



Circulating tumour cells in patients with lung cancer universally indicate poor prognosis

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This meta-analysis demonstrates that the prognostic value of epithelial CTCs is greater than that of mesenchymal CTCs in lung cancer. CTCs indicate poor prognosis in all subtypes of lung cancer; this effect increases with CTC detection thresholds. <https://bit.ly/3dhlY0C>

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Abstract

Background: In lung cancer, the relevance of various circulating tumour cell (CTC) subgroups in different lung cancer subtypes is unclear. We performed a comprehensive meta-analysis to assess the prognostic value of CTCs in the different histological types of lung cancer, with particular respect to CTC subtypes, cut-offs and time points of CTC enumeration.

Methods: We searched MEDLINE, Web of Science and Embase alongside relevant studies evaluating the prognostic value of CTCs in lung cancer patients. A random-effects model was used for meta-analysis, calculating hazard ratios (HRs), 95% confidence intervals and p-values.

Results: 27 studies enrolling 2957 patients were included. CTC detection indicates poor prognosis, especially in small cell lung cancer (SCLC) patients (overall survival HR 3.11, 95% CI 2.59–3.73) and predicts a worse outcome compared to nonsmall cell lung cancer patients. Epithelial CTCs predict a worse outcome for lung cancer than mesenchymal CTCs or epithelial–mesenchymal hybrids.

Conclusion: CTCs indicate poor prognosis in patients with primary lung cancer, with CTCs in SCLC having a more pronounced prognostic effect. The prognostic value of CTCs detected by different markers varies; most evidence is available for the strong negative prognostic effect of epithelial CTCs.

Introduction

With an estimated 1.8 million deaths annually, lung cancer is the world's leading cause of cancer-associated mortality [1]. Although advances in surgical technique and perioperative medicine allow resection of formerly inoperable, locally advanced tumours, the majority of cases are considered inoperable due to functional (limited pulmonary reserve) or oncological (N3 lymph node or distant metastases) reasons. Consequently, only 12% of patients succumb to direct complications of the primary tumour; 37% of patients suffer death from indirect complications of malignant disease (*e.g.* pulmonary embolism, pneumonia) or concomitant lung disease such as COPD; the largest fraction of lung cancer patients (44%) die from complications of metastatic disease [2].

Haematogenous spread dramatically worsens the prognosis of lung cancer and is caused by circulating tumour cells (CTCs). CTCs are tumour cells shed into circulation by the primary tumour or metastatic lesions [3–5]. The detection and isolation of CTCs offer great potential in prognostication, therapy response prediction, liquid biopsy purposes and even the generation of patient-derived pre-clinical models [6–10]. However, CTC detection in the bloodstream is complicated by the rarity of CTCs compared to the



multitude of nonmalignant blood cells (erythrocytes, leukocytes, *etc.*) [11]. Technical solutions attempting to distinguish and separate CTCs from other cells employ physical (such as size, density, deformability) or biological (such as surface proteins) traits, but are limited by the heterogeneity of CTCs [12]. Consequently, no consensus surface marker or physical feature exists to identify and isolate CTCs universally. Therefore, all studies involving CTCs inevitably only focus on a subpopulation of CTCs pre-defined by the identification method used in each particular experiment.

The prognostic value of CTCs in lung cancer has been evaluated in many studies, but the conclusions are controversial [13, 14]. Furthermore, there is a lack of solid evidence as to the role and prognostic relevance of CTCs measured in the different histological types of lung cancer and at different time points during lung cancer, such as pre- or post-treatment CTC measurements.

The role of different CTC subpopulations (*i.e.* epithelial or mesenchymal CTCs) is even less clear [13–40]. Common epithelial markers used for CTC identification include epithelial cell adhesion molecule (EpCAM) and cytokeratins; mesenchymal markers include vimentin and N-cadherin [15, 21, 22, 32]. Other groups have further used stemness markers such as CD133 and ligands δ -like-3 (DLL-3) [12] to characterise CTC subgroups. In addition, to our knowledge, no study has systematically compared the prognostic values of different CTC cut-offs in lung cancer.

Therefore, we performed a comprehensive meta-analysis of all available clinical data to assess the prognostic value of CTCs in the different histological types of lung cancer, with particular respect to different CTC subtypes, cut-offs and time points of CTC enumeration.

Methods

Search strategy and inclusion/exclusion criteria

The MEDLINE, Web of Science and Embase databases were systematically searched in July 2021. The search strategy used the following keywords in various combinations: “circulating tumour cells”, “lung cancer” and “prognosis”. There was no restriction on the year of publication, but the English language was a prerequisite. In addition, we conducted a cited reference search of relevant articles and browsed review articles. The detailed search strategy is listed in the supplementary material (supplement 1, appendix 1).

Two authors (F. Jin and J. Shao) assessed the retrieved studies independently for the inclusion or exclusion of each study according to the following criteria. The inclusion criteria consisted of 1) the studies were about lung cancer patients; 2) CTCs were detected to predict prognosis; 3) progression-free survival (PFS) and/or overall survival (OS) are provided (there is a clear follow-up plan and criteria for the evaluation of tumour recurrence). The exclusion criteria consisted of 1) studies reporting duplicate data or based on the same patient database; 2) incomplete data to calculate hazard ratio (HR) values and 95% confidence intervals; 3) incorrect statistical methods (such as univariable Cox model); 4) sample size was <20. If any information was missing or unclear, the corresponding authors were contacted.

Details of the protocol for this meta-analysis were registered at www.crd.york.ac.uk/PROSPERO/ (identifier CRD42021274871).

Data extraction and assessment of risk of bias

Two authors (F. Jin and L. Zhu) assessed the studies independently and extracted the following data: first author, the country in which the study was performed, publication year, study population details (sample size, age distribution, the proportion of lung cancer subtypes, cancer stage and number of patients at each stage), treatment (including surgery, chemotherapy, radiotherapy), study type, the detection method of CTCs, markers of CTCs, sampling site (peripheral or pulmonary vein), volume of blood sampled, time of sampling (pre- or post-treatment), CTC cut-off line and percentage of positives, and hazard ratio values corresponding to PFS and OS. Any disagreements were resolved by discussion or an independent third person. If the same group published more than one study, the study populations were examined to exclude double reporting of overlapping study cohorts. If the same population was used twice [22, 40] to compare prognostic values at different stages of treatment (pre- or post-) or to use different markers and/or cut-off values, the results were used separately.

The risk of bias was assessed in nonrandomised controlled trials according to the Risk of Bias in Non-Randomised Studies of Interventions (ROBINS-I) assessment tool [41]; the Risk of Bias (RoB2) tool was used in randomised controlled trials [42]. For details of the assessment, please refer to the supplementary material (supplement 1, appendix 2).

Statistical methodology

To statistically assess the prognostic value of CTCs, hazard ratios and their associated standard errors for OS and PFS were extracted from the included studies. If various methods, cut-offs or markers were used to identify CTCs in the same study, these different CTC subpopulations were considered as separate studies in the analysis. The extracted hazard ratios were summarised using meta-package in R (version 4.1.2; R Core Team, Vienna, Austria). Incorrect or incomplete data were recalculated (supplement 1, appendix 3). For example, the 95% confidence intervals of hazard ratios were not provided in the study by NEL *et al.* [15], and thus were recalculated. Conventionally, HR >1 indicates that the CTC-positive group experienced impaired survival compared with the CTC-negative group, but some references [13, 20, 22, 28, 35] reported inverse hazard ratio values (*i.e.* HR >1 indicates favourable survival for CTC-positive patients). These values were recalculated from the original data to be able to compare hazard ratio values throughout this meta-analysis.

The Cochran's Q-test (Chi-squared and p-value) and I^2 measure (with confidence and prediction interval) was used to assess heterogeneity, which describes the proportion of total changes observed between studies. I^2 >25%, >50% and >75% were considered to represent low, moderate and high heterogeneity, respectively [43]. When the Q-test resulted in $p < 0.050$, a random-effects analysis model was used. Otherwise, a fixed-effects analysis model was presented. Reporting a prediction interval (PI) illustrates which range of true effects can be expected in future settings. The prediction intervals crossed the no-effect threshold, indicating that there are settings where those treatments will have no effect or even an effect in the opposite direction [44].

A one-way sensitivity analysis was performed to assess the stability of the results (meta-analysis was repeated by excluding certain studies one by one to explore their effect on the combined effect variable, and the results obtained were compared with the original effect size). Subgroup analyses were used to assess the sources of heterogeneity; if studies did not report corresponding data, they were excluded from subgroup analyses. Pooled effect estimates for each subgroup were tested for statistically significant subgroup differences and $p < 0.100$ was considered a statistically significant subgroup effect [45]. The Benjamini–Hochberg procedure was used to adjust for multiple testing.

Funnel plots and Egger's regression test were used to assess for publication bias (supplementary figure S1; $p < 0.050$ was considered to indicate publication bias). The impact of a single study was evaluated by estimating the hazard ratio within each study. $p < 0.050$ was generally considered statistically significant.

Results

Study characteristics

After searching PubMed, Web of Science and Embase databases with specific keywords, 481 search results written in English were obtained, of which 153 were duplicates. 369 records were excluded for not meeting the inclusion criteria, leaving 112 studies for detailed evaluation. After excluding another 85 studies (of which six were duplicates; three had a sample size of <20; and 76 were not statistically available), 27 studies (2957 patients) were eventually included in the meta-analysis. The literature review process is illustrated in supplementary figure S2.

The details of the demographic and clinical characteristics of the 27 studies are shown in supplementary tables S1 and S2. The studies were published from 2009 to 2021, with sample sizes ranging from 33 to 642 patients. Studies with >100 participants accounted for 33.3% (nine out of 27) of the studies. 15 studies were conducted in Western countries, and the other 12 were from Asia. 19 studies included patients with nonsmall cell lung carcinoma (NSCLC), most of whom (in 11 out of 19 studies) were in stages III–IV. Out of eight studies on small cell lung carcinoma (SCLC), six provided information about the disease stage (limited disease *versus* extensive disease). Treatment consisted of chemotherapy (11 studies), surgery (seven studies), radiation therapy (one study) and tyrosine kinase inhibitors (erlotinib, dabrafenib or gefitinib; two studies). The remaining seven studies reported combinations of these treatments. In 27 studies, CTCs were quantified in peripheral venous blood and the remaining study in the pulmonary vein. The risk of bias was assessed in the 24 nonrandomised controlled trials (supplementary table S3) and three randomised controlled trials (supplementary figure S3).

CTC detection universally indicates poor prognosis

To explore the prognostic value of CTC detection in lung cancer patients, 27 studies (reporting 2957 patients) were included in the analysis. Pooled hazard ratios showed that CTC positivity (regardless of CTC detection method and the individual cut-offs for CTC positivity) is prognostic for both poor OS (HR 2.51, 95% CI 2.06–3.05, $n=30$, $I^2=51\%$, PI 1.18–5.33; $p < 0.010$) and PFS (HR 2.66, 95% CI 2.10–3.37, $n=24$, $I^2=66\%$, PI 1.01–6.98; $p < 0.010$) (figure 1).

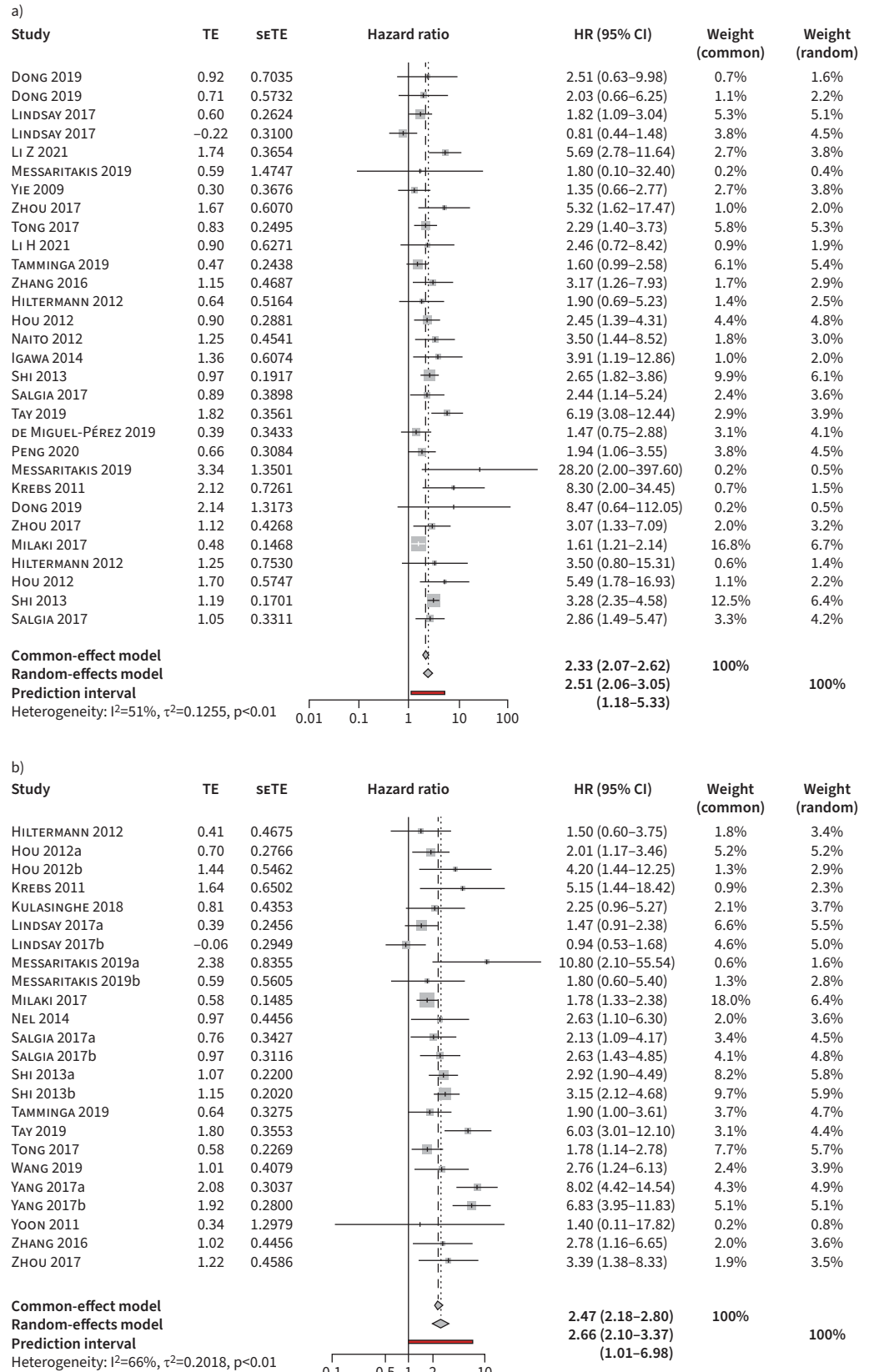


FIGURE 1 Summary estimates of hazard ratio (HR) for a) overall survival and b) progression-free survival. Studies with different circulating tumour cell definitions were analysed separately and marked as “a” and “b”. TE: ln(HR) value.

Sensitivity analysis resulted in a slight difference in OS; hazard ratio estimates ranged from 2.39 (95% CI 1.99–2.88) to 2.60 (95% CI 2.18–3.10) after excluding the studies of TAY *et al.* [21] and LINDSAY *et al.* [13], while removing other studies did not significantly affect hazard ratio. Similar results were obtained for PFS: hazard ratio estimates ranged from 2.49 (95% CI 2.01–3.10) to 2.80 (95% CI 2.24–3.50) after excluding the studies of YANG *et al.* [34] and LINDSAY *et al.* [13] (supplementary figure S4).

Subgroup and sensitivity analyses

We performed subgroup analyses to explore heterogeneity, including the time point of CTC detection, different subtypes and stages of lung cancer, CTC subpopulations, treatment, CTC cut-off values and sample size.

NSCLC versus SCLC

Subgroup analysis according to the histological subtype showed a statistically significant subgroup effect ($p=0.017$), indicating that the subtype of lung cancer significantly influences the prognostic value of CTCs for OS (figure 2). The prognostic effect of CTC detection is greater in SCLC (HR 3.11, 95% CI 2.59–3.73, PI 2.53–3.82; $p=0.560$) than in NSCLC (HR 2.11, 95% CI 1.63–2.73, PI 0.92–4.82; $p<0.050$);

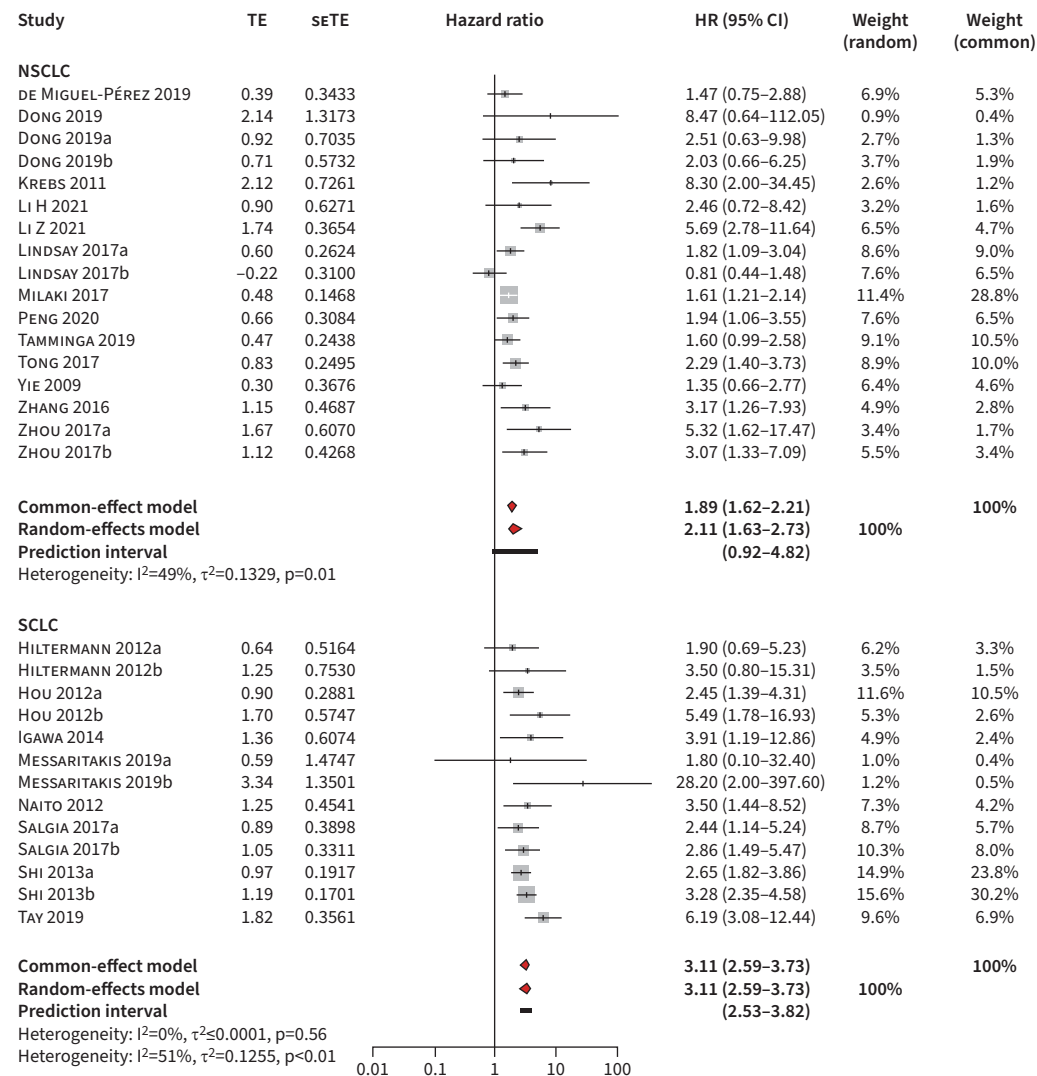


FIGURE 2 Summary estimates of hazard ratio (HR) for overall survival associated with circulating tumour cells (CTCs) detected in nonsmall cell lung cancer (NSCLC) and small cell lung cancer (SCLC) patients. Studies with different CTC definitions were analysed separately and marked as “a” and “b”. TE: ln(HR) value.

therefore, the subgroup effect is quantitative. As the number of trials (more than seven) and participants (>1000) are sufficient in each subgroup, the covariate distribution is not concerning. The heterogeneity is lower within the subgroups than in pooled trials, so the type of lung cancer might explain heterogeneity in the overall analysis.

Epithelial markers versus epithelial–mesenchymal transition hybrids

Mesenchymal markers are often used in conjunction with epithelial markers, most frequently in the CellSearch System [13]. CTCs expressing both epithelial and mesenchymal proteins are considered epithelial–mesenchymal transition (EMT) hybrids, which have been shown to be the putative drivers of metastasis in various malignancies [46–48]. Different markers identify different CTC subpopulations with different biology and inevitably different prognostic values. Therefore, we were interested in the prognostic value of CTCs identified by different markers and compared the prognostic role of CTC subpopulations, defined by commonly used markers and/or their combinations such as epithelial markers (EpCAM, cytokeratins), EMT hybrids markers (epithelial markers+vimentin/ Twist/N-cadherin) and other markers (e.g. survivin, folate receptor).

A subgroup analysis (21 studies involving 2229 patients) was performed to test whether different markers modify the prognostic value for OS (figure 3). The results suggest a statistically significant subgroup effect ($p=0.032$), indicating that the use of different markers to identify CTCs leads to significantly different prognostic values for OS. The detection of CTC with different markers favours shortened OS over prolonged OS, and the effect is greater for purely epithelial CTCs (HR 2.47, 95% CI: 2.15–2.83, PI 1.43–5.11; $p<0.050$) than EMT hybrids (HR 1.37, 95% CI 0.97–1.93, PI 0.28–6.91; $p>0.050$); therefore, the subgroup effect is quantitative. There is a relatively small degree of heterogeneity (36–43%) between the trials, and the heterogeneity is lower within the subgroups than pooled trials, so the subgroup analysis might explain heterogeneity in the overall analysis. As only four trials were included in the EMT hybrids subgroup, we cannot confidently conclude that there is a true subgroup effect.

Cut-offs

Since no standard cut-off value is universally used to define patients as “CTC positive” versus “CTC negative”, the included studies used different cut-offs to define CTC positivity. As this could be a source of heterogeneity, we performed a subgroup analysis (figure 4, supplementary figure S5). If a cut-off value was used in fewer than two trials, it was excluded from the subgroup analysis. The test for subgroup differences showed a statistically significant subgroup effect ($p=0.028$), suggesting that cut-offs significantly modify the prognostic value of CTCs for OS. Hazard ratio values are underestimated if the cut-off is set at 1 CTC per 7.5 mL (36.3% detection rate, 146 out of 402), while the underestimation was no longer present when the cut-off was ≥ 2 CTCs per 7.5 mL (50.7% detection rate, 75 out of 148). However, the relatively small number of trials (three to five trials) and participants (200–400 patients) in each subgroup should be considered when interpreting these results.

Pre-treatment versus post-treatment CTC detection

To investigate and compare the prognostic value of CTC detection before and after treatment, the study was divided into two subgroups (pre- and post-therapeutic sampling time) (figure 5). The test for subgroup differences demonstrated that there is no statistically significant subgroup effect (OS $p=0.640$, PFS $p=0.369$; analysis not presented), suggesting that prognostic values of CTCs detected before and after treatment do not differ. A sufficient number of trials (21 studies) and participants (2635 patients) were included in each subgroup, so the covariate distribution should not be concerning for this subgroup analysis. Sampling time may not be the source of heterogeneity, since I^2 does not appear to be lower within subgroups than in pooled trials.

Treatment

Upon stratification as surgical versus nonsurgical treatment, the test for subgroup differences indicated no statistically significant subgroup effect (OS $p=0.838$; result not presented), suggesting that treatment does not influence the prognostic value of CTCs. However, a smaller number of trials (six studies) and participants (726 patients) contributed data to the surgery subgroup than to the nonsurgery subgroup (15 trials and 1537 patients), meaning that the analysis may not be able to detect subgroup differences.

Tumour stage

Since detailed tumour staging information could not be extracted from the original literature on SCLC, we only analysed studies of NSCLC patients (10 trials reporting 1526 patients) with different Union for International Cancer Control (UICC) stages. In addition, detailed TNM stages or primary tumour size were unavailable for both SCLC and NSCLC. As the information regarding UICC stages was also limited for

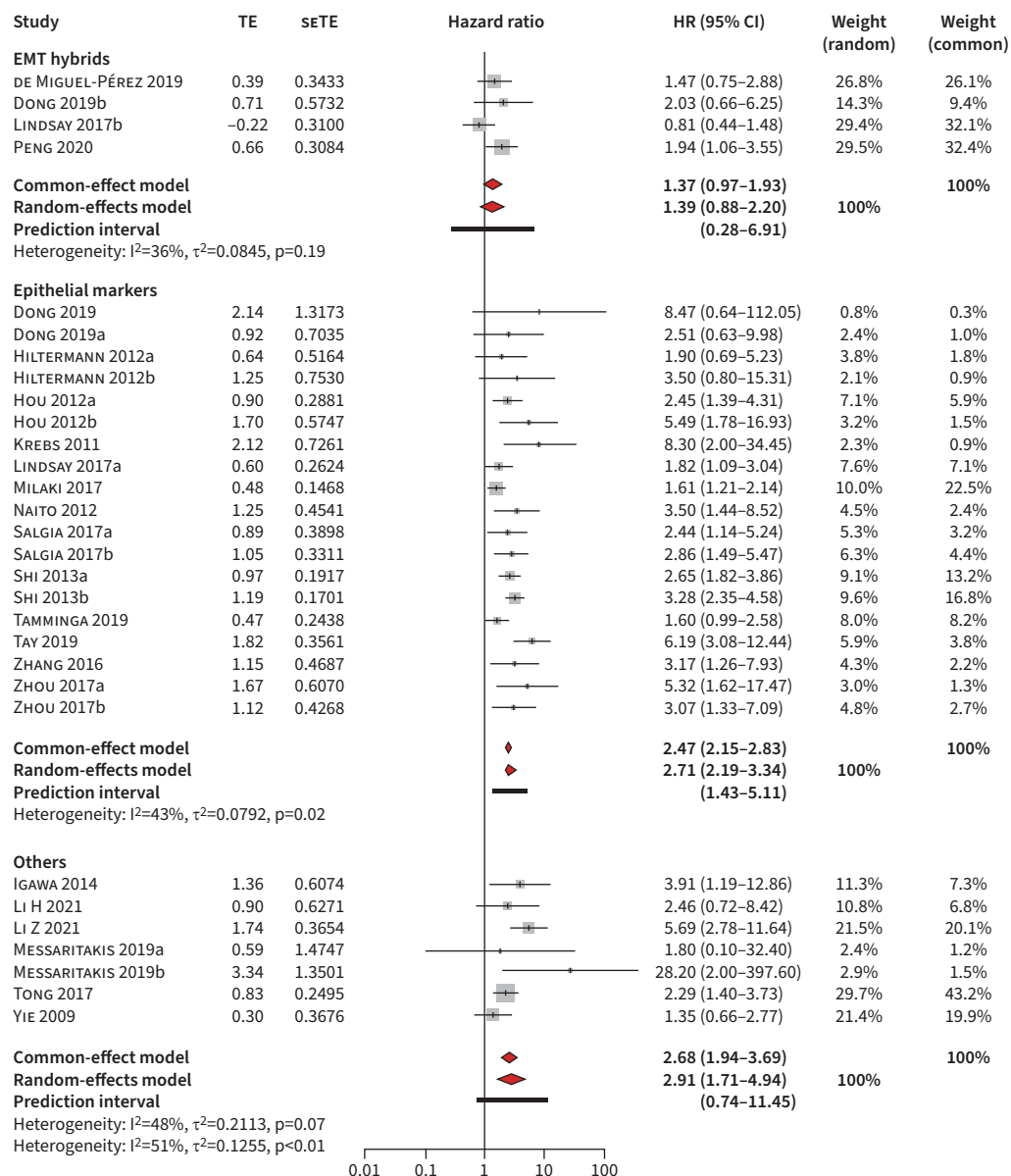


FIGURE 3 Summary estimates of the hazard ratio (HR) of overall survival regarding different markers. Studies with different circulating tumour cell definitions were analysed separately and marked as “a” and “b”. TE: ln(HR) value; EMT: epithelial–mesenchymal transition.

NSCLC, early (UICC I–III) and late (III–IV) stages were analysed in a pooled manner. Interestingly, detection of CTCs indicated a stronger association with poor OS in stage I–III NSCLC (HR 2.79, 95% CI 1.43–5.45; p<0.08) than in stage III–IV disease (HR 2.04, 95% CI 1.45–2.86; p<0.01) (supplementary figure S6). It is worth noting that there is unexplained heterogeneity in these subgroups (stage I–III I²=52%, stage III–IV I²=58%).

Sample size

A subgroup analysis was performed according to the sample size, indicating that the sample size does not modify the prognostic value of CTCs (OS p=0.427). A sufficient number of trials (more than seven studies) and participants (>1000) were included in each subgroup. Surprisingly, the pooled effect was larger in studies with smaller sample sizes (<100 patients; HR 2.71, 95% CI 2.33–3.16, PI 1.74–4.26; p=0.270) than in larger studies (HR 2.25, 95% CI 1.48–3.44, PI 0.62–8.22; p=0.010).

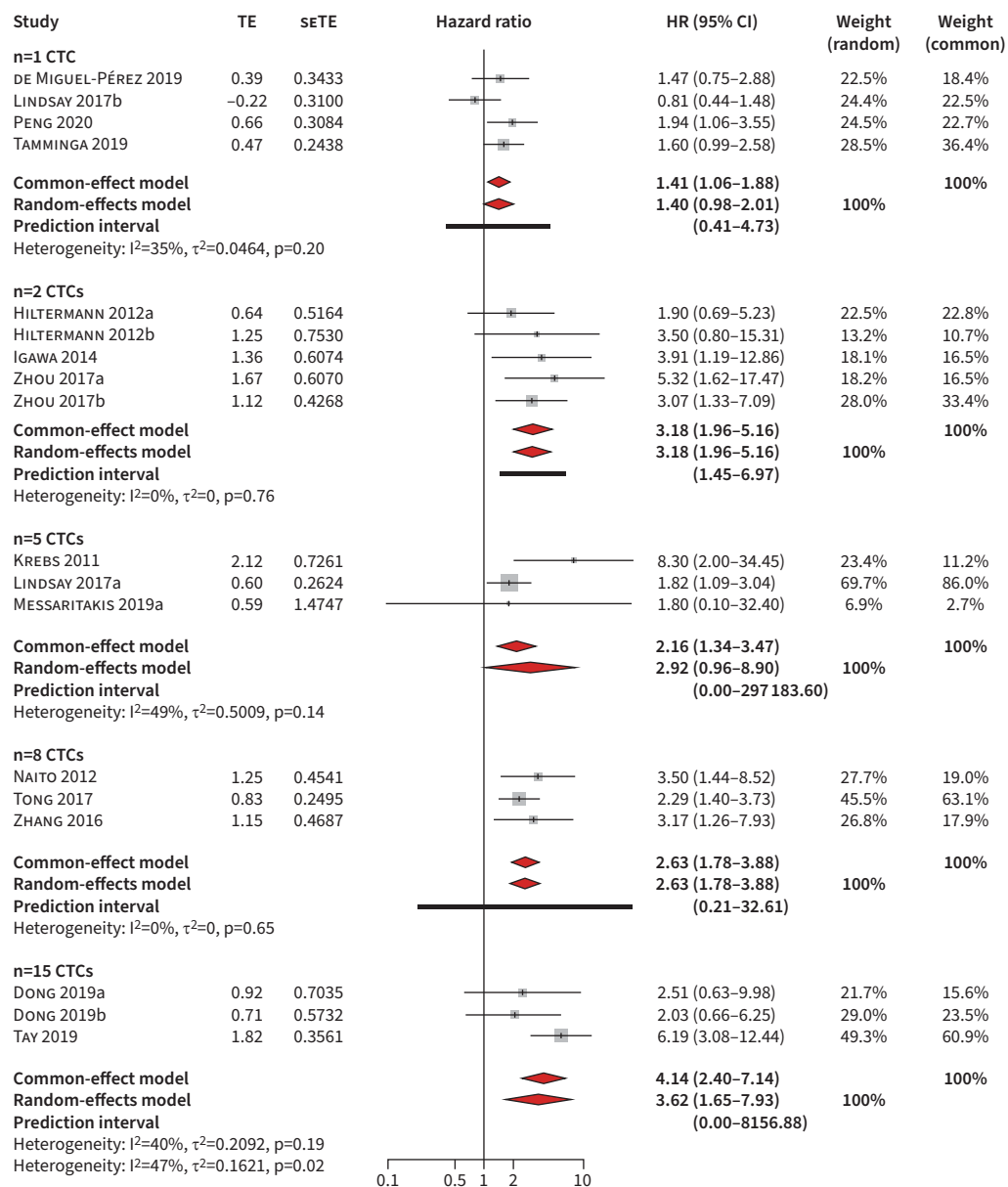


FIGURE 4 Summary estimates of hazard ratio (HR) of overall survival regarding cut-offs. Studies with different circulating tumour cell (CTC) definitions were analysed separately and marked as “a” and “b”. TE: ln(HR) value.

Discussion

Distant metastases are the paramount clinical problem in oncology and a direct result of CTCs [49–51]. Although technically challenging, the detection, enumeration and isolation of CTCs promises significant advantages in prognostication. This liquid biopsy strategy allows repeated molecular analyses as well as *ex vivo* therapeutic assays for response prediction. In addition to CTCs, circulating tumour (ct)DNA (free DNA derived from tumour tissue) is another highly specific serum marker [52]. ctDNA can be present in the absence of detectable circulating tumour cells, and is associated with tumour burden in many malignancies including lymphoma [53, 54]. In lung cancer, persistent ctDNA detection after curative resection is a strong indicator of minimal residual disease and reliably predicts relapse [55, 56]. Aside from its role in minimal residual disease detection, the role of ctDNA as a prognostic or predictive biomarker for patients with lung cancer is still requires further investigation [57]. This study focuses on detecting and enumerating CTCs, *i.e.* intact cells as opposed to cell-free DNA, in lung cancer patients for predictive and prognostic purposes.

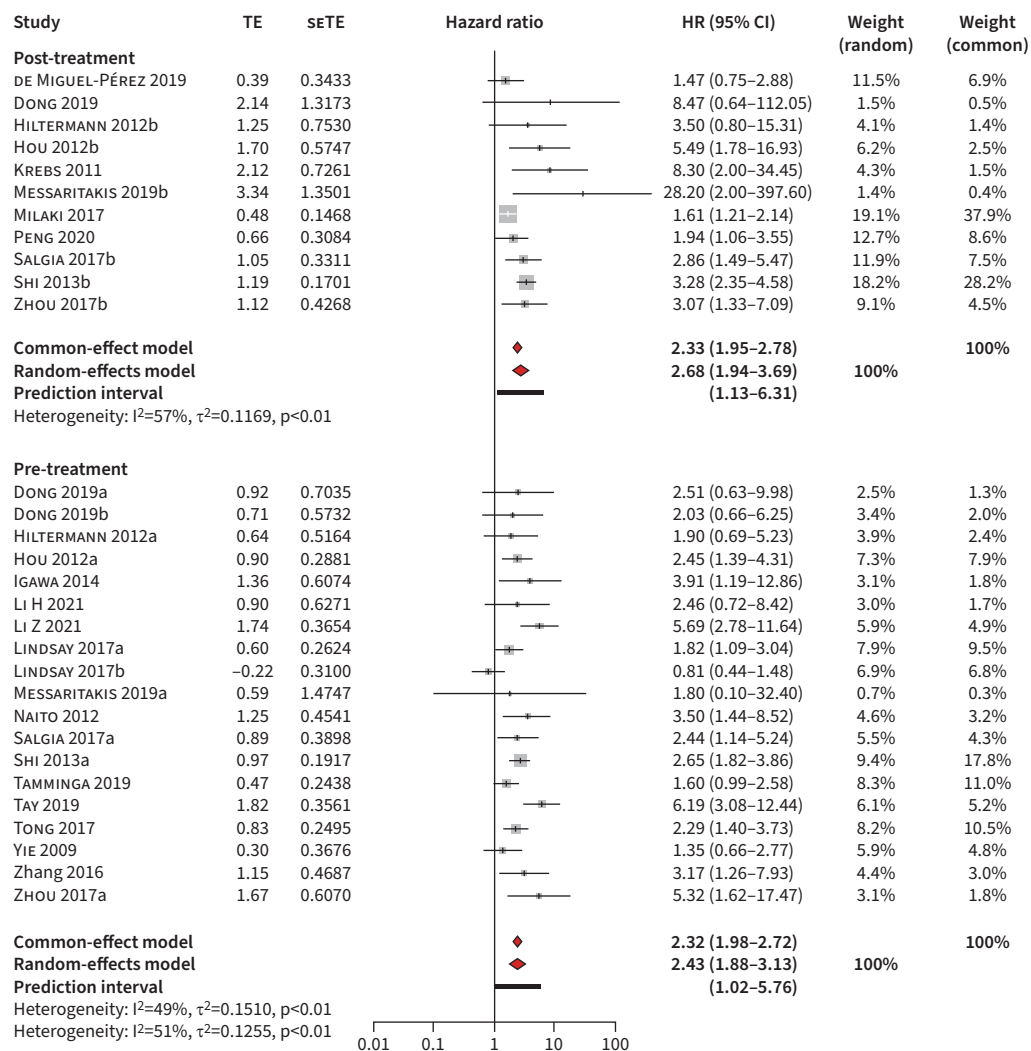


FIGURE 5 Summary estimates for overall survival of subgroup analysis regarding the detecting time. “a” and “b” represent separate analysis of the same study with different circulating tumour cell definitions. HR: hazard ratio; TE: ln(HR) value.

To our knowledge, this is the first study to demonstrate that the histological type of lung cancer significantly influences the prognostic value of CTC for OS. CTC detection in SCLC patients, regardless of CTC markers or cut-offs, suggests a higher risk of shortened OS than in NSCLC patients. This is consistent with the clinicopathological findings of SCLC, where the number of microvessels within the neovascularisation area is higher than NSCLC at the same stage, significantly predicting worse OS [58]. SCLC is a highly metastatic carcinoma with a median survival of only 7–12 months after diagnosis [59, 60]. It has been shown that SCLC patients have an extremely high number of CTCs, and that the number of CTCs in these patients correlates with disease stage and survival [14, 21].

Immunoaffinity-based technologies are among the most frequently used detection methods, usually identifying CTCs based on EpCAM and cytokeratin expression. However, EMT is a crucial mechanism during metastasis, allowing tumour cells to gain invasive abilities required for successful metastasis. This results in a subset of CTCs with downregulated EpCAM and (or) cytokeratin expression, often accompanied by stem cell characteristics [61, 62]. Hence, reliance on epithelial markers alone will inevitably lead to studies focusing on epithelial CTCs only, not investigating nonepithelial CTCs, which perhaps play a more critical role in metastasis. Therefore, the present study evaluated the prognostic value of different subtypes of CTCs in lung cancer and shows that detecting epithelial CTCs implies an unfavourable prognosis in lung cancer. Interestingly, while detecting mesenchymal CTCs also predicts an

unfavourable prognosis, the prognostic value of epithelial CTCs was more pronounced than that of other CTC subgroups. In contrast to studies in pancreatic cancer reporting that EMT hybrids (CTCs expressing both epithelial and mesenchymal markers) are the actual mediators of metastasis [46, 47], we could not demonstrate that EMT hybrid CTCs have a more pronounced impact on survival in lung cancer than purely epithelial or purely mesenchymal CTCs (only one study). However, the vast majority of studies included in this analysis focused on epithelial CTCs, and the available data about mesenchymal CTCs is very limited. In addition, the detection methods are not entirely comparable, therefore these results must be interpreted cautiously.

In addition, due to the lack of a uniform testing technique and corresponding criteria, different studies have chosen various cut-off values to define whether a CTC detection is positive or not. We performed a subgroup analysis and found that different cut-off values significantly modify the prognostic effect of CTC. Compared to ≥ 1 CTCs per 7.5 mL, the prognostic effect increased when ≥ 2 CTCs per 7.5 mL were set as the cut-off. Therefore, different markers and various cut-off values can influence the prognostic effect of CTCs. However, the relatively small number of trials and participants must be considered when interpreting these results.

The presented evidence confirms that the presence of CTCs before and after treatment has a solid prognostic significance for lung cancer patients regardless of the lung cancer subtype, the CTC subtype or the detection method. The persistence of CTCs after completion of treatment (even more so after curative treatment such as surgical resection) strongly predicts early recurrence and poor survival [63, 64]. Although indicators for poor prognosis, CTCs may serve as a source of tumour DNA/RNA for next-generation sequencing-based therapeutic prediction [65] and even enable blood-derived tumour cell lines for *ex vivo* testing of targeted treatments predicted *in silico* [7].

In addition, this work investigated whether the disease stage affects the prognostic value of CTCs. Due to limited data in SCLC, these analyses were performed only in studies enrolling NSCLC patients. In addition, only gross UICC stages as opposed to detailed TNM stages or even information about the tumour size were available for NSCLC. As expected, CTCs had significant prognostic value in patients with advanced NSCLC. In contrast to previous findings [66], the results revealed that CTCs in relatively early stages (I–III) were more prognostic of inferior OS than in advanced stages (III–IV). This may be due to the fact that the detection of CTCs in the early stages of NSCLC implies vascular invasion and potentially distant (micro)metastases, thus strongly predicting a poor prognosis. This is well in line with recent studies reporting that persistent detection of ctDNA after curative resection of NSCLC indicates minimal residual disease and worse prognosis [55, 56], as well as with reports that microvessel density correlates with tumour size and prognosis [67]. An important initial step in haematogenous metastasis (and before that, the occurrence of CTCs) is vascular invasion [68], and numerous experiments have shown that the presence of vascular invasion is an important risk factor for recurrence in patients with early-stage NSCLC [68–70]. Therefore, our findings emphasise the role of CTCs in patients with early-stage NSCLC, which may indicate either residual or (in case of locally ablative treatment such as surgery or radiation therapy) recurrent disease or metastatic activity. Additional studies are needed to determine whether treatment decisions could be based on CTC detection in general or even the detection of specific CTC numbers or subtypes.

The number of CTCs in blood samples obtained intraoperatively from pulmonary veins is much higher than in peripheral blood, most of which are nonmalignant epithelial cells, and a few are CTCs with genomic aberrations [71]. This uneven distribution of CTC in different blood compartments is consistent with our findings in colorectal cancer [3, 10]. Another group showed that the association of CTCs in the pulmonary veins with lung cancer recurrence is not more robust than that in the peripheral system [40]. While there are founder CTCs (*i.e.* CTCs with metastasis-initiating capacities) in both blood compartments, their number may not greatly differ between blood compartments as intraoperatively generated CTCs may not have the metastasis-initiating capacities of the founder subgroup of naturally occurring CTCs [72, 73]. Our study found that high-level CTC detection in the peripheral veins predicted worse OS than in pulmonary veins (detected at the beginning of the operation prior to lobectomy, data not shown). However, given the limited sample size, more data is necessary to determine the role and metastatic capacity of CTCs detected in the pulmonary veins during surgery.

Compared to previous meta-analyses, our study has several unique features. To our knowledge, this is the first study comprehensively assessing the prognostic value of different CTC subtypes in lung cancer. Furthermore, we compared various factors with multiple subgroup analyses to evaluate their impact on the outcome and analysed their subgroup effects according to the essential criteria [45]. Nonetheless, this

meta-analysis inevitably has limitations. We noted moderate heterogeneity and applied random-effects models for more conservative estimates. Although the subgroup analysis revealed that different CTC markers and cut-offs, as well as the histological type of lung cancer, possible the source of heterogeneity and the sensitivity analyses convinced the results were stable, a certain degree of heterogeneity still exists. Also, the limited number of studies (especially randomised controlled trials) should be considered. Nevertheless, the uniformly poor prognostic value of CTC detection found in this meta-analysis in all subgroup analyses provides conclusive evidence for the unfavourable prognostic value of CTCs in lung cancer.

In summary, the current study supports the prognostic value of CTC in different histological types of lung cancer. It also implies that the markers and cut-offs used for CTC identification have a decisive impact on their prognostic value. In addition, CTCs detected both before and treatment predict poor outcomes in lung cancer patients.

Points for clinical practice

- The prognostic value of epithelial CTCs is greater than that of mesenchymal CTCs
- CTCs indicate unfavourable prognosis in all subtypes of lung cancer
- Higher detection thresholds correlate with stronger prognostic value of CTCs

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Data availability: The data and code underlying this article can be shared upon request to the corresponding author.

Conflict of interest: S. Loges reports grants and personal fees from BerGenBio AS, BMS, and Roche, and personal fees from Lilly, Sanofi, Novartis, Boehringer Ingelheim, AstraZeneca, MSD, Sanofi Aventis, Janssen, Takeda and Daiichi-Sankyo outside the submitted work. The other authors reported no disclosures.

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