RESEARCH ARTICLE

Prognostic Significance of Circulating Tumor Cells in Small-Cell Lung Cancer Patients: a Meta-analysis

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Abstract

Circulating tumor cells (CTCs) are believed to be particularly important and a reliable marker of malignancy. However, the prognostic significance of CTCs detected in patients with small cell lung cancer (SCLC) is still unclear. We therefore aimed to assess the prognostic relevance of CTCs using a meta-analysis. We searched PubMed for relevant studies and statistical analyses were conducted to calculate the hazard ratio (HR) and 95% confidence intervals (CIs) using fixed or random-effect models according to the heterogeneity of included studies. A total of 7 papers covering 440 SCLC patients were combined in the final analysis. The meta-analysis revealed that CTCs were significantly associated with shorter overall survival (HR=1.9; 95%CI: 1.19-3.04; Z=2.67; P<0.0001) and progression-free survival (HR=2.6; 95%CI: 1.9-3.54; Z=6.04; P<0.0001). The results thus suggest that the presence of CTCs indicates a poor prognosis in patients with SCLC. Further well-designed prospective studies are required to explore the clinical applications of CTCs in SCLC.

Keywords: Circulating tumor cells - small-cell lung cancer - overall survival - progress-free survival-meta-analysis

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Introduction

Lung cancer is the most common cause of cancer-related death worldwide. In the United States, lung cancer is the second most common cancer in men and women, and it is the leading cause of cancer-related death (Horner et al., 2010). Small cell lung cancer (SCLC) accounts for about 13% of all lung cancer cases with about 29,000 new cases diagnosed in the USA (American Cancer Society, 2009). And in 2013, an estimated 31, 000 new cases of SCLC will occur in the United State (Siegel et al., 2013). SCLC patients are staged according to a two-stage system, which was developed by the Veterans Administration Lung Cancer Study Group, limited-stage disease and extensive-stage disease. In total, 60~70% of SCLC patients present with extensive-stage disease (Govindan et al., 2006). SCLC is highly sensitive to initial chemotherapy and radiotherapy; however, most patients eventually die of recurrent and metastatic disease. Prognosis in SCLC is dismal. Median survival without treatment has been reported as 2-4 months (Kato et al., 1969). Studies have shown that approximately <25% of patients with limited-stage disease and only 1-2% of patients with extensive-stage disease survive for five years (Turrisi et al., 1999; Takada et al., 2002). This is predominantly attributable to the rapid doubling time, high growth fraction, and early development of widespread metastases. Although some prevalent clinical characteristics (gender, age, PS, weight, number of metastatic site, LDH, creatinine level ect.) (Albain et al., 1990; Yip et al., 2000; Foster et al., 2009) are currently used as the prognostic factors in SCLC patient follow-up, the individual value of these tools remains poor. And to date, no histopathological, molecular or genetic prognostic tests are available. Therefore, sensitive prognostic and predictive markers is urgently needed in SCLC.

In SCLC patients, detecting circulating tumor cells (CTCs) may show clinical benefits in diagnosis and treatment. CTCs are tumor cells that are shed from the primary tumor, flowing through the bloodstream and circulate throughout the body. Detection of the presence of CTCs and their characteristics may be used to estimate the risk of metastatic relapse, facilitate stratification of patients to adjuvant therapy, select therapeutic regimens and monitor the efficacy of systemic anticancer therapy (Rolle et al., 2005; Alix-Panabieres et al., 2008; Tanaka et al., 2009). In the recent years, various new CTCs assays are developed and employed for their detection, including immunocytochemistry (ICC), reverse-transcriptase polymerase chain reaction (RT-PCR), and the CellSearch System. Recently, several meta-analyses or studies in several cancer types have demonstrated the prognostic significance of CTCs (Ma et al., 2012; Huang et al., 2013; Turker et al., 2013; Ma et al., 2014). However, in SCLC patients, there still remains controversial regarding the presence of CTCs and the prognosis in SCLC patients. Some studies have reported that CTCs detection in the peripheral blood is significantly associated with shorter

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survival (Hou et al., 2012; Naito et al., 2012). In contrast, other studies have failed to show such an association between the presence of CTCs and a poorer prognosis (Hiltermann et al., 2012; Normanno et al., 2014). Therefore, we conducted a meta-analysis to evaluate the prognostic value of baseline CTCs level before treatment in patients with SCLC.

Materials and Methods

Inclusion and exclusion criteria

All eligible papers focusing on the association between CTCs level and survival time were collected to perform this meta-analysis. This meta-analysis includes articles from studies meeting the following inclusion criteria: (1) the clinical research on baseline CTCs level before treatment in SCLC patients; (2) the research subjects are SCLC patients without any restriction on age or race; and (3) outcome indicators include overall survival (OS) and progress-free survival (PFS). The exclusion criteria are the following: (1) no clear follow-up and survival analysis; (2) no exact data provided or the data can`t be calculated for prognostic evaluation of patients with SCLC.

Literature collection and screening

Primary research articles relating to the CTCs level in patients with SCLC were identified by electronic search of the PubMed on July 15, 2014. We conducted an independent review of citations from PubMed. Keywords were circulating tumor cell (s), small cell lung carcinoma. The search strategy used text terms such as circulating cancer cells, CTCs, blood epithelial cell, and small cell lung carcinoma to identify relevant information as well. Furthermore, relevant papers were identified from cited references of retrieved articles and review articles by a manual search, to ensure that no articles were overlooked.

Data extraction

We recorded the following information from each eligible paper: author's name, patient's country, publication year, and type of study, number of patients, tumor stage, methods of CTCs detection, detection rate, cutoff value of CTCs, clinical outcome and analysis method. Data for multivariate survival analysis reported in the article were included in the present meta-analysis; if these data were not available, then univariate analysis data were included. According to the study objective, we performed an analysis determined whether CTCs level is associated with OS and PFS.

Data analysis

OS and PFS in relation to baseline CTCs level before treatment in SCLC were estimated by the Hazard ratio (HR). HR with its 95% confidence interval (95%CI) was used to combine the data. When described in original articles, we obtained these values directly. Otherwise, HR and 95%CI were calculated as described previously (Parmar et al., 1998). The pooled HR with 95%CI for survival were calculated by fixed or random-effects models. Heterogeneity between studies was evaluated with the Cochran's Q test (chisquared test; χ^2) and by quantifying the inconsistency (I^2). When P was less than 0.05, a random-effects model estimate was used. Otherwise, a fixed-effects model estimate was presented. Robustness of our meta-analysis was verified by one-way sensitivity analysis. Therefore, every single study was deleted, and the pooled HR and 95%CI as well as the tests for heterogeneity of the remaining studies were calculated. Publication bias was assessed using the funnel plot and the Begg's test, and for the possible publication bias, we used trim and fill method to evaluate the influence to the result. All statistical analyses were conducted using the stata12.0. P values less than 0.05 were considered statistically significant.

Results

Literature screening

The systematic literature search yielded a total of 313 literatures (Figure 1). After screening of titles and abstracts, 305 articles were excluded because of irrelevant publications, review articles, duplicates, and overlapped studies. Upon full text review of the remaining 8 articles, a total of one article had to be excluded because the

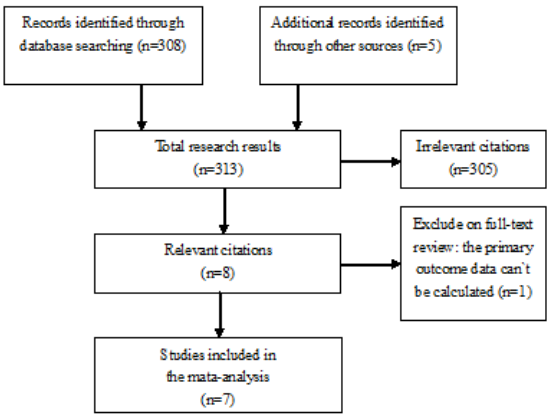

Figure 1. Flow Chart of Selecting the Eligible Publications

Table 1. Main characteristics of the eligible studies

Study	Patients` country	Year	Tumor stage (n)	Type of study	Number of patients	Technique	Cutoff	Positive (%)	Outcome	Analysis
Hou	United Kingdom	2009	LS (35)/ES (53)	prospective	88	CellSearch	300CTCs/7.5ml	86%	OS	univariate
Hou	United Kingdom	2012	LS (31)/ES (66)	prospective	97	CellSearch	50CTCs/ 7.5ml	85%	OS/PFS	multivariate
Naito	Japan	2012	LS (27)/ES (24)	prospective	51	CellSearch	8CTCS/7.5ml	68.60%	OS	multivariate
Hiltermann	Netherlands	2012	LS (21)/ES (38)	prospective	59	CellSearch	2CTCs/7.5ml	84.70%	OS/PFS	multivariate
Shi	China	2013	LS (27)/ES (28)	prospective	55	Rt-PCR	3.8CK-19 positive CTCs/6ml	78.20%	OS/PFS	multivariate
Normanno	Italy	2014	ES	prospective	60	CellSearch	282CTCS/7.5ml	90%	OS	multivariate
Igawa	Japan	2014	LS (8)/ES (22)	prospective	30	OBP-401	2CTCS/7.5ml	96%	OS	multivariate

Abbreviations: LS=limited-stage; ES=extensive-stage; CTCs=circulating tumor cells; OS=overall survival; PFS=progress-free survival; RT-PCR=Reverse-transcriptase polymerase chain reaction

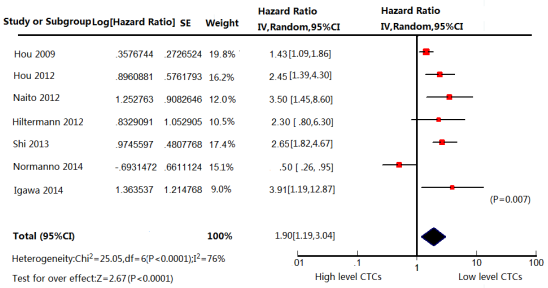

Figure 2. Forest Plot of Overall Survival of SCLC and CTCs Levels, the Horizontal Lines Correspond to the Study-Specific HR and 95%CI, Respectively. The Area of the Squares Reflects the Study Specific Weight. The Diamond Represents the Pooled Results of HR and 95%CI. In this Analysis, Random Effect Model was Used

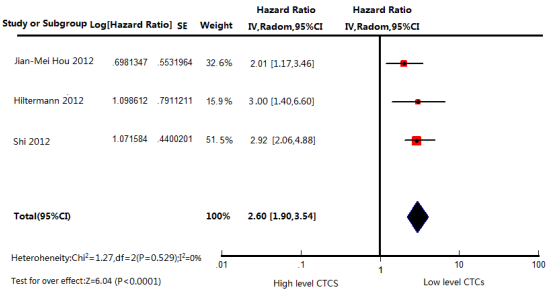

Figure 3. Forest Plot of Progress-Free Survival of SCLC and CTCs Levels. In this analysis, Fixed-Effects Model was Used

primary outcome data can`t be calculated (Oshita et al., 2003). Finally, 7 publications met the criteria for analysis, comprising 440 SCLC patients (Hou et al., 2009; 2012; Naito et al., 2012; Hiltermann et al., 2012; Shi et al., 2013; Igawa et al., 2014; Normanno et al., 2014). The sample size per study ranged from 30 to 97 patients, and studies were published between 2009 and 2014. The main characteristics of the included studies are summarized in Table 1.

Methodology assessment of CTCs level detection

There were three types of methods for assessment of CTCs status in blood specimens: CellSearch, RT-PCR and OBP-401 system. Five studies used CellSearch techniques to evaluate CTCs status, one study used RT-PCR techniques, and 1 study used OBP-401 system. In these 7 studies, the detection rate for CTCs with an average of more than about 68.6%.

The characteristics and treatment of these studies

In these 7 studies, there were 5 studies that reported using chemotherapy and 2 studies reported that the majority of patients were treated with chemotherapy and some patients with radiotherapy and chemotherapy combination of programs.

CTCs level and overall survival

All these 7 studies can be extracted for the HR values and for their 95%CI directly used for the evaluation of the CTCs levels and the OS. Because the heterogeneity across the studies was less than 0.05 (x^2=25.05; I^2=76%), the estimated pooled HR for studies was calculated

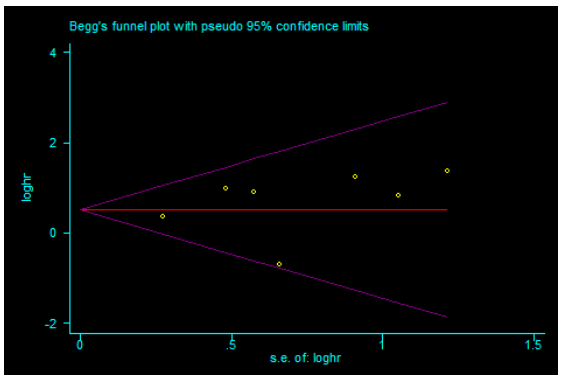

Figure 4. Begg's Funnel Plot for Publication Bias for Studies Reporting OS with Unadjusted HR. Each point represents a separate study for the indicated association. Log OR represents natural logarithm of or. vertical line represents the mean effects size

using a random-effects model. The combined HR of all studies reporting the effect of presence of CTCs on OS by a random-effects model was 1.90 (95%CI 1.19-3.04, *P*=0.007), suggesting that higher baseline CTCs level was associated with a poor OS in SCLC patients (Figure 2). To further evaluate the robustness of our results, we performed one-way sensitivity analysis indicating that none of the included studies had influence on our pooled HR or provoked heterogeneity (data not shown).

CTCs level and progress-free survival

Among these 7 studies, three studies can be extracted for the HR values and for their 95%CI directly used for the evaluation of the value of CTCs levels and the PFS. There was better homogeneity between each study (*P*=0.529; x^2=1.27; I^2=0%). The combined HR for PFS of the patients with high baseline CTCs levels by a fix-effects model was 2.60 (95%CI 1.90-3.54, *P*<0.0001), suggesting that high baseline CTCs levels was associated with a poor PFS in SCLC patients (Figure 3).

Publication bias

We performed the funnel plots and Begg's test to assess the publication bias. The funnel plot (Figure 4) shows that the points evenly distributed, symmetrical, and all the points are within the 95%CI. And Pr>|z|=1.000 (continuity corrected), 1.000>0.05. It indicates there is no publication bias, and the result of the study is credible.

Discussion

In this meta-analysis, we provided strong evidence of an association between the CTCs level detected in the peripheral blood and clinical outcomes in SCLC patients. Previous several small-scale studies showed that the presence of CTCs associated with a poorer survival in SCLC patients. Therefore, a quantitative meta-analysis of the study outcomes was required. The results of our collective evaluation of the publications on SCLC indicate that the presence of CTCs could be a prognostic marker.

Previous studies had demonstrated that CTCs was an ideal tumor marker and can be used in clinical practice, screening of high risk population, early diagnosis,

monitoring of cancer patients and prognosis evaluation. Several independent studies have shown that an increase in CTCs level is prognostic in metastatic breast, prostate, colorectal, and non-small-cell lung cancer (NSCLC) (Cristofanilli et al., 2004; Cohen et al., 2008; de Bono et al., 2008; Krebs et al., 2011). However, a meta-analysis revealed a negative result between pre-treatment CTCs level and OS of patients with SCLC in subgroup analysis (Ma et al., 2012). In our meta-analysis, we demonstrate the prognostic value of baseline CTCs number in SCLC, and the result showed that the presence of CTCs appears to confer a poorer prognosis. Among these seven studies included in this meta-analysis, five studies suggested that CTCs levels can be used for the prognosis evaluation of patients with SCLC. Only two of the studies did not have drawing the same conclusion, we consider that this may be related to the stage of SCLC patients and the CTCs cutoff value. For example, the patients all included with extensive-stage SCLC and the cutoff value was 282 in one study (Normanno et al., 2014). These seven studies have clear diagnostic, inclusion, and exclusion criteria. The patients in this meta-analysis were grouped according to CTCs cutoff value, and the OS and PFS were the main outcome. HR value was a statistical indicator to assess the impact of different levels of CTCs for prognosis of patients with SCLC.

In the present study, we also showed that the use of different approaches, such as RT-PCR, OBP-401, and CellSearch, confirmed that CTCs represent a significant meta-risk for both OS and PFS in SCLC. In these 7 studies, the CellSearch technology was used in the majority of studies for detecting CTCs. And previous studies reported that the detection rate of CTCs by the CellSearch system has been reported to be relatively high, with 67 to 86% of the patients being reported to have≥2 CTCs per 7.5 ml of blood (Oshita et al., 2003; Tanaka et al., 2009; Wu et al., 2009). Although among these 7 studies, different techniques to detect CTCs are used, there was no significant difference in the detection rate.

Regarding the number detected, previous studies reported that different CTCs cutoff value to predict the prognosis in patients with SCLC. These studies explored the prognostic role of CTCs in SCLC and identified different cutoffs that separate patients in favorable and unfavorable groups according to CTCs count. However, there is no optimal CTCs cutoff value for predicting the outcomes in SCLC patients up to now. Therefore, further studies are needed to explore the optimal CTCs cutoff value to determine the prognosis of SCLC patients.

This study had some limitations that are worth noting. First, this meta-analysis was limited to the published scientific publications, and univariate analysis data were also included in the present meta-analysis because multivariate survival analysis data were not available. Therefore, analysis method and the studies origin might explain some of heterogeneity. Moreover, we excluded a paper which can`t be calculated, which may influence the result to some degree. In addition, we did not weigh each study by a quality score, because no such score has received general agreement for applying in a meta-analysis, making more difficult the evaluation of its quality. At last, there was not a uniform cutoff value to define high level CTCs in SCLC patients, which might lead to heterogeneity. And CTCs detection assays varied in our study. In particular, measurements, and experimental design, may have partly influenced the clinical result. In addition to molecular markers, the treatment regime may affect the survival time of SCLC patients. This situation might influence the overall outcome and should be taken into account.

In conclusion, our meta-analysis supports that baseline CTCs level is associated with prognosis in SCLC patients by combining the results of different studies. The patients with high CTCs level may predict poor prognosis. In the future, the detection of CTC at baseline might serve as a tool to guide treatment in SCLC patients. Further adequately designed prospective multi-center studies are required to explore the clinical utility of CTC in SCLC.

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