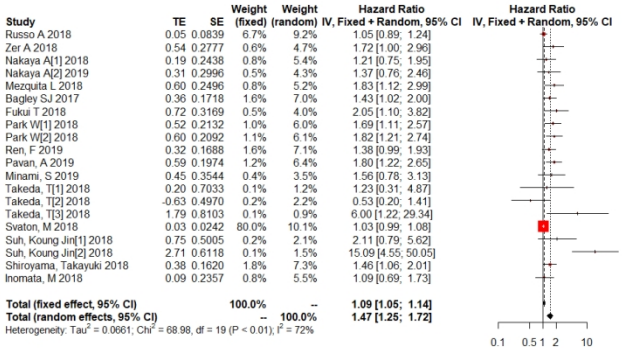


Figure 4 Forest plot of the association between NLR and PFS in patients with lung cancer receiving immunotherapy

396x246mm (96 x 96 DPI)

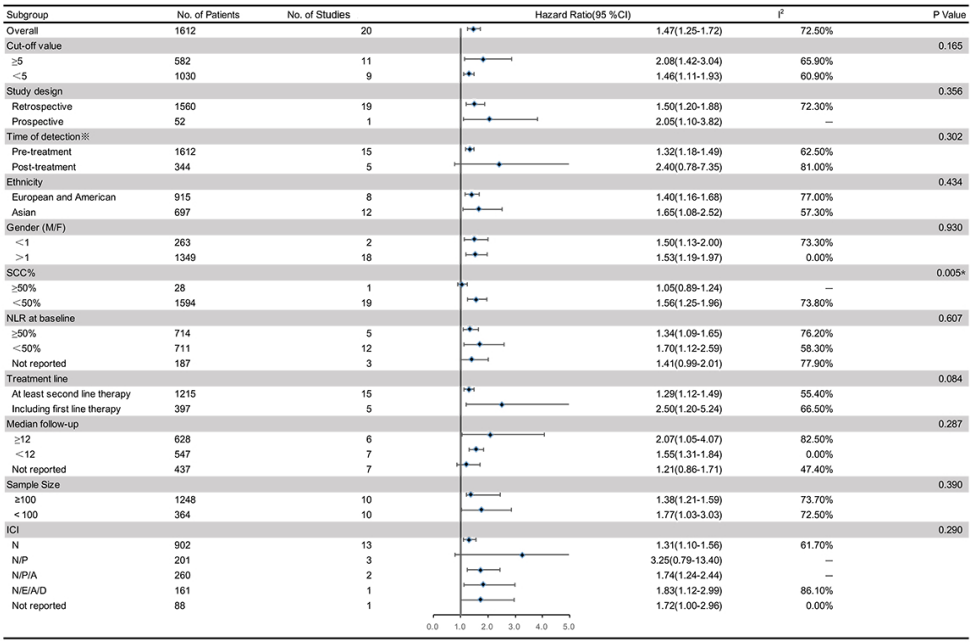


Figure 5 Subgroup analysis of the relationship between the NLR and PFS in patients with lung cancer receiving immunotherapy

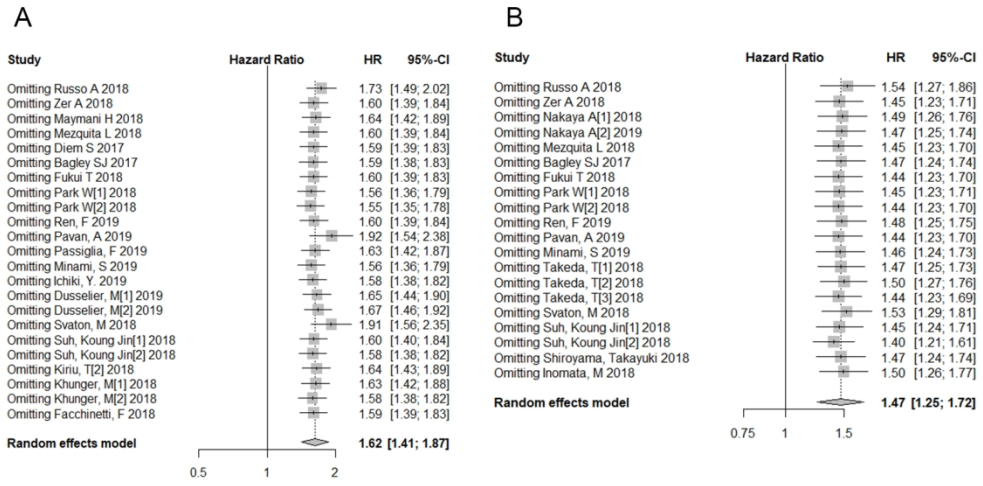


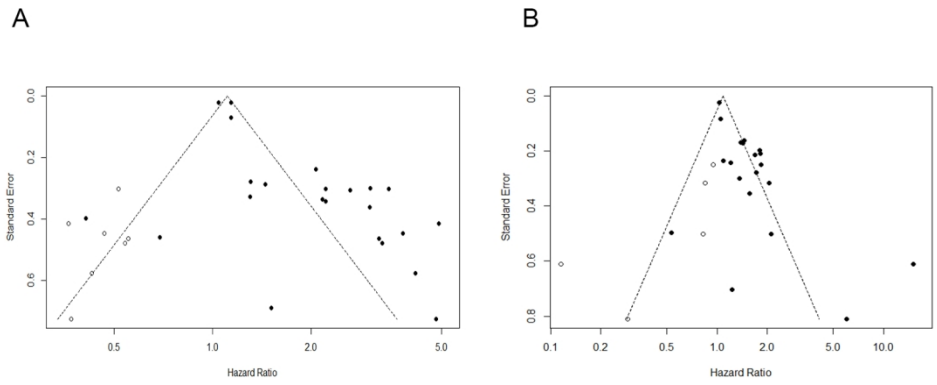
Figure 6 Sensitivity analysis on OS (A) and PFS (B)

143x74mm (300 x 300 DPI)

Supplement Table1 Search strategy for meta-analysis of Correlation of the neutrophil to lymphocyte ratio and clinical outcomes in lung cancer patients receiving immunotherapy: a meta-analysis (PubMed via NLM)

	Search terms: ***neutrophil to lymphocyte ratio and lung cancer patients with immunotherapy***
Population: persons with lung cancer receiving immunotherapy	
1	(((((Cancer of Lung) OR Pulmonary Neoplasms) OR Neoplasms, Lung) OR Lung Neoplasm) OR Neoplasm, Lung) OR Neoplasms, Pulmonary) OR Neoplasm, Pulmonary) OR Pulmonary Neoplasm) OR Lung Cancer) OR Cancer, Lung) OR Cancers, Lung) OR Lung Cancers) OR Pulmonary Cancer) OR Cancer, Pulmonary) OR Cancers, Pulmonary) OR Pulmonary Cancers) OR Cancer of the Lung) OR "Lung Neoplasms"[Mesh])))) AND (("Immunotherapy"[Mesh]) AND Immunotherapies)
Intervention (Expose): neutrophil to lymphocyte ratio	
2	((NLR) OR (neutrophil to lymphocyte ratio) OR neutrophil lymphocyte ratio))
Combined sets	
3	1 and 2
Limits	
4	3 AND English [Language]

[Mesh] = Term from the Medline controlled vocabulary, including terms found below this term in the Mesh hierarchy



150x60mm (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.	2-3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
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.	5
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.	6
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.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5-6 and supplements
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).	5-8
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.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
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.	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7-8
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.	7-8

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).	8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8
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.	8-9
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.	8-9
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).	11-12
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.	8-11
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-11
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11-12
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12
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).	12-13
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).	15-16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
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.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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