RESEARCH COMMUNICATION

Meta-analysis of Circulating Tumor Cells as a Prognostic Marker in Lung Cancer

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Abstract

Introduction: Recent studies have shown that circulating tumor cells (CTCs) play potential roles as diagnostic and prognostic biomarkers with various cancer types. The aim of this study was to comprehensively and quantitatively summarize the evidence for the use of CTCs to predict the survival outcome of lung cancer patients. Materials and Methods: Relevant literature was identified using Medline and EMBASE. Patients' clinical characteristics, overall survival (OS) and progression-free survival (PFS) together with CTC positive rates at different time points (before, during and after treatment) were extracted. A meta-analysis was performed to clarify the prognostic role of CTCs and the correlation between the CTC appearance and clinical characteristics. Results: A total of 12 articles containing survival outcomes and clinical characteristics and 15 articles containing only clinical characteristics were included for the global meta-analysis. The hazard ratio (HR) for OS predicted by pro-treatment CTCs was 2.61 [1.82, 3.74], while the HR for PFS was 2.37 [1.41, 3.99]. The HR for OS predicted by post-treatment CTCs was 4.19 [2.92, 6.00], while the HR for PFS was 4.97 [3.05, 8.11]. Subgroup analyses were conducted according to histological classification and detection method. Odds ratio (OR) showed the appearance of pro-treatment CTCs correlated with the lymph node status, distant metastasis, and TNM staging, while post-treatment CTCs correlated with TNM staging only. Conclusion: Detection of CTCs in the peripheral blood indicates a poor prognosis in patients with lung cancer.

Keywords: Lung cancer - circulating tumor cells - prognosis - meta-analysis

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Introduction

Lung cancer was the most common cancer as well as the leading cause of cancer death. Approximately 1.6 million new cases of lung cancer will be diagnosed and 1.4 million deaths will occur from lung cancer during 2008 (Jemal et al., 2011).

The presence of circulating tumor cells (CTCs) in the blood was first reported by T. R. Ashworth more than a century ago (Ashworth, 1869). The level of detected CTCs was widely used in the diagnosis of breast (Cristofanilli, 2006), colorectal (Cohen et al., 2008), lung (Krebs et al., 2011) and prostate cancers (Helo et al., 2009). The detection of CTCs have been recently developed to reflect the progression and survival of the disease. Many studies reached in a positive conclusion towards the role of CTCs in prognostic prediction of lung cancer. However, some other study stood with the opposite attitude (Chen et al., 2007). Thus, it still remained a question whether CTCs can warn for disease progression and survival earlier and less invasively than conventional methods currently available.

The aim of this study is to comprehensively and quantitatively summarize the evidence for the use of CTCs to predict the clinical results of lung cancer patients.

Materials and Methods

Search strategy

Medline and EMBASE were searched for the last time on Feb 26, 2012. The search strategy included the following keywords variably combined by ''CTCs'', ''circulating tumor cells'' and ''lung cancer''.

Study inclusion/exclusion criteria

Studies were considered eligible if they met all of the following inclusion criteria, (i) discussed patients with lung cancer, (ii) measured the appearance of CTCs in peripheral blood, and (iii) investigated the association between CTCs' appearance rate and survival outcome (overall survival, OS or progression free survival, PFS). Studies wereexcluded based on any of the following criteria, (i) were review articles or letters (ii) analyzed in varioustumors but with no specific results of lung cancer, (iii) lacked keyinformation for analysis with methods developed by Parmar et al. (1998), Williamson et al. (2002), and Tierney et al. (2007).

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Data Extraction

Articles were reviewed independently by two investigators (Ma XL and Xiao ZL) for article inclusion and exclusion. Disagreements were resolved by consensus. Data were extracted from eligible studies by two investigators (Ma XL and Liu L) independently. The primary data were p-value, the Kaplan–Meier survival curves or HR and 95% confidence interval (CI) of survival outcomes. Additional data obtained from the studies included first author, publication year, study size, patients age and sexuality, TNM stage, histological classification, methods to detect CTCs, positive CTCs definition, the attitude conclusion and other clinical characteristics.

Statistical Methods

The logHR and SE (logHR) (SE) were used for aggregation of the survival results, but these statistical variables were not given explicitly in most studies. We calculated the necessary statistics on the basis of available numerical data with methods developed by Parmar, Williamson, and Tierney. We performed meta-analysis in OS and PFS, the subgroup research were given when the article number ≥ 2. Calculation was accomplished by the software designed by Matthew Sydes and Jayne Tierney with these methods (Medical Research Council Clinical Trials Unit, London, UK) (Tierney et al., 2007).

We also examine the correlation between CTCs appearance and the clinical variables including TNM stage, the depth of invasion, lymph node status, distant metastasis, sexuality and smoking status. According to clinical characteristics, Stage I and Stage II were combined and Stage III and Stage IV were combined; pT1 and pT2 were combined and pT3 and pT4 were combined. Odds ratio (OR) was used as the measure index to describe the correlation.

Forrest plots were used to estimate the effect of CTCs appearance on survival outcome and the correlation between CTCs appearance and the clinical variables. Heterogeneity was defined as $p < 0.10$ or $I^2 > 50\%$ (Higgins et al., 2003). When homogeneity was fine ($p \leq 0.10$, $I^2 \leq 50\%$), a fixed effect model was used for secondary analysis. If not, a random effect model was used. An observed HR>1 indicated worse outcome for the positive group relative to the negative group and would be considered statistically significant if the 95% CI did not overlap 1.The Begg's rank correlation also was applied to assess the potential publication bias, $p > 0.05$ was considered that there was no potential publication bias (Begg, 1994). All above calculations were performed using RevMan5.1 (Cochrane collaboration, Oxford, UK) Publication biases were evaluated using the Begg's funnel plot by STATA 11.0 (STATA Corporation, College Station, TX).

Results

Eligible Studies

The initial search yielded 1457 articles. We did another electronic search with the same key words using online EMBASE, which was unable to retrieve additional pertinent references. In all yielded publications including potential ones in reviews, reviewers identified 69 potential studies for full-text review. 42 studies were excluded for follow reasons: they did not mention survival outcomes

Table 1. Baseline Characteristics of Included Studies

Author	year	case	control size	age	male%	III & IV%	histologic cell type squamous cell carcinoma%	treatment	follow up (month)	sampling time
Chen TF	2007	67	unknown	median 62	89.6	91.4	ADC 32 SQC 32 others 3	chemo. and radio.	median 37	before and after TM
Hofman V	2011	208	39	median 63	67.8	34.1	ADC 115 SQC 54 others 39	surg. and chemo. Or untreated	median 24	before TM
Hou JM	2009	50	85	median 67	54	—	—	chemo. and radio.	median 3	before and after TM
Hou JM	2012	97	—	median 68	44.3	—	—	surg. And chemo.	mean 7.4	before and after TM
Liu L	2008	134	186	—	—	73.1	ADC 44 SQC 40 SMC 31 others 19	chemo.	median 30	before TM
Nieva J	2012	28	—	median 64	53.8	—	ADC 21 SQC 5 others 2	chemo. Or biotherapy	median 10	before TM
Sher YP	2005	54	24	median 65	59.3	—	ADC 35 SQC 14 others 5	surg. Or chemo.	85	before TM
Yamashita J	2000	32	—	median 63	31.2	6.2	ADC 29 SQC 2 others 1	surg.	median 12	before and after TM
Yamashita J	2002	103	Unknown	median 68	73.8	26.2	ADC 66 SQC 37	surg.	—	before TM
Kurusu Y	1999	103	32	median 68	73.8	26.3	ADC 66 SQC 37	surg.	—	before and after TM
Yie SM	2009	143	172	median 57	73.4	71.3	ADC 87 SQC 56	surg. And/or chemo.	36	before and after TM
Okumura Y	2009	30	—	median 65	70	23.3	ADC 18 SQC 7 SMC 1 others 4	surg.	median 13	before TM
Hofman V	2010	210	40	median 63	72.3	37.6	ADC 120 SQC 57 others 33	surg. (and chemo.)	median 15	before TM
Hofman V	2010	250	59	median 65	68.9	27.6	ADC 150 SQC 67 others 33	surg.	—	before TM
Krebs MG	2011	101	—	median 67	53.4	100	ADC 31 SQC 32 others 63	chemo. Or untreated	mean 5.4	before and after TM
Sawabata N	2007	9	4	median 58	100	0	ADC 6 SQC 3	surg.	median 14	before TM
Yoon SO	2010	79	—	median 66	60.8	—	ADC 45 SQC 27 others 7	surg.	60	before and after TM
Funaki S	2011	94	—	median 68	59.6	6.4	ADC 71 SQC 14 others 9	surg.	median 13	during TM
Castaldo G	1997	24	unknown	mean 62	87.5	91.7	ADC 9 SQC 12 SMC 3	—	6	before TM
Guo Y	2009	83	30	median 55.9	60.2	63.9	— —	surg.	—	before TM
Peck K	1998	86	62	median 66	66.3	70.9	ADC 47 SQC 17 SMC 15 others 7	surg. And/or chemo. And/or radio.	mean 3.8	before TM
Sheu CC	2006	100	147	median 64	36.1	58	ADC 72 SQC 28	—	—	before TM
Wendel M	2012	78	—	median 64	53.8	83.3	ADC 44 SQC 20 others 14	chemo.	—	before TM
Wu C	2009	47	31	—	—	93.6	ADC 27 SQC 7 SMC 13	chemo.	—	before TM
Farace F	2011	20	—	mean 55.8	55	100	ADC 16 others 4	—	—	before TM
Tanaka F	2009	125	25	—	—	25.6	ADC 85 SQC 22 SMC 9 others 9	surg. Or untreated	—	before TM
Huang TH	2007	51	40	median 58.6	52.9	25.5	ADC 21 SQC 30	surg. Or chemo. Or radio.	—	before TM
Devriese LA	2012	46	46	mean 58	63	100	ADC 30 SQC 8 others 8	chemo. Or biotherapy	—	before TM
Hayes DC	2006	49	25	mean 61.8	49	—	ADC 11 SQC 8 SMC 10 others 20	chemo. Or untreated	—	before TM
Li J	2005	52	5	31-78	67.3	30.8	ADC 30 SQC 22	surg.	—	during TM
Sienel W	2003	62	—	—	72.6	—	ADC 19 SQC 28 others 15	surg. And radio.	median 25	during TM

ADC, adenocarcinoma; AQC, squamous cell carcinoma; chemo., chemotherapy; radio., radio., radiotherapy; surg., surgery; TM, treatment

Table 2. Overview of the Study Design Variables

Author	sampling site/volume	methods	markers	positive definition	outcomes	multivariate	attitude
Chen TF	PB/8ml	RT-PCR	CK19 mRNA	——	OS&PFS	yes	negative
Hofman V	PB/10ml	ISET	——	unfiltrated	OS&PFS	Yes	positive
Hou JM OS&PFS	PB/7.5ml yes	CellSearch positive	EpCAM,keratin 4,5,6,8,10,13,18,DAPI,CD56	all markers+ and CD45-			
Hou JM OS&PFS	PB/7.5ml Yes	CellSearch and ISET positive		EpCAM,CK8,18,19,DAPI	all markers+ and CD45-		
Liu L	PB/5ml	RT-PCR	TSA-9, Keratin 19,Pre-proGRP	1,2 or 3 markers	OS	Yes	positive
Nieva J	PB	IF	CK 1,4–8,10,13,18,19 and DAPI	all markers+ and CD45-	OS	No	positive
Sher YP	PB/3-4ml	RT-PCR	keratin 19, Ubiquitin thiolesterase C	Lc=1,Lc formula in article	OS	No	positive
Yamashita J	PB	RT-PCR	CEA mRNA	——	OS	No	positive
Yamashita J	PB	RT-PCR	CEA mRNA	——	OS	Yes	positive
Kurusu Y	PB	RT-PCR	CEA mRNA	RT-PCR	OS	Yes	positive
Yie SM	PB	RT-PCR based on ELISA	survivin	——	OS	Yes	positive
Okumura Y	PB/7.5ml PV/2.5ml	CellSearch	EP-CAM,DAPI,CK	morphology, all markers+ and CD45-	OS	No	negative
Hofman V	PB/7ml	ISET or CellSearch	EpCAM,DAPI,CK2, 5, 6, 8, 10, 11, 14/15, 18 and 19,vimentin	morphology, all markers+ and CD45-	PFS	Yes	positive
Hofman V	PB/10ml	ISET	——	morphology	——	——	——
Krebs MG	PB/7.5ml	CellSearch	EpCAM,CK8,18,19,DAPI	morphology, all markers+ and CD45-	OS&PFS	Yes	positive
Sawabata N	PB/7.5ml	CellSearch	EpCAM,CK8,18,19,DAPI	morphology, all markers+ and CD45-	PFS	No	——
Yoon SO	PB	RT-PCR	TTF-1,CK19 mRNA	any target	OS	Yes	positive
Funaki S	PV/1ml	ICC	——	anyform (singular or cluster)	——	Yes	positive
Castaldo G	PB	RT-PCR	CEA mRNA	——	——	No	——
Guo Y	PB/3ml	RT-PCR	CK20,CK19,CEA mRNA	1,2 or 3 visible bands by naked eye	——	——	——
Peck K	PB/3-5ml	RT-PCR	CK19 mRNA	——	——	——	——
Sheu CC	PB/≤ 5ml	RT-PCR	17 marker panel	12 out of 17 genes overexpression	——	——	——
Wendel M	PB	CellSesearch	EpCAM,CK8,18,19,DAPI	morphology, all markers+ and CD45-≥2 in 7.5ml of blood	——	——	——
Wu C	PB/7.5ml	IF,IHC	CK 18,CK19,DAPI,	morphology, all markers+ and CD45-≥2 in 7.5ml of blood	——	——	——
Farace F	PB/17.5ml	CellSearch and ISET	EpCAM,CK8,18,19,DAPI	morphology, all markers+ and CD45-	——	——	——
Tanaka F	PB/7.5ml	CellSearch	EpCAM,CK8,18,19,DAPI	morphology, all markers+ and CD45-	——	——	——
Huang TH	PB/8ml	ICC and RT-PCR	CK19mrna, LUNX mRNA	morphology, visible red color of antibody in plasma	——	——	——
Devriese LA	PB/8ml	RT-PCR	EpCAM,CK7,CK19,EGP (epithelial glycoprotein,FN1	any target, quadratic discriminant analysis	——	——	——
Hayes DC	PB/5-8ml	antibody coctail and RT-PCR	——	CD45-,CD66b-,CD36-,glycohorinA-, healthy cut-off values	——	——	——
Li J	PB	RT-PCR	CEA mRNA	——	——	——	——
Sienel W	PV/10ml	ICC	CK8, 18, and 19	any target	——	——	——

PB, peripheral blood; PV, pulmonary blood; RT-PCR, reverse transcriptase polymerase chain reaction; ISET, isolation by size of epithelial tumor cells; IF, immunofluorescence; ICC, immunocytochemistry; IHC, immunohistochemistry; CK, cytokeratin; EpCAM, Epithelial cell adhesion molecule; DAPI, 4',6-diamidino-2-phenylindole; TSA, tumor specific antigen; TTF1, thyroid transcription factor 1; pro-GRP, progastrin releasing peptide; LUNX, lung specific protein X; FN1, fibronectin; CEA, Carcinoembryonic antigen; OS, overall survival; PFS, progression free survival

characterized by CTCs in 30 studies, did not extract enough data to calculate both HR for survival outcome and OR for the correlation in 10 studies, were concerned about disseminated tumor cells (DTCs) in one study (Kubuschok et al., 1999), or used exactly identical cases in Kurusu' study (Kurusu et al.,1999) and Yamashita's study (Yamashita et al., 2002). We finally used the information from both of the two articles and named it Kurusu Y in our list. Okumura's study (Okumura et al., 2009) referred the survival outcome of OS, but we can't calculate the HR (95% CI). Thus, we only extracted the patients' clinical characteristics in this article. Finally, we enrolled 12 (Kurusu et al., 1999; Yamashita et al., 2000; Sher et al., 2005; Chen et al., 2007; Liu et al., 2008; Hou et al., 2009; Hofman et al., 2011; Krebs et al., 2011; Yoon et al., 2011; Hou et al., 2012; Nieva et al., 2012) articles containing survival outcomes and patients' clinical characteristics and 15 articles (Castaldo et al., 1997; Peck et al., 1998; Li et al., 2005; Hayes et al., 2006; Sheu et al., 2006; Huang et al., 2007; Sawabata et al., 2007; Guo et al., 2009; Okumura et al., 2009; Tanaka et al., 2009; Wu et al., 2009; Farace et al., 2011; Devriese et al., 2012; Hofman et al., 2012; Wendel et al., 2012) containing only patients' clinical characteristics in our analysis (Figure 1). These studies were published between the year of 1997 and 2012. The total number of patients included was 2615, ranging from 9 to 250 patients per study (median, 78). HRs on OS, and PFS could be extracted for 11 and 5 studies respectively. Patients' clinical characteristics were listed in Table 1 and an overview of the study design variables were listed in Table 2.

Correlation between CTCs appearance and survival outcome (OS and PFS)

Overall Analyses: The meta-analysis of all studies on OS showed significant prognostic effects on CTCs detected in samples collected before and after treatment. The HR (95% CI) of 9 studies (Kurusu et al., 1999; Yamashita et al., 2000; Sher et al., 2005; Chen et al., 2007; Liu et al., 2008; Hou et al., 2009; Hofman et al., 2012;

Table 3. Meta-analysis of CTCs Prediction Significance of Lung Cancer and Subgroup Analysis

Sampling time	OS					PFS				
Analysis	Study n.	Patient n.	Model	HR (95% CI)	I^2, p	Study n.	Patient n.	Model	HR(95% CI)	I^2, p
Before treatment										
Total	9	773	Random	2.61 [1.82, 3.74]	69%,0.001	4	473	Random	2.37 [1.41, 3.99]	66%,0.03
NSCLC	7	626	Random	2.79 [1.86, 4.17]	53%,0.05	3	376	Random	2.32 [1.09, 4.94]	75%,0.02
SCLC	2	147	Random	2.19 [0.90, 5.34]	89%,0.003	1	97	— —	2.69 [1.62, 4.48]	— —
RT-PCR	5	390	Random	3.04 [1.71, 5.42]	65%,0.02	1	67	— —	1.17 [0.68, 2.03]	— —
ISET	1	208	— —	2.10 [1.34, 3.29]	— —	1	208	— —	2.64 [1.52, 4.57]	— —
CellSearch	2	127	Random	2.19 [0.90, 5.34]	89%,0.003	2	198	Fixed	3.17 [1.89, 5.33]	17%,0.27
After treatment										
Total	5	447	Fixed	4.19 [2.92, 6.00]	37%,0.18	3	265	Fixed	4.97 [3.05, 8.11]	44%,0.17
NSCLC	4	350	Fixed	3.85 [2.63, 5.63]	33%,0.21	2	168	Random	5.90 [1.80, 19.38]	70%,0.07
SCLC	0	— —	— —	— —	— —	1	97	— —	6.30 [2.19, 18.14]	— —
RT-PCR	3	249	Fixed	3.48 [2.34, 5.16]	0%,0.69	1	67	— —	3.53 [1.88, 6.60]	
ISET	0	— —	— —	— —	— —	0	— —	— —	— —	— —
CellSearch	1	97	— —	8.67 [2.84, 26.50]	— —	1	97	— —	6.30 [2.19, 18.14]	— —

Legends:Analyses and subgroup analyses were performed according to different sampling time. Subgroup analyses were focused on stratifion by histological classification (NSCLC or SCLC) and method used to detect CTCs (RT-PCR, ISET OR CellSearch). OS, overall survival; PFS, progression free survival; n., number; HR, hazard ratio; CI, confidence interval; ADC, adenocarcinoma; AQC, squamous cell carcinoma; RT-PCR, reverse transcriptase polymerase chain reaction; ISET, isolation by size of epithelial tumor cells

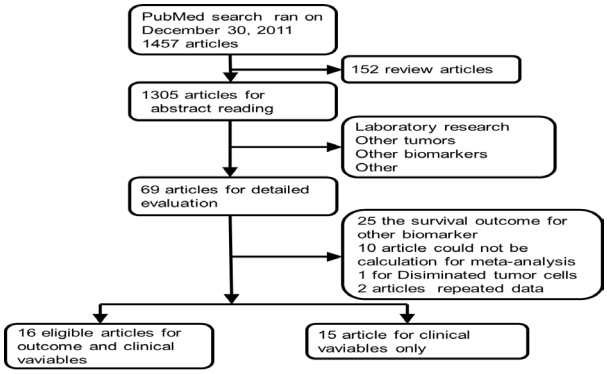

Figure 1. Selection of Studies

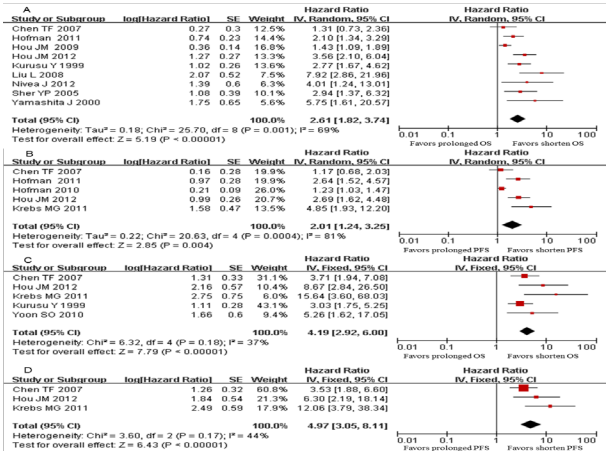

Figure 2. Estimated Hazard Ratios (HRs) Summary for (A) overall survival with circulating tumor cells detected in pro-treatment peripheral blood, (B) progression free survival with circulating tumor cells detected in pro-treatment peripheral blood, (C) overall survival with circulating tumor cells detected in post-treatment peripheral blood and (D) progression free survival with circulating tumor cells detected in post-treatment peripheral blood

Hou et al., 2012; Nieva et al., 2012) before treatment was 2.61 [1.82, 3.74] (n=773, I^2 = 69%, P=0.001), and the HR (95% CI) of 5 studies (Kurusu et al., 1999; Chen et al., 2007; Krebs et al., 2011; Yoon et al., 2011; Hou et al., 2012) after treatment was 4.19 [2.92, 6.00] (n=447, I^2 = 37%, P = 0.18). Pooled analysis of all studies (Chen et al., 2007; Hofman et al., 2011; Krebs et al., 2011; Hou et al., 2012) on PFS showed that the presences of CTCs in peripheral blood collected before and after treatment were associated with poor survival outcome again. The pooled HRs were 2.37 [1.41, 3.99] (n = 473, I^2 = 66%, P = 0.03) and 4.97 [3.05, 8.11] (n = 265, I^2 = 44%, P = 0.17), respectively (Figure 2, Table 3).

Subgroup analysis: As several studies collected samples at various time points, we separately summarized them according to the time points in subgroup analyses stratified by either of patients' clinical characteristics we analyzed. When there was more one study focusing on a subgroup, we conducted a meta-analysis and listed the result in Table 3; otherwise, we listed the result of the original study without analysis.

We first evaluated the prognostic significance of CTCs in NSCLC and SCLC. Studies (Kurusu et al., 1999; Yamashita et al., 2000; Sher et al., 2005; Chen et al., 2007; Liu et al., 2008; Hofman et al., 2012; Nieva et al., 2012) dealing with NSCLC pro-treatment samples yielded HRs [95% CI] for both OS and PFS, OS: 2.79 [1.86, 4.17] (n=626, I^2 = 53%, p=0.05) and PFS: 2.32 [1.09, 4.94] (n=376, I^2=75%, p=0.02). Studies(Hou et al., 2009, 2012) dealing with SCLC pro-treatment samples only yielded poor insignificance HR value of OS and were not sufficient to calculate HR of PFS (n=1). Studies (Kurusu et al., 1999; Chen et al., 2007; Krebs et al., 2011; Yoon et al., 2011) dealing with NSCLC post-treatment samples were analyzed for HR of OS (3.85 [2.63, 5.63], n=350, I^2 = 37%, p = 0.18) and PFS (5.90 [1.80, 19.38], n=168, I^2 = 70%, p = 0.07). There were no sufficient studies for the subgroup analysis of SCLC samples.

As shown by the subgroup analysis stratified by method used to identify CTCs in peripheral blood, we found OS prediction effect of CTCs in the analyses of studies applied RT-PCR (shown in Table 2) using samples collected before treatment (HR = 3.04 [1.71, 5.42], n=390, I^2 = 65%, p =

Table 4. Meta-analyses of CTCs Appearance Odds Ratios in Patients Classified by Different Clinical Characteristics

Sampling time	Analysis	Study n.	Patient n.	Model	OR(95% CI)	p value	Heterogeneity (I^2, p)	Conclusion
Before treatment	TNM stage (III/IV vs. I/II)	16	1361	Random	1.91 [1.07, 3.39]	0.03	62%, 0.0006	positive
	The depth of invasion (pT3/pT4 vs. pT1/pT2)	6	472	Random	1.52 [0.41, 5.72]	0.53	84%, <0.00001	negative
	Lymph node (N3/N4 vs.N1/N2)	8	653	Fixed	2.27 [1.54, 3.35]	<0.0001	37%, 0.14	positive
	Distant metastasis (yes vs. no)	15	1299	Random	2.59 [1.33, 5.04]	0.005	70%, <0.0001	positive
	Sexuality (male vs. female)	9	609	Fixed	1.19 [0.81, 1.75]	0.37	34%, 0.15	negative
	Histological differentiation (ADC vs. SQC)	16	1115	Random	1.25 [0.84, 1.87]	0.28	42%, 0.04	negative
	Smoking (yes vs.no)	3	206	Fixed	1.76 [0.93, 3.33]	0.08	0%, 0.93	negative
During treatment	TNM stage (III/IV vs. I/II)	4	208	Fixed	2.79 [1.13, 6.85]	0.03	47%, 0.13	positive
	The depth of invasion (pT3/pT4 vs. pT1/pT2)	2	156	Fixed	1.25 [0.35, 4.45]	0.73	0%, 0.58	negative
	Lymph node status (N3/N4 vs.N1/N2)	2	156	Fixed	0.83 [0.26, 2.61]	0.75	26%, 0.24	negative
	Distant metastasis (yes vs. no)	3	176	Fixed	1.61 [0.28, 9.29]	0.59	0%, 0.83	negative
	Sexuality (male vs. female)	3	186	Fixed	1.46 [0.73, 2.96]	0.29	0%, 0.67	negative
	Histological differentiation (ADC vs. SQC)	5	218	Fixed	0.47 [0.24, 0.95]	0.04	13%, 0.33	positive
After treatment	TNM stage (III/IV vs. I/II)	4	250	Fixed	4.86 [2.29, 10.29]	<0.0001	0%, 0.53	positive
	The depth of invasion (pT3/pT4 vs. pT1/pT2)	2	115	Fixed	1.58 [0.63, 3.94]	0.33	0%, 0.48	negative
	Lymph node status (N3/N4 vs.N1/N2)	2	115	Random	2.01 [0.36, 11.21]	0.42	62%, 0.10	negative
	Sexuality (male vs. female)	2	115	Random	1.11 [0.15, 7.97]	0.92	70%, 0.07	negative
	Histological differentiation (ADC vs. SQC)	2	113	Random	1.92 [0.49, 7.54]	0.35	59%, 0.12	negative

OR, odds ratio; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; vs., versus

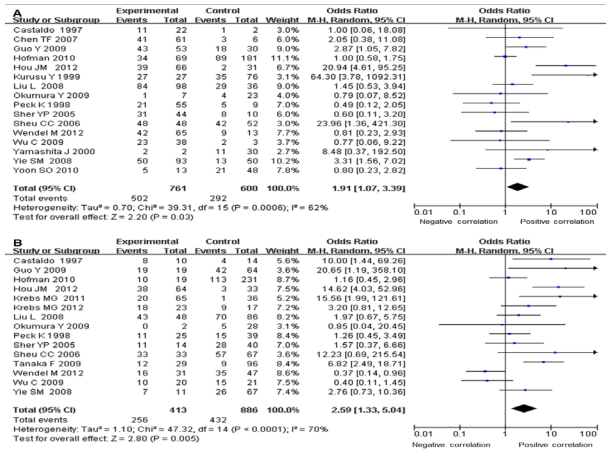

Figure 3. Estimated Odds Ratios (ORs) Summary for Correlation of (A) circulating tumor cells appearance and TNM staging, (B) circulating tumor cells appearance and distant metastasis

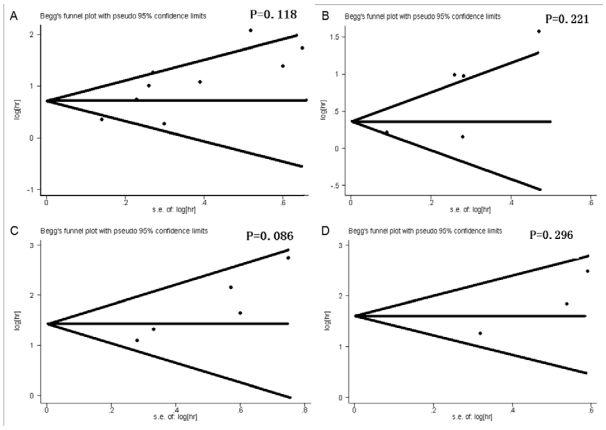

Figure 4. Funnel Plots of Publication Bias Summary for Corresponding Meta-analysis in Figure 2. Orderly, they are Funnel plots of publication bias for meta-analysis of hazard ratios (HRs) for (A) overall survival with circulating tumor cells detected in pro-treatment peripheral blood, (B) progression free survival with circulating tumor cells detected in pro-treatment peripheral blood, (C) overall survival with circulating tumor cells detected in post-treatment peripheral blood and (D) progression free survival with circulating tumor cells detected in post-treatment peripheral blood

0.02) and after treatment (OS: HR=3.48 [2.34, 5.16], n= 249, $I^2 = 0$, p = 0.69). The only significant result of meta-analysis was HR for PFS predicted by samples collected before treatment processed by CellSearch (HR = 3.17 [1.89, 5.33], n = 198, $I^2 = 17\%$, p = 0.27). Further data concerning subgroup analysis were summarized in Table 3.

Correlation between CTCs appearance in peripheral blood and clinical characteristics

We stratified the studies (Peck et al., 1998; Kurusu et al., 1999; Yamashita et al., 2000; Sher et al., 2005; Sheu et al., 2006; Chen et al., 2007; Liu et al., 2008; Okumura et al., 2009; Tanaka et al., 2009; Hofman et al., 2011; Jemal et al., 2011; Krebs et al., 2011; Yoon et al., 2011; Hou et al., 2012; Wendel et al., 2012), (Castaldo et al., 1997; Li et al., 2005; Hayes et al., 2006; Huang et al., 2007; Sawabata et al., 2007; Guo et al., 2009; Wu et al., 2009; Farace et al., 2011; Devriese et al., 2012; Hofman et al., 2012) to observe the correlation between each clinical characteristic and CTCs appearance in peripheral blood in lung cancer patients. As shown in table 4, CTCs were more likely to show up in peripheral blood in III/IV lung cancer patients than I/II patients using samples collected from all their time points, especially using post-treatment samples (OR= 4.86 [2.29, 10.29], p < 0.0001) (Figure 3). Similar results were received only when lymph node status and distant metastasis were stratifying factors using post-treatment samples (Figure 3). When we stratified the studies by sexuality, smoking status or histological differentiation (adenocarcinoma versus squamous cancinoma), correlation between clinical characteristics and CTCs appearance was weak or insignificant (Table 4).

Assessment of publication bias

As shown in Figure 4, Begg's test was used to examine publication bias. No significant publication biases were found in results of HRs for OS both using samples collected before and after treatment (P = 0.118 and P = 0.221 respectively). As for PFS, we obtained similar

results (P = 0.086 and P = 0.296 when using samples before and after treatment respectively).

Discussion

As we know, it was the first time that a comprehensive and detailed meta-analysis revealed the prognostic role of CTCs for lung cancer. CTCs expression was confirmed with a poor survival outcome according to the evidence-based medicine in our study.

Our results revealed CTCs' prognostic value in lung cancer (Table 3), which was in agreement with the recent meta-analysis in colorectal cancer (Rahbari et al., 2010), breast cancer (Zhao et al., 2011), melanoma (Mocellin et al., 2006) and prostate cancer (Wang et al., 2011). As referred in Hayes (Hayes et al., 2001), a prognostic factor with RR > 2 is considered as useful practical value. Fortunately, all the pooled HRs were above 2.0 in our study. These results indicated that detected CTCs appearance in peripheral blood of lung cancer patients could predict their prognosis practically.

Comparing the results yielded in studies using samples collected before and after treatments, we could find out that the HRs for survival outcome were significantly higher in post-treatment group (4.19 [OS] and 4.97 [PFS]) than those pro-treatment (2.61 [OS] and 2.01 [PFS]). These results indicated that the post-treatment detection of CTCs was more persuasive than that at baseline, which recommended us detecting CTCs after treatment rather than before to predict patients' survival. Furthermore, four studies (Yamashita et al., 2000; Chen et al., 2007; Krebs et al., 2011; Hou et al., 2012) examined CTCs on the respectively identical populations both before and after treatment CTCs support our finding with higher HRs after treatment.

In SCLC subgroup analysis using random mode, we noticed that 2 included studies had significant results (1.43 [1.09, 1.89] and 3.56 [2.10, 6.04]), but they reached a conclusion of negative (HR 2.19 [0.90, 5.34]). This could be explained by an HR compensation on confidence interval on the smaller side when a random model was applied, which leads to an overlap with 1 (Hedges & Vevea, 1998). This puzzle could be solved when much more studies were conducted to confirm clinical value of the CTCs tested in SCLC. For there were not always sufficient subgroup studies, when grouping studies by different detecting methods, the HRs could be only obtained in OS prediction by pro- and post-treatment CTCs detected by RT-PCR and PFS prediction by post-treatment CTCs detected by CellSearch. Thus, we could not reach in a conclusion which method was more accurate in detection of CTCs of prognostic value. However, Hofman's study (Hofman et al., 2011) showed that HR value was higher using CellSearch than that of ISET in clinical research consisted of 208 patients. Future study could pay attention to this question to optimize the detection method.

In the correlation study of CTCs appearance with patients' clinical characteristics, the ORs revealed that pro-treatment CTCs appearance was correlated with TNM staging, lymph node status and distant metastasis. No significant or weak correlation had been observed with the depth of invasion, sexuality, histological differentiation and smoking status. Experimental studies had proven CTCs was correlated to distant metastasis former (Kim et al., 2009). Hou JM and colleagues summarized that CTCs is a factor that promotes metastasis as well (Hou et al., 2011). Coupled with a gradually increase OR of TNM staging through treatment, the detection of post-treatment CTCs had a potential ability in earlier, less invasive and more reliable discovery of disease progression in the follow up. Similarly, Tanaka et al. (2009) demonstrated that CTCs as a diagnostic marker in lung cancer,showed good sensitivity and specificity in distinguishing clinical stage. Lymph node status and happened distant metastasis were associated with pro-treatment CTCs but not during or after. This might be explained by that these clinical factors were obtained before treatment, whereas CTCs detection during or after treatment might be affected by the treatment.

Besides, the limitation still existed in the present detection method. As referred in Pantel K's study (Pantel and Alix-Panabieres, 2010), CTCs positive rate detected by identification of EpCAM in patients with happened distant metastasis were lower than that in non-metastasis patients. They hypothesized that it was the epithelial-mesenchymal transition (EMT) that led to a decline in the EpCAM expression. Thus, CTCs of an EMT phenotype could be missed by current detection methods.Intriguingly, we found that the positive rate of CTCs after treatment was smaller than that before treatment in all the studies referred (Yamashita et al., 2000; Chen et al., 2007; Krebs et al., 2011; Hou et al., 2012). This might be explained by platelet's role in promoting EMT with the influence of surgery which leads to local platelet accumulation (Labelle et al., 2011).

Significant heterogeneity was found in the meta-analysis of the prognostic role of CTCs collected before treatment (69%, 0.001).When we divided studies into subgroups of NSCLC and SCLC, the heterogeneity could not be eliminated (53%, 0.05). To exclude technique biases, subgroup analyses were performed for the most frequently used methods, RT-PCR, CellSearch and ISET (Pantel and Alix-Panabieres, 2010). This suggested that the techniques were unlikely to be a source of biases. Therefore, histological classification and detection methods were not major sources of heterogeneity. This could be explained by different cut-off values and different composition of NSCLC in each study. The meta-analysis performed in subgroup of post-treatment had revealed a fine homogeneity in both OS and PFS.

A potential source of biases was related to the HRs and 95% CI extrapolation. Once the key information was not provided by the authors, we calculated them from the data available in the article. Once there was no sufficient information for calculation, we extracted them from the survival curves. Multivariate survival analysis reported in the article was included in the our analysis; if these data were not available, we extracted univariate data instead. These results should be confirmed by an adequately designed prospective study. Furthermore, there was also some tiny bias derived from the software

we used, designed by Matthew Sydes and Jayne Tierney. This was because this software retained only percentile when calculated the logHR and SE. However, when we verified the data again by STATA 11.0, only minimal bias was observed. The publication biases were additional problem for the meta-analysis. Fortunately, the Begg's test showed no significant publication bias ($p > 0.05$).

In conclusion, the meta-analysis suggested that the both pro- and post-treatment CTCs appearance in peripheral blood were associated with poor prognosis in lung cancer patients. It was of more significance using CTCs to predict survival after treatment. In addition, the detection of post-treatment CTCs had a potential ability in earlier, less invasive and more reliable discovery of disease progression in the follow up. These results should be confirmed by adequately multi-center designed prospective studies in future.

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