# Prognostic value of circulating endothelial cells in non-small cell lung cancer patients: a systematic review and meta-analysis

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**Background:** Circulating endothelial cells (CECs) have been indicated as a potential biomarker of vascular damage in a variety of cancers. Several studies have revealed CECs may reflect the extent of tumor angiogenesis; however, the role of CECs in the prognosis of non-small cell lung cancer (NSCLC) is undetermined to date. A meta-analysis has been prepared to determine whether the base level of CECs and the changes of CECs after therapy (ΔCECs: post-therapeutic value minus the pre- therapeutic value) could be considered as a prognostic tool for patients with NSCLC.
**Methods:** Systematic reviews of studies published before April 30 2015 were conducted on the association between the levels of CECs or ΔCECs and the prognosis of NSCLC in several data bases. Hazard ratios (HRs) and the 95% confidence intervals (CIs) were used to collate the data. Similarly, heterogeneity and publication bias were also evaluated.
**Results:** A total of nine studies, containing eight prospective studies and one retrospective study, involving 515 patients was identified. Patients with higher level of CECs counts at baseline were associated with longer progression-free survival (PFS) (HR 0.71, 95% CI: 0.529-0.891). ΔCECs could also be considered a prognostic indicator in NSCLC patients (HR 0.575, 95% CI: 0.401-0.75). The former and the later are without a significant heterogeneity in the data ($I^2$=21.2% and 0.0%, P=0.274 and 0.870, respectively). However, there was no correlation between the base level of CECs and the overall survival (OS) (HR 0.914, 95% CI: 0.560-1.267, $I^2$=43.6%, P=0.150).
**Conclusions:** Higher levels of CECs counts at baseline and the ensuing decrease after therapy demonstrated a positive correlation with longer PFS in NSCLC patients. But this phenomenon has not been found in OS. From a certain perspective, CECs counts and ΔCECs could be potential prognostic indicators for NSCLC patients.

**Keywords:** Circulating endothelial cells (CECs); the change of circulating endothelial cells (ΔCECs); prognostic; non-small cell lung cancer (NSCLC)

## Introduction

Lung cancer is one of the most common cancers and accounts for more deaths than any other cancer in both men and women (1). Survival rates for lung cancer remain critically low, the 5-year survival rate for men with lung cancer is 14% compared with 19% for women (2). Current research into lung cancer is mainly focused on noninvasive molecular targets which are used to predict the therapeutic effect of lung cancer treatment and the prognosis of patients with lung cancer.

Circulating endothelial cells (CECs) are mature viable cells that are shed from the endothelium, circulate within the bloodstream and still exhibit proliferative capacity despite their terminal differentiation (3). An alteration in CECs has been associated with several diseases, such as acute coronary syndromes (4), hereditary hemorrhagic telangiectasia (5), anti-neutrophil cytoplasmic antibodies (ANCA) associated vasculitis (6), etc. The hypothesis (7) that tumor growth is angiogenesis dependent was initially proposed in 1971 based on the observation that the expansion of a tumor mass was limited in the absence of angiogenesis. Subsequently, a large amount of experimental data substantiated the hypothesis. In 2001 Mancuso *et al*. (8) detected increased amounts of CECs in breast cancer for the first time using flow cytometry (FCM). Up to now, CECs have been put forward as promising biomarkers for diagnosis and prognosis of different types of cancers, including, but not limited to, colorectal cancer (9), prostate cancer (10) and pancreatic carcinoma (11).

In 2014 Mehran *et al*. found that levels of CECs were higher in mice with tumors than without tumors. Then Mehran found the same phenomenon in clinical lung cancer patients compared with normal healthy volunteers (12), so Mehran suggested that CECs are more numerous in cancer patients than in healthy subjects. Soon thereafter several studies supported their conclusion (12-14). However to-date, the relationship between the level of CECs and the diagnosis of non-small cell lung cancer (NSCLC) has had no definitive study. Moreover, the high level of CECs and the change of CECs (ΔCECs) have been reported to be correlated with both poor or good prognosis, partially since a single study might be insufficient to detect a possible small effect of CECs and ΔCECs level on NSCLC prognosis, especially when the sample size is statistically small. In this study, a systematic review and meta-analysis to estimate the effect of CECs and ΔCECs level on the survival of NSCLC patients were conducted. It was planned to analyze the prognostic value from the two aspects of progression-free survival (PFS) and overall survival (OS) in patients with NSCLC.

## Methodology

### *Publication search*

The electronic databases of PubMed and Web of Science were searched for relevant article from the date of the publications inception until April 30, 2015. The keyword used for the search strategy was "circulating endothelial cells". A subsequent, more refined search included the terms "cancer" OR "tumor". Further filter conditions included:

(I) only complete studies (abstracts only were excluded) in English; and (II) reports necessary to extract the required indicators. This study was planned, conducted, and reported in adherence to the professional standards of quality for reporting meta-analyses (15).

### *Inclusion criteria*

Studies included in this meta-analysis had to meet all the following criteria: (I) all the patients were diagnosed with NSCLC by histopathology; (II) the ability to evaluate the correlation between CECs or ΔCECs levels and prognosis of NSCLC patients including PFS or OS; and (III) the study directly reported the hazard ratios (HRs) and their 95% confidence intervals (CIs), or provided sufficient information allowing for approximations of HRs and the 95% CIs. If multiple publications from the same study population were available, the most recent and detailed study was eligible for inclusion in the meta-analysis.

### *Data extraction*

Each article was reviewed independently by two authors: Liu Y and Ye W. If there was a difference of opinion, the articles were discussed with a third author: Yuan D. The general data collected from each study were the first author's name, study country, publication year, type of study, number of patients, therapy schedule, methodology of CECs collection, cut-off value, points of the collection, the cell surface markers, and HR with 95% CI, which were used to measure the effective value.

### *Quality assessment*

The quality of each study was assessed by the same two investigators using "The Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses" (16). Given the variability in the quality of related studies searched in the initial literature search, these studies were considered to be high quality if the scores achieved a level of six or more.

### *Statistical methods*

The level of CECs or ΔCECs was considered "high" or "low" according to the cutoff values used in each study. The association between CECs or ΔCECs level and the clinical outcomes was evaluated using the HR of high CECs
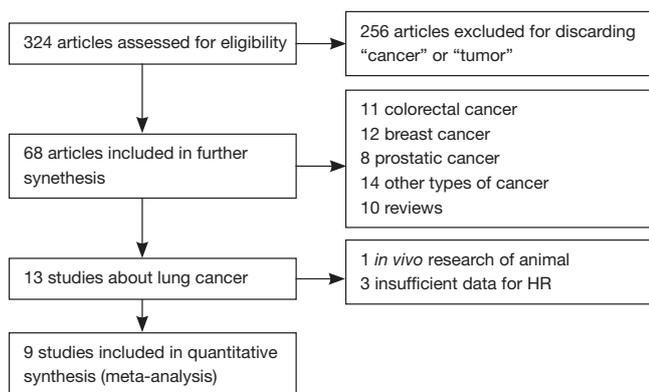
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**Figure 1** Flow diagram of study selection. HR, hazard ratio.

or ΔCECs level patients over low CECs or ΔCECs level patients and their 95% CI, the HR <1 and 95% CI that did not overlap with 1 implied a good prognosis for the patient with a high level, while the HR >1 and 95% CI that did not overlap with 1 signified poor survival. When these statistical variables were described in text or tables, we obtained them directly from each trial publication. If the data were not given directly, the available data were calculated from Kaplan-Meier curves (using Engauge Digitizer version 4.1) and the HR and 95% CI were calculated using the methods reported by Tierney *et al*. (17). The Mantel-Haenszel test was used to test significance, and P<0.05 was considered statistically significant. Statistical heterogeneity was assessed by the visual inspection of forest plots, by performing the $\chi^2$ test, and by calculating the $I^2$ value. Heterogeneity was considered significant if the P value is less than 0.1. The value of $I^2$ is used to assess the degree of heterogeneity ($I^2 < 25\%$ no heterogeneity; $I^2 = 25\text{-}50\%$ moderate heterogeneity; $I^2 > 50\%$ large or extreme heterogeneity).

*Publication bias evaluation*

Potential publication bias was evaluated using Begg's test (18) and Egger's test (19) and standard error was plotted against log (HR) to form a simple scatterplot. P<0.05 was considered to be representative of a significant statistical publication bias. All of the statistical analyses were performed with STATA 11.0 (StataCorp, College Station, TX, USA). All P values were two tailed.

**Results**

*Study selection and characteristics*

Our initial search identified 324 citations from a search of

the above databases using the search strategy as previously described. Two hundred and fifty-six reports were excluded as they were not research about cancers or tumors when we scanned the abstracts. Subsequently, in the remaining 68 research papers, included 11 colorectal cancer, 12 breast cancer, 8 prostatic cancer, 10 reviews, and 14 other cancer papers and finally 13 reports were about lung cancer. After in depth analysis of these 13 reports, one report focused on animal experiment and three reports did not provided sufficient data about HR, thus there additional four reports were excluded from the selection. Thus, nine reports fit the selection criteria for this research (*Figure 1*), including eight prospective studies and one retrospective study.

The clinical characteristics of these nine included studies eligible for the meta-analysis are summarized in *Table 1*; two studies evaluated patients from Spain, one from Japan, two from Syria, one from France, and three from China. They were primarily published between 2009 and 2015 (*Table 1*). The nine studies, which involved 772 NSCLC patients, with the sample sizes ranging from 31 to 151 patients (mean 85.8). Two hundred and fifty-seven patients were not accepted into the sample population or were lost during follow-up before the disease progressed. The number completing the entire survey was 515.

*Therapy schedule and sample collection*

The standard courses of treatment were double-agent platinum-based chemotherapy (14,24,25), chemotherapy combined with anti-angiogenesis (20-22,26), chemotherapy combined with radiotherapy (13), and surgery (23). All studies obtained the peripheral blood (PB) to separate the CECs in NSCLC patients. The points of collection and the cut-off value were different for each study (*Table 2*). In addition, HR values in two datasets were directly extracted from original data, and seven were extrapolated from survival curves.

*Baseline CECs counts and PFS in NSCLC patients*

Six studies including 387 patients reported data on baseline CECs counts and PFS in NSCLC. One report (14) implied that patients who had a higher baseline CECs count possessed a longer PFS. However, one study (23) suggested that a high CECs count at baseline significantly correlated with shorter PFS. The balance of the four studies (13,24-26) insisted that there was no significant correlation between baseline CECs count and PFS. Finally, combined data

**Table 1** Main characteristics of eligible studies

| First author | Year | Country | Type of study | No. of patients | Therapy schedule |
|---|---|---|---|---|---|
| Kawaishi M (14) | 2009 | Japan | Prospective | 31 | Paclitaxel (200 mg/m$^2$) + carboplatin (area under the curve of 6), Q3W |
| Chu TQ (20) | 2012 | China | Retrospective | 122 | Paclitaxel (175 mg/m$^2$, d1) + carboplatin (area under the curve =5, d1) + endostatin or placebo on days 8-21, Q3W |
| Fleitas T (21) | 2012 | Spain | Prospective | 60 | Double-agent platinum-based chemotherapy ± bevacizumab, Q3W |
| Wang J (22) | 2013 | China | Prospective | 74 | Navelbine (25 mg/m$^2$, days 1, 8) + cisplatin (25 mg/m$^2$, days 2-4) ± endostatin (7.5 mg/m$^2$, days 1-14), Q3-4W |
| Ilie M (23) | 2014 | France | Prospective | 74 | Surgery |
| Najjar F (13) | 2014 | Syria | Prospective | 151 | Double-agent platinum-based chemotherapy + radiotherapy (if this was necessary according to tumor stage), Q3W |
| Sánchez Hernández A (24) | 2015 | Spain | Prospective | 69 | Cisplatin (75 mg/m$^2$) + pemetrexed (500 mg/m$^2$) or carboplatin (area under the curve 5-6) + pemetrexed (500 mg/m$^2$), Q3W |
| Najjar F (25) | 2015 | Syria | Prospective | 89 | Maximum-tolerated dose of double-agent platinum-based chemotherapy, Q3W |
| Yuan DM (26) | 2015 | China | Prospective | 102 | Double-agent platinum-based chemotherapy ± endostar (7.5 mg/m$^2$), Q3W |

**Table 2** Main items of the sample

| First author | Method | Sample | CECs or ΔCECs | Surface biomarker | Cut-off value | Point of collection |
|---|---|---|---|---|---|---|
| Kawaishi M (14) | IMS | Peripheral blood | CECs | CD146+/CD105+/ DAPI+/CD45− | NA; 400 cells/4 mL | At baseline, 8 and 22 days after starting chemotherapy |
| Chu TQ (20) | FCM | Peripheral blood | CECs, ΔCECs | CD146+/CD133−/ CD45− | AUC =0.759; 0.58/μL | At baseline and at weeks 3 and 6 |
| Fleitas T (21) | IMS | Peripheral blood | CECs | CD146+ | 75$^{th}$ percentile; 152 cells/mL | At baseline, 3 weeks after the third cycle of treatment |
| Wang J (22) | FCM | Peripheral blood | ΔCECs | CD146+/CD105+/ CD45− | NA | Two days before the beginning of each therapeutic cycle, on the 8$^{th}$ day after completion of the last cycle |
| Ilie M (23) | IMS | Peripheral blood | CECs | CD146+/CD105+/ DAPI+/CD45− | 75$^{th}$ percentile; NA | At least 15 days before surgery |
| Najjar F (13) | IMS | Peripheral blood | CECs | CD146+ | 75$^{th}$ percentile; 120 cells/mL | At baseline, after every therapy |
| Sánchez Hernández A (24) | IMS | Peripheral blood | CECs | CD146+/CD105+/ DAPI+/CD45− | Mean; 152.79 cells/4 mL | At baseline, before the second and third cycles of treatment |
| Najjar F (25) | IMS | Peripheral blood | CECs, ΔCECs | CD146+ | 75$^{th}$ percentile; 362 cells/4 mL | At baseline, 3 weeks after the third cycle of chemotherapy (day 60) |
| Yuan DM (26) | FCM | Peripheral blood | CECs, ΔCECs | CD146+/CD31+/ CD45− | AUC =0.736; 210 cells/10$^5$ cells | At baseline and before the third cycle of chemotherapy |

CECs, circulating endothelial cells; ΔCECs, the change of circulating endothelial cells; IMS, immunomagnetic separation; NA, not applicable; FCM, flow cytometry; AUC, area under the curve.
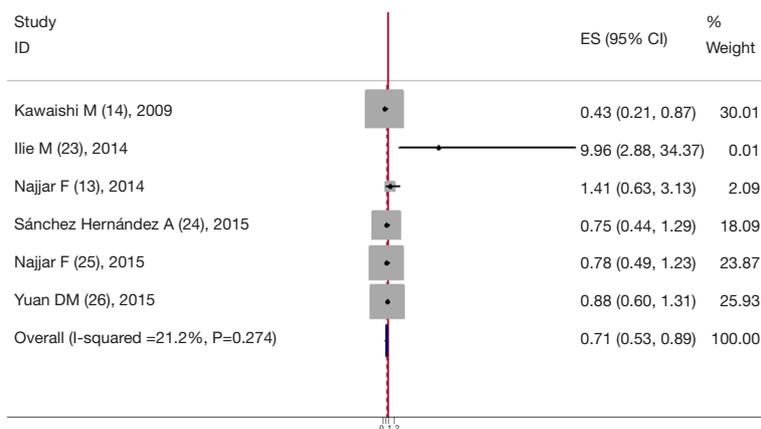
**Figure 2** Meta-analysis (forest plot) of studies assessing baseline CECs counts of PFS in NSCLC patients. CECs, circulating endothelial cells; PFS, progression-free survival; NSCLC, non-small cell lung cancer; ES, effect size; CI, confidence interval.
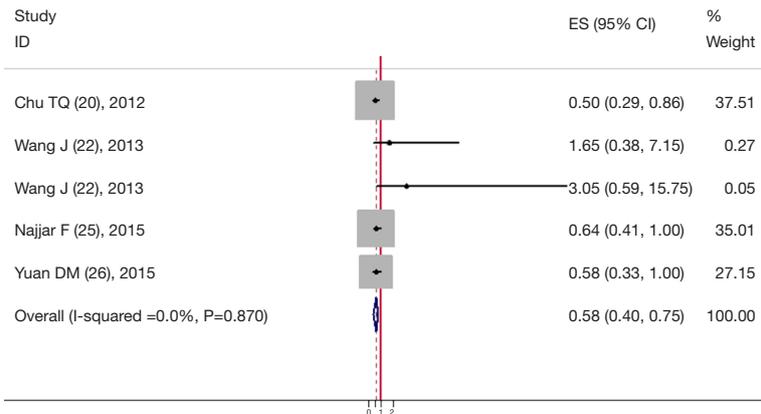


**Figure 3** Meta-analysis (forest plot) of studies assessing post-therapeutic changes of CECs counts of PFS in NSCLC patients. CECs, circulating endothelial cells; PFS, progression-free survival; NSCLC, non-small cell lung cancer; ES, effect size; CI, confidence interval.

from all six studies suggested that high level of baseline CECs counts and long PFS had a positive relationship with a pooled HR estimate of 0.71 (95% CI: 0.529-0.891) (*Figure 2*). The heterogeneity between the studies was not significant ($I^2$=21.2%, P=0.274) in either analysis.

### ∆CECs levels and PFS in NSCLC patients

Four studies including 273 patients with NSCLC, reported data on the change of CECs after therapy. One study was categorized randomly into two segments undergoing separate regimens of chemotherapy with Rh-endostatin or single chemotherapy, and followed-up to disease progression, respectively. So, five related studies were included about ∆CECs level and PFS. Three related studies (20,25,26) maintained that greater reduction of CECs significantly

correlated with longer median PFS, the other related study (22) indicated that there was no correlation between ∆CECs level and PFS. Combined data from these studies showed patients with a high percentage change in CECs counts after therapy had significantly longer PFS than those with low percentage change. The pooled HR estimate was 0.575 (95% CI: 0.401-0.75) (*Figure 3*). As well, there was no heterogeneity between the reports with $I^2$=0.00%, P=0.742.

### Baseline CECs counts and OS in NSCLC patients

In total, four reports were involved in baseline CECs counts and OS in patients with NSCLC. In the context of a single group, two (21,23) of these studies with high CECs counts at baseline had a significantly worse OS, with a P value of 0.006 and 0.04, respectively. The other two experiments did not
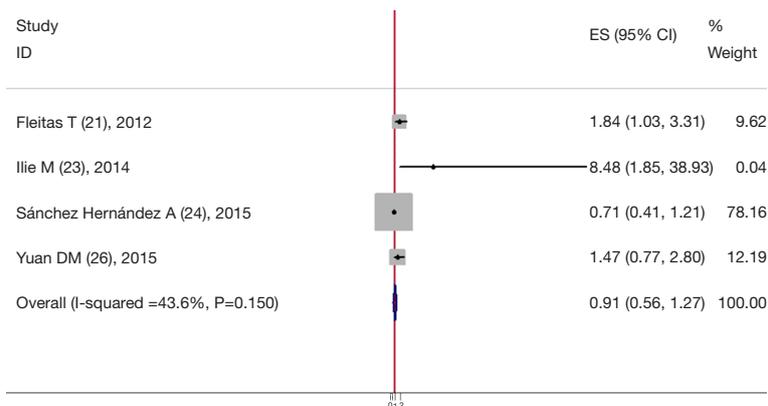
**Figure 4** Meta-analysis (forest plot) of studies assessing baseline CECs counts of OS in NSCLC patients. CECs, circulating endothelial cells; OS, overall survival; NSCLC, non-small cell lung cancer; ES, effect size; CI, confidence interval.
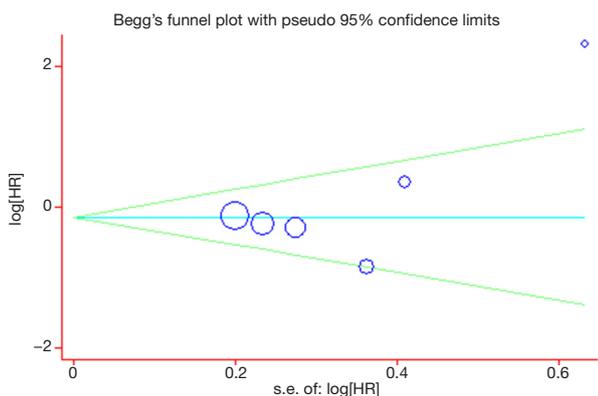


**Figure 5** Funnel plot of studies assessing baseline CECs counts of PFS in NSCLC patients. CECs, circulating endothelial cells; PFS, progression-free survival; NSCLC, non-small cell lung cancer; HR, hazard ratio.
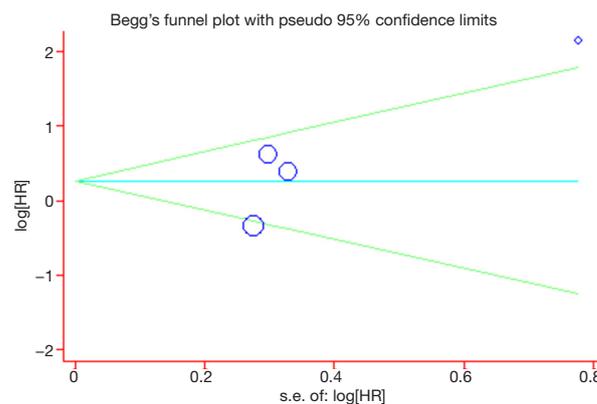


**Figure 6** Funnel plot of studies assessing baseline CECs counts of OS in NSCLC patients. CECs, circulating endothelial cells; PFS, progression-free survival; NSCLC, non-small cell lung cancer; HR, hazard ratio.

find a correlation between CECs baseline levels and the OS in patients with NSCLC; however, when these studies which had been analyzed were merged with each other; the combined HR was 0.914, 95% CI was 0.560-1.267. And there was only small heterogeneity ($I^2$=43.6%, P=0.150) (*Figure 4*).

### *Publication bias*

A Begg's funnel plot was prepared for the visual assessment of obvious publication bias for the included studies in CECs or ΔCECs levels. There was no overt evidence of significant publication bias for the studies included in our meta-analysis by the P value ($P_{CECs-PFS}$=0.462; $P_{\Delta CECs-PFS}$=0.221 and $P_{CECs-OS}$=0.308). An Egger's test was then employed for the formal evaluation and statistical significance was deemed when

the P<0.05. The $P_{CECs-PFS}$=0.177 (*Figure 5*) and the $P_{CECs-OS}$=0.210 (*Figure 6*), indicated that there were no significant publication bias among these studies, which concerned the base CECs counts of the PFS or OS in NSCLC patients. Alternatively, there did exist statistical differences on publication bias between the studies on the change of CECs after therapy and PFS, as the $P_{\Delta CECs-PFS}$=0.043 (*Figure 7*).

### Discussion

In recent years, personalized pharmacotherapy was more and more popular in oncology. Some researchers had examined the major subtype of driver mutations that have been identified in NSCLC and summarized the relevant therapies, in order to contribute to the personalized
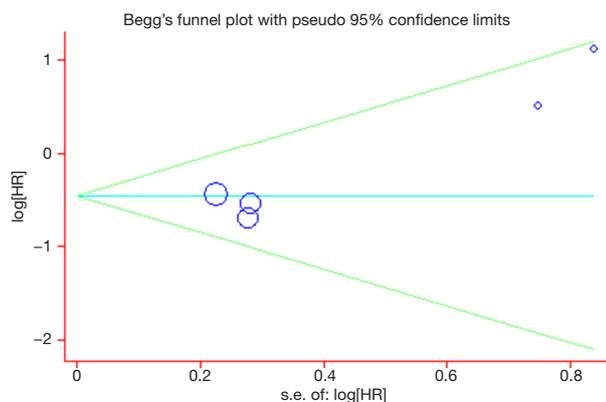
**Figure 7** Funnel plot of studies assessing post-therapeutic changes of CECs counts of PFS in NSCLC patients. CECs, circulating endothelial cells; PFS, progression-free survival; NSCLC, non-small cell lung cancer; HR, hazard ratio.

treatment (27). After the diagnosis and treatment, increasing attention is being paid to the prognostic biomarkers in NSCLC patients, such as Ki67, carcinoembryonic antigen (CEA), neuron-specific enolase (NSE) etc. CEC is a new prediction of these people. Generally speaking, in healthy donor's PB can also detect the existence of the CECs, but the level is very low with typical counts being 0-20 cells per ml of blood (28). The present meta-analysis is the first study to systematically evaluate the association between CECs or ΔCECs and prognosis in NSCLC patients. Our study combined the outcomes of 515 NSCLC patients from nine reports, which revealed a longer PFS in NSCLC patients having higher levels of baseline CECs and ΔCECs. Although, the base level of CECs has no effect on OS, Chu *et al.* (20) and Yuan *et al.* (26) showed that the median survival of patients with greater CECs reduction was significantly longer than those with lower reductions. As these indexes were inconformity in each research, collocated and correlated data was not further analyzed.

Originally three techniques are being used to detect CECs, including reverse transcription-polymerase chain reaction (RT-PCR), immunomagnetic separation (IMS) and FCM (29), but as preclinical data is conflicting, using RT-PCR to quantify CECs has been largely abandoned (30), so the latter two are the commonly used method. Similarly, the IMS and FCM depend on CD146 driven immunomagnetic isolation. At times, further identification also needs the expression of additional endothelial markers such as 2-(4-amidinophenyl)-1 Hindole-6-carboxam-idine (DAPI) or CD105 (31). This meta-analysis included studies

using IMS or FCM to count CECs, in these studies with CD146 being the primary positive marker on the CECs surface along with other morphological characteristics comprising CD105+, DAPI+, CD31+, CD133– or CD45–, reference to *Table 2*. Method and the choice of surface markers result in the different measuring values, this will bring different cut-off values. In these nine studies, the basis of cut-off values included the mean, the 75th percentile, the optimal area under receiver operating characteristic (ROC) curve and two entire studies had no mention of the terms. Cut-off value of research directly influence the grouping and the outcome.

Angiogenesis is considered to be an absolute prerequisite for malignant tumour growth and metastasis (32). Because the incomplete basement membrane, tumor blood vessels are immature and are therefore prone to rupture (33). So, anti-angiogenesis therapy has emerged. Since the first vascular endothelial growth factor (VEGF)-targeting drug (bevacizumab) was approved by the US Food and Drug Administration in 2004, the anti-angiogenic drugs have proliferated rapidly. Despite anti-angiogenic drugs prolonging the survival of patients with lung, colon, and renal carcinomas (34-36). Four reports involving CECs counts and anti-angiogenesis therapy (20-22,26) are included in this meta-analysis. The anti-angiogenesis drugs include Rh-endostatin and bevacizumab, the related data demonstrated that the CECs counts of the anti-angiogenesis therapy group decreased significantly compared with the control group. Moreover, in the majority of studies, it was found that CECs levels significantly decreased after chemotherapy in partial response (PR) patients, with contrasting results being obtained in progressive disease (PD) patients (14,20,22,24-26). The other study implied that the amounts of CECs at baseline in the patients who showed PD were significantly lower than in PR patients (14). This revealed that the CECs counts appeared to be a promising predictive maker of the clinical efficacy of chemotherapy, especially combined with anti-angiogenesis therapy.

In addition, one study was collected from preoperative blood samples from 74 patients who underwent resection for NSCLC (23), the data displayed high levels of CECs at baseline significantly correlated with shorter PFS and OS (P<0.001, P=0.005, respectively) of NSCLC patients. This is contrary to other studies, temporary surgical stimulation might affect the vascular system disorder, or the duration of follow-up can also affect this.

As far as the data is concerned, no meta-analysis regarding this relationship has been published to-date. Previous studies

have reported inconsistent and conflicting results about the association between the CECs counts at baseline or the change of CECs levels after therapy and the prognosis of NSCLC. The limitations in size and follow-up proved to be insufficient in one individual study, this meta-analysis made up for this defect to a certain extent. Nevertheless, several limitations of this meta-analysis should be indicated. First, in terms of meta-analysis, some inevitable publication bias may exist, because the failure of researchers to submit negative studies for publication, or negative results are often rejected by journals, whereas positive results tend to be accepted (37). Second, since some HRs were not directly reported in the studies, it was necessary to calculate HR from the data provided in the papers or extrapolate them from the survival curves. The estimated HR might be less reliable than the data obtained directly from published statistics. Third, the patient's general conditions of these studies are also the source of bias, such as race, age, complication, the sensitivity of the response to drugs and so on. Therefore, further high quality studies are needed, which include a large number of incident NSCLC cases with sufficient follow-up time, and information available on all patients and the relationship between CECs counts and the diagnosis or OS of the NSCLC.

## Conclusions

In summary, results of our meta-analysis suggest that CECs counts at baseline and ΔCECs levels could be used to determine the prognosis of patients with NSCLC and predict the effectiveness of the treatment without the limitations; especially it might be more ideal than the baseline CECs counts as a prognostic factor in patients with NSCLC that the reduction of CECs after therapy.

## Acknowledgements

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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