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

Xue-Lei Ma1&, Zhi-Lan Xiao1&, Lei Liu1&, Xiao-Xiao Liu1, Wen Nie1, Ping Li1, Nian-Yong Chen1, Yu-Quan Wei1

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

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 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 were excluded based on any of the following criteria, (i) were review articles or letters (ii) analyzed in various tumors but with no specific results of lung cancer, (iii) lacked key information for analysis with methods developed by Parmar et al. (1998), Williamson et al. (2002), and Tierney et al. (2007).
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 and 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 and others. Disagreements were resolved by consensus.

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 stage, the depth of invasion, lymph node status, distant appearance and the clinical variables including TNM stage, histological classification, methods to detect CTCs, positive CTCs definition, the attitude conclusion and other clinical characteristics.

Table 1. Baseline Characteristics of Included Studies

| Author   | year | case | control size | age | male% | III & IV% | histologic cell type | 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 | chemo. and radio. | 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   | 68           | median 66 | 44.3 |    | —                     | chemo.     | median 7 before and after TM |              |
| Liu L    | 2008 | 134  | 186          | median 76 | —   | 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 | chemo. and radio. | median 12 before and after TM |              |
| Yamashita J | 2000   | 32   | —            | median 63 | 31.2 | 6.2 | ADC 29 SQC 2 others 1 | chemo.     | median 12 before and after TM |              |
| Yamashita J | 2002   | 103  | Unknown      | median 68 | 73.8 | 26.2 | ADC 66 SQC 37 | —                     | —              |              |
| Kuranu Y | 1999 | 103  | 32           | median 68 | 73.8 | 26.3 | ADC 66 SQC 37 | —                     | —              |              |
| Yie SM   | 2009 | 143  | 172          | median 57 | 73.4 | 71.3 | ADC 87 SQC 56 | —                     | —              |              |
| Okumura Y | 2009   | 30   | —            | median 65 | 70  | 23.3 | ADC 18 SQC 7 SMC 1 others 4 | chemo. and radio. | median 13 before TM |              |
| Hofman V | 2010 | 210  | 40           | median 63 | 72.3 | 37.6 | ADC 120 SQC 57 others 33 | chemo. and radio. | median 15 before TM |              |
| Hofman V | 2010 | 250  | 59           | median 65 | 68.9 | 27.6 | ADC 150 SQC 57 others 33 | chemo. and radio. | median 13 before TM |              |
| Krebs MG | 2011 | 101  | —            | median 67 | 53.4 | 100 | ADC 31 SQC 32 others 63 | chemo. And/or radio. | median 5.4 before and after TM |              |
| Sawabata N | 2007   | 9    | 4            | median 58 | 100  | 0   | ADC 6 SQC 3 | chemo. and radio. | median 14 before TM |              |
| Yoon SO  | 2010 | 79   | —            | median 66 | 60.8 | —   | ADC 45 SQC 27 others 7 | chemo.     | median 60 before and after TM |              |
| Funaki S  | 2011 | 94   | —            | median 68 | 59.6 | 6.4 | ADC 71 SQC 14 others 9 | chemo. and radio. | median 6 during TM |              |
| Castaldo G | 1997 | 24   | unknown      | median 62 | 87.5 | 91.7 | ADC 9 SQC 12 SMC 3 | —                     | —              |              |
| Guo Y    | 2009 | 83   | 30           | median 55.9 | 60.2 | 63.9 | ADC 47 SQC 17 SMC 15 others 7 | chemo. and radio. | median 3.8 before and after TM |              |
| Peck K   | 1998 | 86   | 62           | median 66 | 63.7 | 70.9 | ADC 47 SQC 17 SMC 15 others 7 | chemo. and radio. | median 10 before TM |              |
| Sheu CD  | 2006 | 100  | 147          | median 64 | 36.1 | 58  | ADC 72 SQC 28 | chemo. and radio. | —              | —              |
| Wendel M | 2012 | 78   | —            | median 59 | 53.8 | 83.3 | ADC 44 SQC 20 others 14 | chemo.     | —              | —              |
| Wu C     | 2009 | 47   | 31           | median 66 | 93.6 | 93.6 | ADC 27 SQC 7 SMC 13 | chemo.     | —              | —              |
| Fastacre F | 2011 | 20   | —            | mean 55.8 | 55  | 100 | ADC 16 others 4 | chemo. and radio. | —              | —              |
| Tanaka F | 2011 | 25   | 25           | —                     | 25.6 | 25.6 | ADC 85 SQC 22 SMC others 9 | chemo.     | —              | —              |
| Huang TH | 2007 | 51   | 40           | median 58.6 | 52.9 | 25.5 | ADC 21 SQC 30 | chemo. and radio. | —              | —              |
| Devriende LA | 2012 | 46   | 46           | mean 58 | 63  | 100 | ADC 30 SQC 8 others 8 | chemo. Or biotherapy | —              | —              |
| Hayes DC | 2006 | 49   | 25           | mean 61.8 | 49  | —   | ADC 11 SQC 8 SMC 10 others 20 | chemo. Or biotherapy | —              | —              |
| Li J     | 2005 | 52   | 31-78        | median 76 | 67.3 | 30.8 | ADC 30 SQC 22 | —                     | —              | —              |
| Sienel W | 2003 | 62   | —            | median 55.9 | 60.2 | 63.9 | ADC 47 SQC 17 SMC 15 others 7 | chemo. and radio. | median 10 before TM |              |

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

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

1138 Asian Pacific Journal of Cancer Prevention, Vol 13, 2012
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., 2000; Sher et al., 2005; Chen et al., 2007; Sawabata et al., 2007; Huang et al., 2009; Hofman et al., 2011; Devriese et al., 2012; Hofman et al., 2012; Wendel et al., 2012) containing only patients’ clinical characteristics and OS. 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; 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.

# Table 2. Overview of the Study Design Variables

| Author | Sampling site | Volume | Methods | Markers | Positive Definition | Outcomes | Multivariate Analysis |
|--------|---------------|--------|---------|---------|---------------------|----------|----------------------|
| Chen TF | PB/8ml        | RT-PCR | CK19 mRNA | ——   | OS&PFS       | yes     | negative             |
| Hofman V | PB/10ml      | ISET   | ——   | ——   | OS&PFS       | yes     | positive             |
| Hou JM  | PB/7.5ml      | CellSearch | EpCAM, keratin 4, 5, 6, 8, 10, 13, 18, DAPI, CD56 | all markers+ and CD45- | OS&PFS | yes     | positive             |
| OS&PFS | PB/7.5ml      | CellSearch | EpCAM, CK8, 18, 19, DAPI | all markers+ and CD45- | OS&PFS | yes     | positive             |
| Hou JM  | PB/7.5ml      | CellSearch and ISET | EpCAM, CK8, 18, 19, DAPI | all markers+ and CD45- | OS&PFS | yes     | positive             |
| Liu L   | PB/5ml        | RT-PCR | TSA-9, Keratin 19, Pro-proGRP | 1.2 or 3 markers | OS | Yes     | positive             |
| Nieva J | PB/1ml        | IF     | CK 1.4, 8.10,13,18, and DAPI | all markers+ and CD45- | OS | No      | positive             |
| Sher YP | PB/3-4ml      | RT-PCR | keratin 19, Ubiquitin thiolesterase C | Lec=Lec 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             |
| Kuruus Y | PB/RT-PCR | CEA mRNA | ——   | OS   | Yes     | positive             |
| Yie SM  | PB/RT-PCR     | survivin | ——   | OS   | Yes     | positive             |
| Okumura Y | PB/7.5ml      | CellSearch | EP-DAPCAM, DAPI, CK | morphology, all markers+ and CD45- | OS | No      | negative             |
| Hofman V | PB/7ml       | ISET or CellSearch | EpCAM, DAPI, CK2, 5, 6, 8, 10, 19, vimentin | morphology, all markers+ and CD45- | PFS | Yes     | positive             |
| Hofman V | PB/10ml      | ISET   | ——   | ——   | PFS      | Yes     | positive             |
| 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- | OS&PFS | No      | positive             |
| Yoon SO | PB/RT-PCR     | TTF-1, CK19 mRNA | any target | OS | Yes     | positive             |
| Funaki S | PV/1ml        | ICC    | ——   | anyform (singular or cluster) | —— | ——      | ——             |
| Castaldo G | PB/RT-PCR  | CEA mRNA | ——   | —— | ——      | ——           |
| Guo Y   | PB/3ml        | RT-PCR | CK20, CEA mRNA | 1.2 or 3 visible bands by naked eye | PFS | ——      | ——             |
| Peck K  | PB/3-5ml      | RT-PCR | CK19 mRNA | any target | —— | ——      | ——             |
| Sheu CC | PB/8ml        | RT-PCR | CEA mRNA | ——   | ——      | ——             |
| Wendel M | PB/CellSearch | EpCAM, CK8, 18, 19, DAPI | morphology, all markers+ and CD45- | —— | ——      | ——             |
| Wu C    | PB/7.5ml      | RT-PCR | CEA mRNA | ——   | ——      | ——             |
| Farace F | PB/17.5ml     | CellSearch | 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/RT-PCR     | CK19 mRNA, LUNX mRNA | any target | —— | ——      | ——             |
| Devriese LA | PB/RT-PCR  | EpCAM, CK7, CK19, EGP (epithelial glycoprotein, FN1) | any target, quadratic discriminant analysis | —— | ——      | ——             |
| Hayes DC | PB/5-8nl      | antibody | coxal and RT-PCR | —— | ——      | ——             |
| Li J    | PB/RT-PCR     | CEA mRNA | ——   | —— | ——      | ——             |
| Sienel W | PV/10ml      | ICC    | CK8, 18, 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.
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) | I², p | Study n. | Patient n. | Model | HR (95% CI) | I², 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 | Random | 2.69 [1.62, 4.48] | --- |
| RT-PCR | 5 | 390 | Random | 3.04 [1.71, 5.42] | 65%, 0.02 | 1 | 67 | Fixed | 1.17 [0.68, 2.03] | --- |
| ISET | 1 | 208 | --- | 2.10 [1.34, 3.29] | --- | 1 | 208 | Fixed | 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 stratification by histological classification (NSCLC or SCLC) and methods 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.
more likely to show up in peripheral blood in III/IV lung cancer patients. As shown in Table 4, CTCs were characteristic and CTCs appearance in peripheral blood was weak or insignificant (Table 3). The only significant result of meta-analysis was HR for PFS predicted by samples collected before treatment processed by CellSearch (HR = 3.17 [2.04, 4.97], p = 0.0001) (Figure 3). 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; Okamura 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; Shen et al., 2006; Chen et al., 2007; Liu et al., 2008; Okumura et al., 2009; Yamashita et al., 2000; Sher et al., 2005; Sheu 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 (Table 4). When we stratified the studies by sexuality, smoking status or histological differentiation (adenocarcinoma versus squamous carcinomma), 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 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 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 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 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

---

**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 (I², p) | Conclusion |
|---------------|-------------------------------------------|----------|------------|-------|------------|---------|----------------------|------------|
| Before        | 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%, <0.0001         | 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        | TNM stage (III/IV vs. I/II)               | 4        | 208        | Fixed | 2.79 [1.13, 6.85] | 0.03    | 47%, 0.13            | positive   |
| treatment     | 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%, 0.76             | negative   |
|               | Histological differentiation (ADC vs. SQC) | 5        | 218        | Fixed | 0.47 [0.24, 0.95] | 0.04    | 13%, 0.33            | positive   |
| After         | TNM stage (III/IV vs. I/II)               | 4        | 250        | Fixed | 4.86 [2.29, 10.29] | 0.0001  | 0%, 0.53             | positive   |
| treatment     | 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.
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 ORs 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.

Acknowledgements

The author(s) declare that they have no competing interests.

References

Ashworth TR (1869). A case of cancer in which cells similar to those in the tumours were seen in the blood after death. 14,146-149.

Begg CB (1994). Publication bias. 25, 299-409.

Castaldo G, Tomaiuolo R, Sanduzzi A, et al (1997). Lung cancer metastatic cells detected in blood by reverse transcriptase-polymerase chain reaction and dot-blot analysis. J Clin Oncol, 15, 3388-93.

Chen TF, Jiang GL, Fu XL, et al (2007). CK19 mRNA expression measured by reverse-transcription polymerase chain reaction (RT-PCR) in the peripheral blood of patients with non-small cell lung cancer treated by chemo-radiation: an independent prognostic factor. Lung Cancer, 56, 105-14.

Cohen SJ, Punt CJ, Iannotti N, et al (2008). Relationship of circulating tumour cells to tumour response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. J Clin Oncol, 26, 3213-21.

Cristofanilli M (2006). Circulating tumor cells, disease progression, and survival in metastatic breast cancer. Semin Oncol, 33, S9-14.

Devriese LA, Bosma AJ, de Heuvel MM, v Heemskeren W, Voest EE, Schellens JH (2012). Circulating tumor cell detection in advanced non-small cell lung cancer patients by multi-marker QPCR analysis. Lung Cancer, 75, 242-7.

Farace F, Massard C, Vimond N, et al (2011). A direct comparison of CellSearch and ISET for circulating tumour-cell detection in patients with metastatic carcinomas. Br J Cancer, 105, 847-53.

Guo Y, Wang J, Huang P (2009). [Clinical Significance of CK20, CK19, CEA mRNAs in Peripheral Blood from Lung Cancer Patients.]. Zhongguo Fei Ai Za Zhi, 12, 1013-7.

Hayes DC, Secrist H, Bangur CS, et al (2006). Multigene real-time PCR detection of circulating tumor cells in peripheral blood of lung cancer patients. Anticancer Res, 26, 1567-75.

Hayes DF, Isaacs C, Stearns V (2001). Prognostic factors in breast cancer: current and new predictors of metastasis. J Mammary Gland Biol Neoplasia, 6, 375-92.

Hedges LV, Vevea JL (1998). Fixed-and random-effects models in meta-analysis. Psychol Methods, 3, 486.

Helo P, Cronin AM, Danila DC, et al (2009). Circulating prostate tumor cells detected by reverse transcription-PCR in men with localized or castration-refractory prostate cancer: concordance with CellSearch assay and association with bone metastases and with survival. Clin Chem, 55, 765-73.

Higgins JP, Thompson SG, Deeks JJ, Altman DG (2003). Measuring inconsistency in meta-analyses. BMJ, 327, 557-60.

Hofman V, Bonnetaud C, Ilie MI, et al (2011). Preoperative circulating tumor cell detection using the isolation by size of epithelial tumour cell method for patients with lung cancer is a new prognostic biomarker. Clin Cancer Res, 17, 827-35.

Hofman V, Ilie MI, Long E, et al (2011). Detection of circulating tumor cells as a prognostic factor in patients undergoing radical surgery for non-small-cell lung carcinoma: comparison of the efficacy of the CellSearch Assay and the isolation by size of epithelial tumor cell method. Int J Cancer, 129, 1651-60.

Hofman V, Long E, Ilie M, et al (2012). Morphological analysis of circulating tumour cells in patients undergoing surgery for non-small cell lung carcinoma using the isolation by size of epithelial tumour cell (ISET) method. Cytopathology, 23, 30-8.

Hofman V, Long E, Ilie M, et al (2012). Morphological analysis of circulating tumour cells in patients undergoing surgery for non-small cell lung carcinoma using the isolation by size of epithelial tumour cell (ISET) method. Cytopathology, 23, 30-8.

Hou JM, Greystoke A, Lancashire L, et al (2009). Evaluation of circulating tumour cells and serological cell death biomarkers in small cell lung cancer patients undergoing chemotherapy. Am J Pathol, 175, 808-16.

Hou JM, Krebs M, Ward T, et al (2011). Circulating tumor cells as a window on metastasis biology in lung cancer. Am J Pathol, 178, 989-96.

Hou JM, Krebs MG, Lancashire L., et al (2012). Clinical significance and molecular characteristics of circulating tumor cells and circulating tumor microemboli in patients with small-cell lung cancer. J Clin Oncol, 30, 525-32.

Huang TH, Wang Z, Li Q, Li FR, Qi H, Zhou SX (2007). [Clinical significance of enrichment and detection of circulating tumor cells in NSCLC patients with immunomagnetic beads]. Zhonghua Zhong Liu Za Zhi, 29, 676-80.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. CA Cancer J Clin, 61, 69-90.

Kim MY, Oskarsson T, Acharyya S, et al (2009). Tumor self-seeding by circulating cancer cells. Cell, 139, 1315-26.

Krebs MG, Sloane R, Priest L, et al (2011). Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol, 29, 1556-63.

Krebs MG, Sloane R, Priest L, et al (2011). Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. J Clin Oncol, 29, 1556-63.

Kubushek B, Passlick B, Izbicki JR, Thetter O, PanteI K (1999). Disseminated tumor cells in lymph nodes as a determinant for survival in surgically resected non-small-cell lung cancer. J Clin Oncol, 17,19-24.

Kurusu Y, Yamashita J, Ogawa M (1999). Detection of circulating tumor cells by reverse transcriptase-polymerase chain reaction in patients with resectable non-small-cell lung cancer. Surgery, 126, 820-6.

Kurusu Y, Yamashita J, Ogawa M (1999). Detection of circulating tumor cells by reverse transcriptase-polymerase chain reaction in patients with resectable non-small-cell lung cancer. Surgery, 126, 820-6.

Labelle M, Begum S, Hynes RO (2011). Direct signaling between platelets and cancer cells induces an epithelial-
mesenchymal-like transition and promotes metastasis. Cancer Cell, 20, 576-90.

Li J, Sun YE, Sheng QM, Yan LD, Lu XC (2005). [Detection of blood dissemination during the operation of lung cancer and its significance]. Zhonghua Wai Ke Za Zhi, 43, 76-9.

Liu L, Liao GQ, He P, et al (2008). Detection of circulating cancer cells in lung cancer patients with a panel of marker genes. Biochem Biophys Res Commun, 372, 756-60.

Mocellin S, Hoang D, Ambrosi A, Nitti D, Rossi CR (2006). The prognostic value of circulating tumor cells in patients with melanoma: a systematic review and meta-analysis. Clin Cancer Res, 12, 4605-13.

Nieva J, Wendel M, Luttgen MS, et al (2012). High-definition imaging of circulating tumor cells and associated cellular events in non-small cell lung cancer patients: a longitudinal analysis. Phys Biol, 9, 016004.

Okumura Y, Tanaka F, Yoneda K, et al (2009). Circulating tumor cells in pulmonary venous blood of primary lung cancer patients. Ann Thorac Surg, 87, 1669-75.

Pantel K, Alix-Panabieres C (2010). Circulating tumour cells in cancer patients: challenges and perspectives. Trends Mol Med, 16, 398-406.

Parmar MK, Torri V, Stewart L (1998). Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med, 17, 2815-34.

Peck K, Sher YP, Shih JY, et al (1998). Detection and quantitation of circulating cancer cells in the peripheral blood of lung cancer patients. Cancer Res, 58, 2761-5.

Rahbari NN, Aigner M, Thorlund K, et al (2010). Meta-analysis shows that detection of circulating tumor cells indicates poor prognosis in patients with colorectal cancer. Gastroenterology, 138, 1714-26.

Sawabata N, Okumura M, Utsumi T, et al (2007). Circulating tumor cells in peripheral blood caused by surgical manipulation of non-small-cell lung cancer: pilot study using an immunocytoLOGY method. Gen Thorac Cardiovasc Surg, 55, 189-92.

Sher YP, Shih JY, Yang PC, et al (2005). Prognosis of non-small cell lung cancer patients by detecting circulating cancer cells in the peripheral blood with multiple marker genes. Clin Cancer Res, 11, 173-9.

Sheu CC, Yu YP, Tsai JR, et al (2006). Development of a membrane array-based multimarker assay for detection of circulating cancer cells in patients with non-small cell lung cancer. Int J Cancer, 119, 1419-26.

Tanaka F, Yoneda K, Kondo N, et al (2009). Circulating tumor cell as a diagnostic marker in primary lung cancer. Clin Cancer Res, 15, 6980-6.

Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. Trials, 8, 16.

Wang FB, Yang XQ, Yang S, et al (2011). A Higher Number of Circulating Tumor Cells (CTC) in Peripheral Blood Indicates Poor Prognosis in Prostate Cancer Patients - A Meta-analysis. Asian Pac J Cancer Prev, 12, 2629-35.

Wendel M, Bazhenova L, Boshuizen R, et al (2012). Fluid biopsy for circulating tumor cell identification in patients with early-and late-stage non-small cell lung cancer: a glimpse into lung cancer biology. Phys Biol, 9, 016005.

Williamson PR, Smith CT, Hutton JL, Marson AG (2002). Aggregate data meta-analysis with time-to-event outcomes. Stat Med, 21, 3337-51.

Wu C, Hao H, Li L, et al (2009). Preliminary investigation of the clinical significance of detecting circulating tumor cells enriched from lung cancer patients. J Thorac Oncol, 4, 30-6.

Yamashita J, Matsuoka A, Kurusu Y, et al (2002). Preoperative evidence of circulating tumor cells by means of reverse transcriptase-polymerase chain reaction for carcinoembryonic antigen messenger RNA is an independent predictor of survival in non-small cell lung cancer: a prospective study. J Thorac Cardiovasc Surg, 124, 299-305.

Yamashita JI, Kurusu Y, Fujino N, Saisyoji T, Ogawa M (2000). Detection of circulating tumor cells in patients with non-small cell lung cancer undergoing lobectomy by video-assisted thoracic surgery: a potential hazard for intraoperative hematogenous tumor cell dissemination. J Thorac Cardiovasc Surg, 119, 899-905.

Yoon SO, Kim YT, Jung KC, et al (2011). TTF-1 mRNA-positive circulating tumor cells in the peripheral blood predict poor prognosis in surgically resected non-small cell lung cancer patients. Lung Cancer, 71, 209-16.

Zhao S, Liu Y, Zhang Q, et al (2011). The prognostic role of circulating tumor cells (CTCs) detected by RT-PCR in breast cancer: a meta-analysis of published literature. Breast Cancer Res Treat, 130, 809-16.