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Quantitative Analysis of Circulating Cell-Free DNA for Correlation with Lung Cancer Survival: A Systematic Review and Meta-Analysis

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ABSTRACT

Introduction: Despite the growing interest in circulating cell-free DNA (cfDNA), no conclusive evidence exists on the value of quantitative analysis of cfDNA for the prediction of lung cancer survival. We performed a systematic review and meta-analysis of primary studies to estimate the impact of higher baseline cfDNA levels on survival outcomes of patients with lung cancer.

Methods: A comprehensive search was performed using the PubMed, Web of Knowledge, and Cochrane databases up to March 2016. The methodologic quality of identified studies was assessed by the Newcastle-Ottawa scale. Potential sources of heterogeneity were investigated via subgroup and sensitivity analyses, while publication bias was evaluated by funnel plot and Egger's test.

Results: Among the 17 studies identified, 16 studies (n = 1723 patients) and 5 studies (n = 640) were included in the meta-analysis of overall survival (OS) and progression-free survival (PFS), respectively. Despite the fact that the association with PFS did not reach statistical significance (hazard ratio 1.12% [95% confidence interval 0.91–1.37), the pooled analysis for OS showed evidence of an increased risk of death in patients with higher baseline cfDNA levels (hazard ratio 1.76 [95% confidence interval 1.38–2.25]; $p < 0.001$). Further subgroup and sensitivity analyses confirmed this relationship, although significant between-study heterogeneity was still detected in most comparisons. The Egger's test revealed no statistical evidence of publication bias in the results.

Conclusion: Our findings support the clinical validity of quantitative analysis of cfDNA for the prediction of lung cancer survival. Nevertheless, the establishment of a robust standardized method for determination of optimal cutoff thresholds is required to define the clinical relevance of cfDNA quantification for lung cancer management.

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Keywords: Circulating cell-free DNA; Meta-analysis; NSCLC; Survival

Introduction

Lung cancer is the leading cause of cancer-related death in the world, with more than 1 million deaths annually.[1] NSCLC and small cell lung cancer (SCLC) represent the two major histologies, the former accounting for approximately 85% of all diagnosed lung malignancies.[2] Despite a deeper understanding on the biology of lung cancer and the introduction of innovative therapeutic agents, the overall prognosis remains poor, with an average 5-year survival of approximately 15%. Late diagnosis still represents about 80% of cases.[3] Indeed, if lung cancer is detected early, when the tumor is still localized, survival increases to more than 50%.[4] Therefore, the identification of reliable diagnostic and prognostic markers would greatly improve the potential for early detection, prognosis prediction, and personalized treatments.

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Raised levels of circulating cell-free DNA (cfDNA) in cancer patients were first reported in 1977 by Leon et al.[5] and have now been reported in many cancer types, including lung cancer.[6] Although the precise mechanism of DNA release into the blood has not been fully elucidated, it is clear that much of it is derived from apoptotic and necrotic tumor cells.[7] Given that circulating DNA as a biomarker is easily accessible, reliable, and detectable early in the disease course,[8] quantitative detection of cfDNA, in either plasma or serum, has been proposed in lung cancer patients as a promising tool for diagnostic purposes.[9–11] The value of quantitative analysis of cfDNA as a screening tool for lung cancer has been recently summarized by a meta-analysis that found a diagnostic accuracy not lower than conventional circulating biomarkers for lung cancer screening.[12] Several studies have also investigated the value of quantitative analysis of circulating cfDNA for survival prediction in cancer patients. However, contrasting results have been reported on the impact of higher baseline cfDNA levels on lung cancer survival, with some studies reporting an increased risk of death[13–15] and other authors failing to replicate this finding.[16,17]

Despite an increasing number of studies focusing on circulating cfDNA, no conclusive evidence exists on the value of quantitative analysis of cfDNA for prediction of lung cancer survival, and no meta-analysis has been conducted to clarify this issue. The aim of the present study was to fill this gap by performing a systematic review and meta-analysis of relevant studies to accurately estimate the impact of higher baseline cfDNA levels on survival outcomes of patients with lung cancer. Quality assessment of primary studies and the potential for publication bias were considered part of the meta-analytic process. In addition, subgroup and sensitivity analyses were conducted to explore possible explanations for heterogeneity and to examine the impact of potential confounders on the pooled estimates.

Materials and Methods

Search Strategy and Eligible Criteria

The protocol for this review was published in the PROSPERO database[18] of prospectively registered systematic reviews (database registration CRD42016035965). PubMed, Web of Knowledge, and Cochrane Library databases were searched up to March 2016 using the Boolean combination of the following key terms: lung cancer AND (survival or prognosis) AND (cell-free DNA OR circulating DNA or circulating-free DNA OR circulating cell free DNA OR cfDNA OR total circulating free DNA OR extracellular DNA). We searched for primary studies evaluating the impact of higher baseline levels of cfDNA on survival outcomes. Inclusion criteria were: 1) human studies focusing on lung cancer; 2) studies exploring the relation between baseline cfDNA levels and 1 or more of the following time to events outcomes: overall survival (OS), progression-free survival (PFS), disease-free survival (DFS), or time to progression (TTP). Exclusion criteria were as follows: 1) reviews, meeting abstracts, case studies, and editorials; 2) not human studies; 3) circulating tumor cells; 4) qualitative analysis on circulating tumor DNA; 5) circulating RNA; and 6) microsatellite studies. There were no language restrictions. The retrieved studies were then read in their entirety to assess their appropriateness for inclusion. All references cited in the eligible studies were also reviewed to identify additional published works that were not initially retrieved. If 2 or more studies shared part of the same patient population, the more complete or the one with the larger sample size was included. If survival estimates were not reported or calculable from the original published data, corresponding authors were contacted via e-mail and studies were excluded if relevant data were not provided. All studies were independently analyzed by two reviewers (S.T. and S.C.), and any discrepancies in data extraction were resolved through consensus.

Data Extraction

A standardized form was used for each study from which the following information was extracted: the first author's last name, year of publication, study location, total number of enrolled lung cancer patients and those included in survival analysis, type of lung cancer and number of patients for each stage, treatment methods, DNA source, detection method, and cutoff threshold of baseline cfDNA levels. For each survival outcome reported, the hazard ratio (HR) as well as its 95% confidence interval (CI) was extracted from the study report where possible; otherwise, HRs and 95% CIs were estimated using the method described by Parmar et al.[19] or extrapolated from Kaplan-Meier curves by using KurvE software (Internovi di Scarpellini, Daniele SAS, Cesena, Italy). When HRs and 95% CIs were extracted from the Kaplan-Meier curve, we also attempted to check the results with the authors, because there is a potential to overestimate the true number of events.[20] When HR was adjusted for covariates, it was also extracted along details of the corresponding covariates (Table 1; Supplementary Table 1). All studies have been independently analyzed by two reviewers (S.T. and S.C.), and any discrepancies have been resolved through consensus.

Study Quality

We assessed the quality of studies included in the systematic review by using the Newcastle-Ottawa scale (NOS) for nonrandomized studies (available at: http://www.ohri.ca/programs/clinical-epidemiology/oxford-asp). The studies were judged on three major

components: 1) selection of the groups of study (0–4 points), 2) comparability of cohorts (0–2 points), and 3) assessment of the outcome or exposure (0–3 points). The maximum score could be 9 points, representing the highest methodologic quality. Studies with a NOS score greater than 7 were considered of higher quality. Two reviewers (S.T. and S.C.) independently assessed the quality of each study, and disagreements were resolved through consensus.

Statistical Analysis

OS was defined as the primary survival outcome of interest, while PFS, TTP, or DFS were considered secondary outcomes. In order to conduct robust meta-analyses, we pooled survival outcomes that were reported in $\geq$3 independent studies. For each time to event outcome, adjusted HR was combined if these were available; otherwise, unadjusted estimates were used. Outcome estimates were pooled using the random effects (DerSimonian and Laird) model because it takes into account any difference among studies even if there is no statistical heterogeneity.[21] In case of lack of heterogeneity, the random effects model coincides with the fixed effect model.[22] We estimated the between-study heterogeneity across all eligible comparisons by using the χ^2–based Cochran's Q statistic (significant for $p < 0.10$).[23] We also reported the I^2 index (range 0–100%), which quantifies heterogeneity regardless of the number of studies. Where the I^2 estimate was 50% or more, we interpreted this as indicating the presence of high levels of heterogeneity.[24] Leave-one-out sensitive meta-analysis was performed to assess the contribution of each study to the pooled estimate by excluding individual results one at a time and recalculating the pooled OR estimates for the remaining results. To assess the robustness of overall findings and to further explore possible reasons for heterogeneity, subgroup and sensitivity analyses were conducted when relevant data were reported in at least three independent studies. The presence and extent of publication bias and small-study effects was evaluated graphically by drawing funnel plots for each outcome measure and statistically by means of Egger's standard regression test.[25] An Egger's test p value less than 0.10 was considered to indicate statistically significant publication bias. All pooled analyses were performed using ProMeta software (version 2; Internovi di Scarpellini, Daniele SAS, Cesena, Italy), and the significance of pooled estimates was set at p value less than 0.05.

Results

Characteristics of Identified Studies

The keywords used to search PubMed, Web of Knowledge, and Cochrane databases yielded 507 hits, and 153 were duplicates. By screening titles and abstracts, 254 records were then excluded and 100 potential studies remained available for full-text screening. After removal of 83 studies not fulfilling the inclusion criteria, 17 studies were finally included in the systematic review.[13–17,26–37] The detailed flowchart of the literature review process with reasons of study exclusion is shown in Figure 1. The identified studies were published between 1995 and 2016, with sample sizes ranging from 22 to 446 lung cancer patients. Approximately one-third of the studies (6/17; 35.3%) included more than 100 patients in the survival analysis.[13,14,16,17,32,37]

Details on the demographic and clinical characteristics of the 17 studies are shown in Table 1. Among them, 12 studies were conducted in Europe,[13–15,26–29,31,34–37] three in East Asian countries,[16,32,33] one in north America,[17] and one in India.[30] All manuscripts were written in English with the exception of a manuscript in Spanish.[27] Sixteen studies included patients with NSCLC only,[13–17,27–37] most of which were at stage III/IV only,[14,16,17,27,30,32–35,37] while one study[26] included both NSCLC and SCLC patients. Treatment methods included chemotherapy in 11 studies,[13–17,27,30,32,33,35,37] surgery in two,[28,29] radiotherapy in one,[36] and different combinations of chemotherapy, radiotherapy, or surgery in three studies.[26,31,34] cfDNA was obtained from plasma in 15 studies,[14–17,26,28–37] from serum in a single study,[27] and from both plasma and serum in one other study.[13] Among the different methods of cfDNA detection, quantitative real-time polymerase chain reaction (qPCR) was the most commonly used, being applied in 12 studies,[13–15,17,28,29,31–36] of whom five by means of the human telomerase reverse transcriptase (hTERT) gene amplification.[14,15,28,29,36] HR estimate was reported in the text or provided by the corresponding author in 11 studies,[14–17,28,32–37] while it was extrapolated in six studies.[13,26,27,29–31] HRs and 95% CIs for OS were obtained in 16 studies,[13–17,26,28–37] PFS in five,[16,17,32,35,37] DFS in two,[28,36] and TTP in two studies.[14,27] Adjusted HRs were available in 8 studies.[13–16,32–37] The quality of identified studies was evaluated according to NOS and results are shown in Table 2. The quality score ranged from 6 to 8 (median 7). The seven studies[13,14,16,32–34,37] with NOS scores greater than 7 were considered of higher quality.

Quantitative Data Synthesis

A total of 16 studies[13–17,26,28–37] including 1723 patients with lung cancer were involved in the meta-analysis for the relationship between baseline levels of plasma cfDNA and OS (Fig. 2*A* and Table 3). Despite significant heterogeneity among studies ($p < 0.0001$; $I^2 = 66\%$), the pooled HR showed an increased risk of death in patients with higher baseline cfDNA levels (HR 1.76 [95% CI

Table 1. Characteristics of Studies Evaluating the Impact of Higher Baseline Circulating Free DNA Levels on Survival Outcomes of Patients with Lung Cancer

Authors (y)	Country	No. of Cases/No. Analyzed for Survival	Cancer Type (Stage, n)	Treatment Methods	DNA Source	Detection Method (Gene)	Cutting Point	Survival Analysis	Mos. of Follow-Up	Source of HR
Fournié et al.[26] (1995)	France	68/65	SCLC (22) and NSCLC (46)	Chemotherapy or RT or surgery	Plasma	Nick translation DNA labeling	100 ng/mL	OS	≤23	Extrapolated^{UV}
Gautschi et al.[13] (2004)	Switzerland	185/163	NSCLC (I-II, 19; III, 62; and IV, 104)	Chemotherapy	Plasma serum	qPCR (GAPDH)	10 ng/mL (plasma); 50 ng/mL (serum)	OS	≤26	Extrapolated^{MV}
Camps Herrero et al.[27] (2005)	Spain	78/74	NSCLC (IIIB-IV, 34 and IV, 44)	Chemotherapy	Serum	Spectrophotometry	500 ng/mL	TTP	≤42	Extrapolated^{UV}
Ludovini et al.[28] (2008)	Italy	76/76	NSCLC (I, 20; II, 40; IIIA, 11; and IIIB, 5)	Surgery	Plasma	qPCR (hTERT)	3.25 ng/mL	OS and DFS	≤24 (median 23)	Reported^{UV}
Sozzi et al.[29] (2009)	Italy	38/34	NSCLC (I, 24 and II-IV, 14)	Surgery	Plasma	qPCR (hTERT)	Tertiles (3rd vs two lowest)	OS	≤60	Extrapolated^{UV}
Kumar et al.[30] (2010)	India	100/42	NSCLC (III, 61 and IV, 39)	Chemotherapy	Plasma	ELISA	Tertiles (2nd vs 1st; 3rd vs 1st)	OS	≤28 (median 11)	Extrapolated^{UV}
van der Drift et al.[31] (2010)	The Netherlands	46/46	NSCLC (I, 11; II, 6; III, 12; IV, 15; and unknown, 2)	Chemotherapy, surgery, or RT	Plasma	qPCR (β-globin)	Tertiles (2nd vs 1st; 3rd vs 1st)	OS	≤79	Extrapolated^{UV}
Lee et al.[32] (2011)	Republic of Korea	134/134	NSCLC (IIIB, 12 and IV, 122)	Chemotherapy	Plasma	qPCR (β-actin)	Tertiles (2nd vs 1st; 3rd vs 1st)	OS and PFS	≤45 (median 36)	OS: reported^{MV}; PFS: extrapolated^{UV}
Sirera et al.[14] (2011)	Spain	446/446	NSCLC (IIIB, 70 and IV, 376)	Chemotherapy	Plasma	qPCR (hTERT)	Median (49.8 ng/mL)	OS and TTP	≤40 (median 9.7)	Reported^{MV}
Catarino et al.[15] (2012)	Portugal	104/73	NSCLC (I-IV)	Chemotherapy	Plasma	qPCR (hTERT)	20 ng/mL	OS	≤35 (median 9)	Reported^{MV}
Vinayanuwattikun et al.[33] (2013)	Thailand	58/58	NSCLC (IIIB, 6 and IV, 52)	Chemotherapy	Plasma	qPCR (GAPDH)	4.5 ng/mL	OS	≤36 (median 10.8)	Reported^{MV}
Nygaard et al.[34] (2014)	Denmark	53/53	NSCLC (III, 17 and IV, 36)	Chemotherapy or RT	Plasma	qPCR (PPIA)	75th percentile	OS	≤40	Reported^{MV}
Dowler Nygaard et al.[35] (2014)	Denmark	58/58	NSCLC (III, 6 and IV, 52)	Chemotherapy	Plasma	qPCR (β2M)	75th percentile	OS and PFS	≤22	Reported^{UV}

(continued)

Table 1. Continued

Authors (y)	Country	No. of Cases/No. Analyzed for Survival	Cancer Type (Stage, n)	Treatment Methods	DNA Source	Detection Method (Gene)	Cutting Point	Survival Analysis	Mos. of Follow-Up	Source of HR
Wang et al.[16] (2014)	China	134/134	NSCLC (IIIB, 19 and IV, 115)	Chemotherapy	Plasma	Spectrophotometry	Mean (5.82 ng/mL)	OS and PFS	≤31	Reported[MV]
Bortolin et al.[36] (2015)	Italy	22/22	NSCLC (I)	Stereotactic body RT	Plasma	qPCR (hTERT)	75th percentile (8.4 ng/mL)	OS and DFS	≤47 (median 24.7)	Reported[UVa]
Tissot et al.[37] (2015)	France	218/218	NSCLC (IIIB, 42 and IV, 176)	Chemotherapy	Plasma	Fluorometry	Tertiles (3rd vs two lowest)	OS and PFS	≤80 (median 42.3)	Reported[MVa]
Li et al.[17] (2016)	United States	182/101	NSCLC (IIIB-IV)	Chemotherapy	Plasma	qPCR (β-actin)	Median (7.0 ng/mL)	OS and PFS	≤120	Reported[UVa]

[a]HRs and 95% confidence intervals were provided by the corresponding author after being contacted.

β2M, β2 microglobulin; DFS, disease-free survival; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HR, hazard ratio; hTERT, human telomerase reverse transcriptase; MV, multivariate analysis; OS, overall survival; PFS, progression-free survival; PPIA, peptidylprolyl isomerase A; RT, radiotherapy; qPCR, quantitative real-time polymerase chain reaction; RT, radiotherapy; SCLC, small cell lung cancer; TTP, time to tumor progression; UV, univariate analysis.

1.38–2.25]). Exclusion of any single result from the pooled analysis (i.e., leave-one-out sensitivity meta-analysis) did not substantially alter the overall result (Fig. 1*A*; Supplemental Fig. 1). The pooled HR estimates ranged from 1.59 (95% CI 1.30–1.95) when the Fourniè et al.[26] study was excluded from the analysis to 1.86 (95% CI 1.45–2.38) when the Wang et al.[16] study was omitted.

To further assess the robustness of overall findings and explore possible reasons for the observed heterogeneity, we conducted subgroup analyses including study location, source of HR estimate, method of survival analysis, cfDNA detection method, cutoff threshold of baseline cfDNA levels, sample size, and NOS score. As shown in Table 3, significant between-study heterogeneity was detected across all comparisons, except among studies using tertile- or quartile-based cutting points of cfDNA levels ($p = 0.61$; $I^2 = 0$), in which association with OS was still significant (HR 1.70 [95% CI 1.38–2.10]; $p < 0.001$). It is also of note that significant association with OS was observed across all other comparisons, except among studies located in Eastern countries (HR 1.53 [95% CI 0.99–2.37]; $p = 0.054$) and not using qPCR for cfDNA detection (HR 1.51 [95% CI 0.84–2.71]; $p = 0.167$). Three sensitivity analyses were also carried out according to the following inclusion criteria: chemotherapy,[13–17,30,32,33,35,37] advanced NSCLC patients (i.e., stage IIIB/IV),[14,16,17,32,33,37] and qPCR based on hTERT gene amplification.[14,15,28,29,36] Significant association with OS was retained in all sensitivity analyses (Table 3), despite nonsignificant heterogeneity detected only among studies assessing cfDNA by qPCR of hTERT gene amplification ($p = 0.345$; $I^2 = 11$). Similar conclusions were drawn when overall or subgroup analyses were restricted to studies recruiting only patients with NSCLC (Table 1; Supplementary Table 2).

A total of five studies[16,17,32,35,37] including 640 patients with NSCLC were involved in the meta-analysis for the relationship between baseline levels of plasma cfDNA and PFS (Fig. 2*B* and Table 3). The pooled HR showed no association of baseline cfDNA with PFS (HR 1.12 [95% CI 0.91–1.37]; $p = 0.29$) and no significant between-study heterogeneity ($p = 0.23$; $I^2 = 28\%$). Nevertheless, leave-one-out sensitivity meta-analysis suggested lack of robustness of the overall result, given that a borderline significant association with PFS was detected after exclusion of Wang et al.[16] from the pooled analysis (HR 1.21 [95% CI 1.00–1.45]; $p = 0.049$) (Fig. 1*B* and Supplementary Fig. 1). Meta-analyses for TTP[14,27] and DFS[28,36] were not performed because these outcomes were reported in fewer than 3 studies.

Publication Bias

Funnel plot and Egger's test were performed to evaluate publication bias for OS or PFS. The shape of

both funnel plots appeared approximately symmetrical (Fig. 3*A* and *B*), and the *p* values of Egger's test for OS and PFS were 0.199 and 0.642, respectively, suggesting no evidence for publication bias.

Discussion

The recent development of sensitive and accurate methods of detection and quantitative analysis of cfDNA has given rise to a growing interest in this field.[38] Although the precise mechanism remains to be established, the finding that cfDNA mostly derives from lysis of necrotic and apoptotic tumor cells[7,39] has provided the rationale for its use as a surrogate source of tumor DNA. In the last decade, a number of studies have been conducted to assess the value of quantitative analysis of cfDNA for the prediction of lung cancer survival, but the results have been so far inconclusive. In order to elucidate this issue and contribute to a broader discussion on the role of cfDNA as a biomarker, we conducted a systematic revision with meta-analysis of primary studies to estimate the impact of baseline cfDNA levels on survival outcomes of patients with lung cancer.

Our findings show for the first time evidence that increased baseline cfDNA levels in plasma of patients with lung cancer are associated with shorter OS, a result that supports clinical validity of cfDNA quantification for prediction of lung cancer survival. Although cutoff thresholds of plasma cfDNA varied widely across

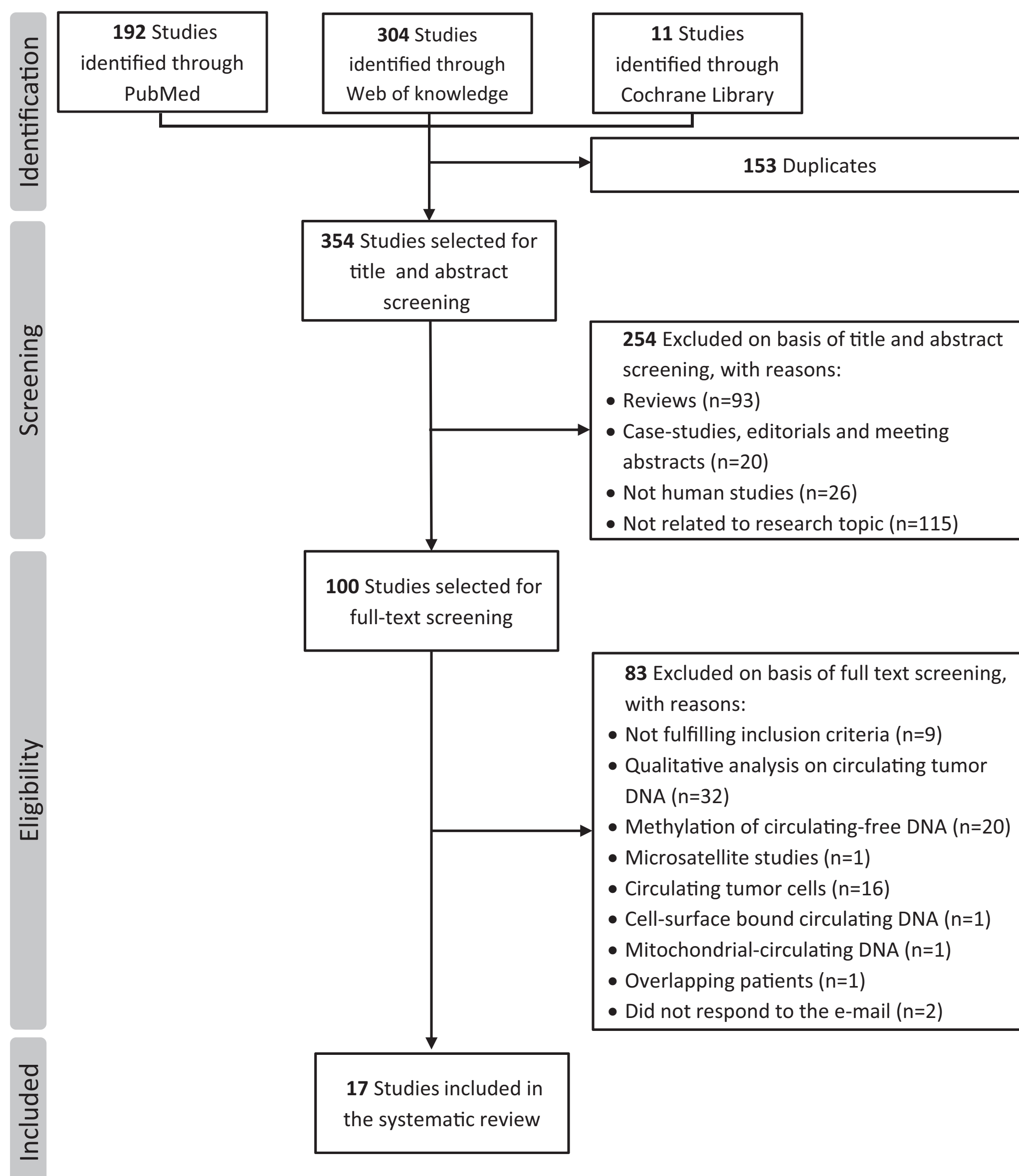
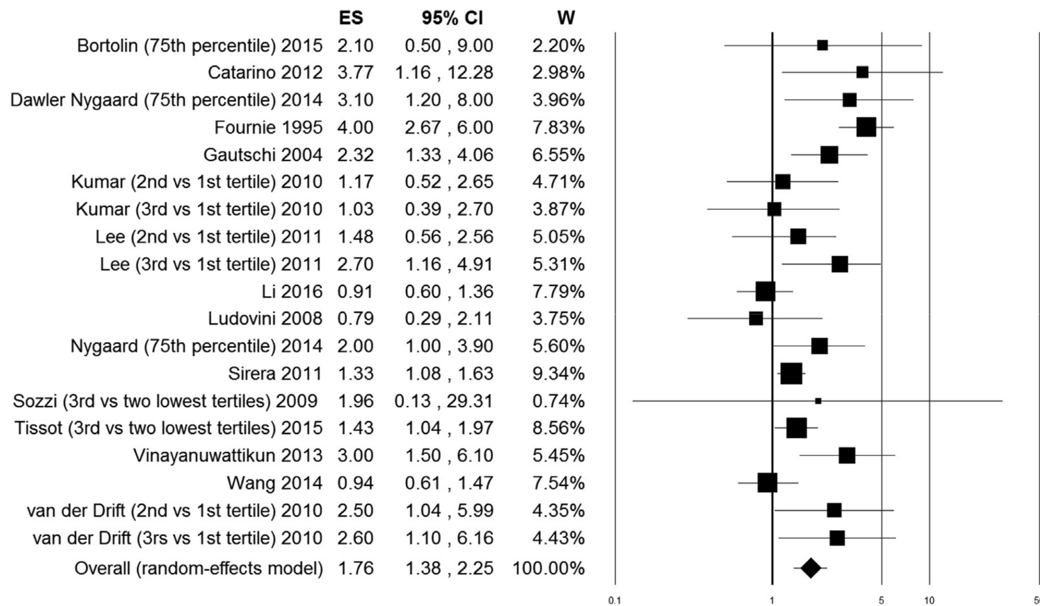


Figure 1. Flowchart of literature search and study selection.

Table 2. Newcastle-Ottawa Quality Assessment of Studies Included in the Systematic Review

Authors (y)	Selection				Comparability		Outcome			Total Score
					Comparability of Cohorts on the Basis of the Design or Analysis					
	Representativeness of the Exposed Cohort	Selection of the Nonexposed Cohort	Ascertainment of Exposure	Demonstration that Outcome of Interest Was Not Present at Start of Study	Age	Any Other Additional Factor	Assessment of Outcome	Was Follow-Up Long Enough for Outcomes to Occur	Adequacy of Follow-Up of Cohorts	
Fournié et al.[26] (1995)	*	*	*				*	*	*	6
Gautschi et al.[13] (2004)	*	*	*		*	*	*	*	*	8
Camps Herrero et al.[27] (2005)	*	*	*	*			*	*	*	7
Ludovini et al.[28] (2008)	*	*	*				*	*	*	6
Sozzi et al.[29] (2009)	*	*	*	*			*	*	*	7
Kumar et al.[30] (2010)	*	*	*	*			*	*		6
van der Drift et al.[31] (2010)	*	*	*	*			*	*	*	7
Lee et al.[32] (2011)	*	*	*		*	*	*	*	*	8
Sirera et al.[14] (2011)	*	*	*	*		*	*	*	*	8
Catarino et al.[15] (2012)	*	*	*	*		*	*	*		7
Vinayanuwattikun et al.[33] (2013)	*	*	*		*	*	*	*	*	8
Nygaard et al.[34] (2014)	*	*	*	*		*	*	*	*	8
Dowler Nygaard et al.[35] (2014)	*	*	*	*			*	*	*	7
Wang et al.[16] (2014)	*	*	*		*	*	*	*	*	8
Bortolin et al.[36] (2015)	*	*	*				*	*	*	6
Tissot et al.[37] (2015)	*	*	*		*	*	*	*	*	8
Li et al.[17] (2016)	*	*	*	*			*	*	*	7



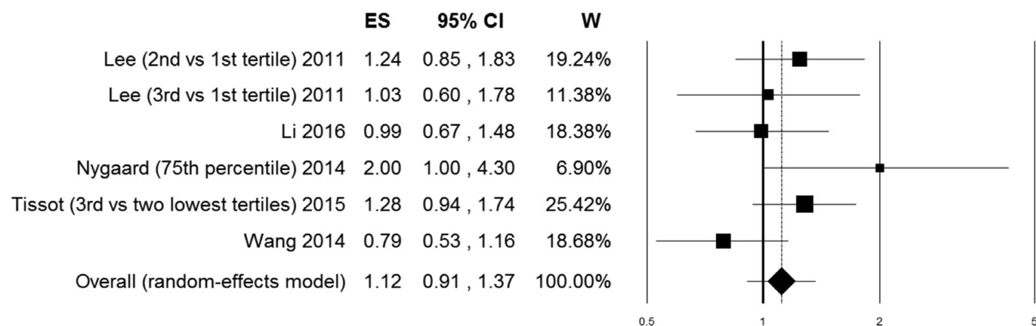


Figure 2. Forest plot of the impact of higher baseline levels of plasma circulating free DNA on overall survival (*A*) or progression-free survival (*B*) in patients with lung cancer. The summary hazard ratio is represented by the diamond, where the center of the diamond indicates the hazard ratio and the ends of the diamond correspond to the 95% confidence interval. Pooled hazard ratio estimates are from the random effects model.

studies, our pooled analysis for OS is consistent with findings of the majority of included studies. The robustness of the pooled OS analysis was first tested by leave-one-out meta-analysis, which showed that no individual study had an excessive influence on the association with OS. Then, several subgroup and sensitivity analyses were conducted to explore possible explanations for the observed heterogeneity and to examine the impact of potential confounders on the pooled OS estimate. Despite substantial heterogeneity was still detected across most comparisons, these further analyses did not substantially alter the overall result of our analysis. It is worthy to note that lack of significant heterogeneity was detected only among studies that used the real-time qPCR based on the hTERT gene amplification ($p = 0.345$; $I^2 = 11\%$), or among those using tertile- or quartile-based methods for cutoff determination ($p = 0.610$; $I^2 = 0\%$). These observations raise the possibility that the technique used for cfDNA detection and the method of cutoff determination may be the main sources of the observed heterogeneity. In recent years, with the rapid development of PCR-based techniques, the methods used in early studies for detection and quantification of cfDNA levels have been gradually replaced by qPCR because of high accuracy, reproducibility, and time effectiveness. However, despite most of the identified studies determining cfDNA levels by qPCR,[13–15,17,28,29,31–36] different reference genes were used, including hTERT,[14,15,28,29,36] glyceraldehyde 3-phosphate dehydrogenase,[13,33] or β-actin[17,32] and a wide range of cutoff thresholds. Additional investigation is therefore warranted to establish a consensus on preanalytical and analytical protocols for cfDNA analysis[40,41] and the optimal cutoff

Table 3. Summary of Random Effect Meta-Analyses for the Relationship Between Baseline Levels of Plasma Circulating Free DNA (cfDNA) and Survival Outcomes of Patients with Lung Cancer

Group or Subgroup	Studies Included	Cases	Test of Association, HR (95% CI)	*p* Value	Test of Heterogeneity (*p* Value Q-test, I^2%)	
Overall survival						
All studies	16	1723	1.76 (1.38-2.25)	<0.001	<0.001	66
Subgroup analyses						
Study location						
Western	12	1355	1.89 (1.40-2.57)	<0.001	<0.001	71
Eastern	4	368	1.53 (0.99-2.37)	0.054	0.043	56
Source of HR						
Reported	11	1373	1.52 (1.20-1.93)	0.001	0.015	53
Data extrapolated	5	350	2.24 (1.48-3.38)	<0.001	0.067	49
Survival analysis						
Multivariate	8	1279	1.68 (1.31-2.16)	<0.001	0.026	54
Univariate	8	444	1.76 (1.07-2.87)	0.025	<0.001	73
Detection method						
qPCR	12	1264	1.84 (1.41-2.39)	<0.001	0.019	49
Other	4	459	1.51 (0.84-2.72)	0.167	<0.001	85
Cutoff threshold						
Tertile- or quartile-based	8	607	1.70 (1.38-2.10)	<0.001	0.610	0
Other	8	1116	1.71 (1.12-2.63)	0.014	<0.001	84
Sample size						
≥100	6	1196	1.37 (1.08-1.75)	0.010	0.036	56
<100	10	527	2.23 (1.61-3.08)	<0.001	0.074	40
NOS score						
>7	7	1206	1.62 (1.27-2.07)	0.001	0.037	53
≤7	9	517	1.86 (1.17-2.97)	0.009	<0.001	71
Sensitivity analyses						
Chemotherapy only	10	1427	1.53 (1.21-1.94)	<0.001	0.011	55
Stage IIIB/IV NSCLC	6	1091	1.39 (1.07-1.80)	0.013	0.021	60
qPCR (hTERT)	5	651	1.40 (1.00-1.97)	0.049	0.345	11
Progression-free survival						
All studies	5	640	1.12 (0.91-1.37)	0.29	0.23	28

CI, confidence interval; HR, hazard ratio; hTERT, human telomerase reverse transcriptase; NOS, Newcastle-Ottawa scale; qPCR, quantitative real-time polymerase chain reaction; RT, radiotherapy.

values for patients' stratification. On the other hand, it should be noted that the association with OS failed to reach statistical significance in studies not using qPCR[16,26,30,37] or that considered only East Asian populations.[16,30,32,33] However, we cannot exclude that these results may be related to the limited number of patients included in these analyses. The same conclusion could be drawn for the nonsignificant association of plasma cfDNA with PFS, for which additional studies are still needed to provide conclusive evidence of a relationship.

Our findings should be interpreted in light of the following limitations and considerations. First, substantial between-study heterogeneity was found in the meta-analysis of OS. This is probably explained by the lack of standardization of the cfDNA test, with major differences regarding method of quantification and cutoff thresholds. Despite these two aspects being identified as potential explanatory variables, other factors could also explain the observed between-study heterogeneity. For instance, circulating cfDNA levels may reflect not only changes in circulating tumor DNA but also reflect medical conditions or patient characteristics that may lead to an increase in cfDNA concentration.[42] Second, about two-thirds of the identified studies involved less than 100 patients with lung cancer in the survival analysis; caution is needed in the interpretation of the pooled HR estimates. In this regard, alternative approaches to the classical random effects meta-analysis, such as bootstrapping or its Bayesian

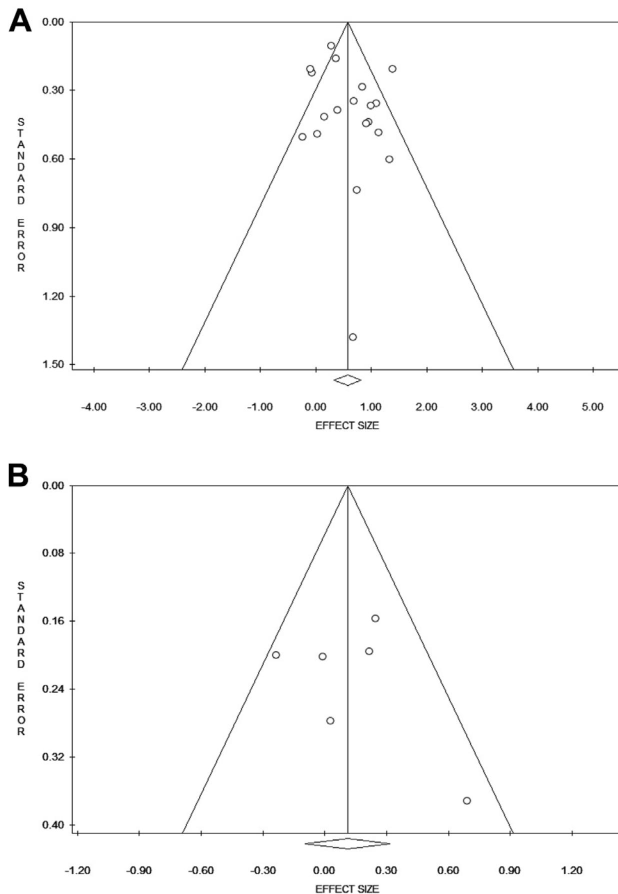


Figure 3. Funnel plot for detection of publication bias in the meta-analysis of overall survival (*A*; Egger's *p* value = 0.199) or progression-free survival (*B*; Egger's *p* value = 0.642).

analogue, may be used in order to provide more robust estimates and CIs. Third, although we attempted to contact the corresponding authors of potentially relevant papers, some of them were unavailable to provide data requested for inclusion in the meta-analysis and this might have affected the results. In addition, we could not evaluate the impact of baseline cfDNA levels on survival and objective response rate after specific cancer treatments because of an extreme intra- and interstudy heterogeneity in terms of chemotherapy drugs and regimens.

In summary, the current meta-analysis shows for the first time evidence of an increased risk of death in patients with lung cancer who have higher baseline cfDNA levels, thereby supporting clinical validity of quantitative analysis of cfDNA for prediction of lung cancer survival. Nevertheless, the establishment of a robust, well standardized method for detection and quantification of cfDNA and determination of the optimal cutoff thresholds are still required to define the clinical relevance of cfDNA quantification for lung cancer management.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at http://dx.doi.org/10.1016/j.jtho.2016.08.002.

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