Circulating Tumor Cells Correlate with Recurrence in Stage III Small-cell Lung Cancer after Systemic Chemoradiotherapy and Prophylactic Cranial Irradiation

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Received February 20, 2014; accepted July 18, 2014

Objective: We investigated the correlation between circulating tumor cells and the incidence of brain metastases as a first site of recurrence among patients with small-cell lung cancer after systemic chemoradiotherapy and prophylactic cranial irradiation. In addition, we assessed the contribution of circulating tumor cells for planning the appropriate total dose of prophylactic cranial irradiation for small-cell lung cancer.

Methods: Patients (n = 112) with diagnosed Stage III small-cell lung cancer were treated with four cycles of platinum-based regimen and concurrent chest irradiation, and then prophylactic cranial irradiation. Blood samples for circulating tumor cell analysis were obtained before the initiation of chemotherapy and after the first and fourth cycle of chemotherapy.

Results: Circulating tumor cells after the first cycle of chemotherapy correlated with tumor response after completion of chemotherapy (P = 0.012). Patients with brain as the first site suffered a higher rate of further metastases to other organs, and local recurrence, compared with those whose first site was the other organs (P < 0.001), and their survival rates were worse. Circulating tumor cells at baseline were the sole independent prognostic factor for specific progression-free survival. Receiver operating characteristic curves based on median specific progression-free survival revealed a circulating tumor cell cutoff at baseline of 218, and circulating tumor cells ≤218 at baseline correlated with significantly higher progression-free survival (P = 0.007), specific progression-free survival (P = 0.001) and overall survival (P = 0.001).

Conclusions: Circulating tumor cells prior to the initiation of chemotherapy are a valuable predictor of specific progression-free survival in Stage III small-cell lung cancer. For patients with circulating tumor cells >218, prophylactic cranial irradiation at a total dose of 30 Gy in 15 fractions is insufficient.

Key words: small-cell lung cancer – circulating tumor cell – prophylactic cranial irradiation – prognosis
INTRODUCTION

Brain metastasis is commonly seen in small-cell lung cancer (SCLC) due to the aggressive nature of the malignancy (1), and patient’s prognosis remains poor with a short survival of only 3–6 months (2). Conventional treatment, including chemotherapy, has been employed for both initial and salvage treatment in these patients (1). However, the central nervous system has proved to be relatively refractory to chemotherapy, as the blood–brain barrier protects subclinical metastases from cytotoxic drugs (2).

Prophylactic cranial irradiation (PCI) was developed to prevent tumors in the brain, and when initially introduced in the early 1970s, overall survival (OS) improved. For PCI-treated SCLC patients in complete remission, the reported incidence of brain metastases was significantly lower (33.3%) than the incidence in untreated patients (58.6%), and over a 3-year period disease-free survival and OS (22.3 and 20.7%, respectively) also improved compared with untreated patients (13.5 and 15.3%) (3). Currently, PCI is widely applied in limited-stage SCLC patients who achieve a complete response to initial systemic treatment, and for extensive-stage patients who suffer from complicated central nervous system symptoms. Both applications provide favorable control of brain metastasis and OS benefits (4). Nonetheless, cerebral recurrence can still occur after PCI, with a relapse rate of 18.9% in limited-stage SCLC and 15.9% in extensive-stage (5). Thus an effective method to predict disease relapse, as well as guidance to determine an appropriate radiation dose of PCI, is urgently needed.

Circulating tumor cells (CTCs) originate from a primary tumor, and their metastatic potential reflect that of the source. Circulation in the bloodstream allows CTCs to migrate to distant organs, including the brain, and facilitates malignancy at a second site (6). Previous studies have quantified CTC amounts to assess the prognosis of various metastatic diseases, including prostate, breast and colorectal cancers (7–9). The CellSearch System (Veridex, Raritan, NJ, USA) is a proven method for the identification and quantification of CTCs of epithelial origin in whole blood (7–9). However, it has not been determined whether CTC counts, taken at specific time-points before and during a course of chemotherapy, may be associated with specific recurrence patterns in SCLC.

In this study, we first investigated the efficacy of CTC counts for predicting SCLC recurrence, and especially the incidence of brain metastasis. In addition, we evaluated the potential of CTC counts to appropriately plan an effective dose of PCI for SCLC.

PATIENTS AND METHODS

Patients and Treatment Plans

The institutional review board of Shandong Tumor Hospital approved this study. Patients at the hospital who had received a diagnosis of Stage III SCLC from November 2006 to February 2010 were prospectively recruited. Each patient provided written informed consent to participate.

For inclusion, each patient had received a comprehensive immunohistochemical confirmation of Stage III SCLC, was older than 18 years, with a performance score ≤3, and in a physical condition that allowed first-line chemotherapy. Routine clinical information was collected at the time of diagnosis, including medical history and physical examination, complete blood cell counts and chemistry panel, chest and upper abdomen computed tomography, brain magnetic resonance imaging, and 18F-fluorodeoxyglucose positron emission tomography/computed tomography. Patients were staged according to the American Joint Committee on Cancer tumor-node-metastasis (TNM) staging for SCLC (seventh edition) (10).

All patients underwent combined concurrent chemoradiotherapy per the National Comprehensive Cancer Network (NCCN) guidelines for SCLC (11). The chemotherapy regimen consisted of platinum (cisplatin or carboplatin) with either etoposide or irinotecan administered every 3 weeks for four cycles. Chest irradiation was initiated within a week after the second cycle of chemotherapy, with 30 daily fractions of 1.8 or 2.0 Gy. A typical PCI dose of 30 Gy in 15 fractions was subsequently delivered over 3 weeks after the completion of the platinum-based cytotoxic treatment (1). Computed tomography scans were obtained after the second and fourth cycles of chemotherapy for tumor response assessment, and every 9–12 weeks thereafter for further follow-up. Tumor size and response to therapy were independently re-evaluated after the fourth cycle of chemotherapy, in accordance with Response Evaluation Criteria in Solid Tumor (RECIST, version. 1.1) (12).

Complete disappearance of all measurable and evaluable disease was considered a complete response. Partial response was defined as a 50% decrease in the sum of the three-dimensional diameters of all measurable lesions. Progressive disease was a 50% increase in the sum of three-dimensional diameters of all measurable lesions, or worsening of evaluable disease or the appearance of new lesions. All conditions that did not qualify as complete response, partial response or progressive disease were considered stable disease.

Progression-free survival (PFS) was defined as the period from the introduction of chemotherapy to tumor progression. OS was the period from the initiation of chemotherapy to death. The period of specific progression-free survival (SPFS) extended from the initiation of chemotherapy to cerebral metastasis, in patients whose first site of recurrence was the brain.

CTC Quantification

A 7.5 ml blood sample was drawn from each patient into a 10 ml Cellsave preservative tube (Veridex, Raritan, NJ, USA) before the initiation of chemotherapy, and after the first and fourth cycles of chemotherapy. Blood tubes were stored at room temperature and centrally processed within 96 h of collection using the CellSearch system (Veridex, Raritan, NJ,
USA), which consists of the CellPrep system, the CellSearch Epithelial Cell Kit (for the measurement of CTCs) and the CellSpotter Analyzer. The automatic system concentrates cells that express the epithelial cell adhesion membrane (EpCAM) antigen. It then specifically identifies and isolates CTC nucleated cells expressing cytokeratins 8/18 or 19 and lacking the leukocyte antigen CD45 (13), as well as presenting cytomorphologic characteristics of tumor cells. After image acquisition, CTC quantification was carried out on the CellTracks Analyzer II (14). Final CTC evaluation was performed by two independent investigators who were blinded to the patient clinical information.

The CTC count before treatment was defined as the baseline value, recorded as the number of CTCs per 7.5 ml of blood.

Statistical Analyses

Descriptive statistics for clinical and CTC variables are presented as median and interquartile range (IQR), or the number with percentage as appropriate. Descriptive statistics for PFS and OS are shown as median and 95% confidence interval (95% CI). The correlation between CTC counts and tumor response after systemic chemoradiotherapy was analyzed via Spearman’s rank correlation test.

Cox regression analysis was used to develop the univariate and multivariate models describing the association of independent variables with SPFS. Independent variable analyses included gender, age, Eastern Cooperative Oncology Group performance status, disease stage and CTC counts. OS and PFS curves were obtained using Kaplan–Meier methods, and differences between groups were assessed using the log-rank test.

The CTC cutoff was determined using receiver operating characteristic (ROC) curves based on whether the brain was the first site of recurrence. Differences in rates were analyzed using the χ² test. P values of ≤0.05 were considered statistically significant. All analyses were performed using SPSS 18 software (SPSS, Chicago, IL, USA).

RESULTS

Patient Characteristics

One hundred and twenty-nine patients were enrolled in this study, among which 112 patients (67 men and 45 women, all Han Chinese) completed the described treatment and were eligible for survival analyses. Among those who did not complete the treatment, 12 patients declined therapy due to financial or personal reasons, 2 patients left the cohort because of Grade 4 marrow depression and another 3 were lost to follow-up after chemotherapy. The median age was 58.5 years (IQR 49–69 years).

TNM classification revealed 70 patients with Stage IIIA disease and 42 with Stage IIIB. At the time of final analysis, 89 (79%) patients had died and 23 (21%) patients were living. Median follow-up for surviving patients was 25 months (range 5–66 months).

Tumor Response and Recurrence

After systemic chemoradiotherapy and subsequent PCI, among the 112 patients 48 (43%), 34 (30%) and 30 (27%) showed complete response, partial response and stable disease, respectively. There was no case of progressive disease.

Disease recurrences were observed in 105 patients after systemic treatment, among whom the brain was the first site of metastasis in 40 (38%), despite the administration of PCI. According to the pattern of failure, the 105 patients could be divided into four groups. Groups A and B comprised patients with the brain as the first site of recurrence, with those in Group A (n = 27) also experiencing further metastases to other organs or local recurrence and Group B (n = 13) without further metastasis or local recurrence. Groups C and D consisted of patients with organs other than the brain as the first site of recurrence, with those in Group C (n = 19) also experiencing further metastases to other locations and Group D (n = 46) with no further metastasis.

Of the 40 patients with the brain as the first site of recurrence (Groups A and B), 27 (67.5%) suffered further metastases to other organs. However, among the 65 patients with organs other than the brain as the first site of recurrence (Groups C and D), only 19 (29.2%) showed further metastases. The difference was statistically significant between the two groups (P < 0.001).

Of the 105 patients with local, regional or distant disease recurrence, 104 received palliative radiation with a total dose of 5000–6000 cGy in 25–30 fractions to the site of relapse. For patients who developed brain metastasis within 6 months after initiating treatment (seven patients), 3000–4000 cGy in 15–20 fractions was administered. Patients who showed brain metastasis after 6 months (40 patients) were administered whole-brain radiation of 2000 cGy, and then 3000–3600 cGy in 15–18 fractions. Concurrent palliative chemotherapy consisting of docetaxel/gemcitabine combined with a platinum-based regimen was applied as second line therapy for 93 patients. Fifty-nine received a CPT-11 combined with the platinum-based regimen for further refractory diseases.

CTC Test Characteristics

In this cohort of 112 patients, two or more CTCs (IQR 16–341) per 7.5 ml blood sample were detected in 87 (78%) patients prior to the initiation of treatment. Another 8 (7%) patients showed one CTC, and the remaining 17 (15%) patients had no CTCs. The median CTC count was 19.5 (IQR 2–247.25). The median CTC count of patients with Stage IIIA disease was 17 (IQR 3–25), and the median CTC count of patients with Stage IIIB disease was 82 (IQR 2–468). No significant difference in CTC enumeration was observed among patients with different disease stages (P = 0.103).
Relative to the CTC count at baseline, CTCs decreased in most patients after the first cycle of chemotherapy (80%, \( P < 0.001 \)). An even higher proportion of patients (84%) showed CTC counts declining from baseline after the fourth cycle of chemotherapy (\( P < 0.001 \)).

**Correlation Analyses**

CTCs after the first cycle of chemotherapy correlated with tumor response after four cycles of chemotherapy (\( P = 0.012 \)). No statistically significant correlation was found between CTCs at baseline (or after four cycles of chemotherapy) and tumor response after the fourth cycle of chemotherapy (\( P = 0.174 \) and \( P = 0.097 \), respectively).

**Univariate Survival Analyses**

The differences in PFS and OS among the four groups (A, B, C and D) were statistically significant (\( P = 0.018 \) and \( P = 0.023 \), respectively). The PFS and OS of A + B were significantly longer than that of C + D (\( P = 0.013 \) and \( P = 0.011 \); Table 1, Figs. 1–3). The PFS and OS of patients whose first site of recurrence was an organ other than the brain (group C + D, 21 and 28 months, respectively) were longer than that of patients whose first site of recurrence was the brain (A + B, 12 and 22 months). There was no significant difference in PFS or OS between Groups A and B (\( P = 0.150, P = 0.307 \), respectively, Fig. 2).

**Univariate and Multivariate Cox Proportional Hazards Regression Analyses**

In the univariate analyses (Table 2), the CTC count at baseline was a valuable predictor for SPFS. Multivariate Cox proportional hazards regression analysis showed that CTCs at baseline were an independent prognostic factor for SPFS, taking into account disease stage, CTCs after one cycle of chemotherapy, CTCs after four cycles of chemotherapy, tumor response, gender, age and performance status (Table 2).

ROC analysis (area under the curve = 0.688; 95% CI, 0.568–0.807) revealed that at baseline 547 CTCs per 7.5 ml blood sample (the highest quartile) showed good specificity for the brain as the first site of recurrence (90.8%), but sensitivity was poor (22.5%; Fig. 4). At 218 CTCs (the median quartile), specificity improved to 81.5% and sensitivity decreased to 40%. At 19 CTCs (the lowest quartile), specificity was 53.8% and sensitivity was 62.5%. Thus, for further evaluations, 218 CTCs at baseline was chosen as the cutoff value.

The median PFS, SPFS and OS in patients divided by CTCs at baseline above and below 218 are shown in Table 3. The differences in PFS, SPFS and OS were statistically significant (\( P = 0.007, P = 0.001, P = 0.001 \), respectively; Table 3; Table 1, Figs. 1–3).
Patients presenting CTCs ≤ 218 at baseline had longer PFS, SPFS and OS.

**DISCUSSION**

This study is the first prospective evaluation of CTC count as a predictor of prognosis for Stage III SCLC patients after systemic therapy and subsequent PCI. The level of CTCs at baseline was an independent prognostic factor for SPFS in both univariate and multivariate analysis.

In our study, two or more CTCs were detected in 72% of the enrolled patients before the initiation of therapy. This is similar to previous studies that focused on patients with advanced SCLC (6, 15, 16). The CTC count at baseline varied greatly among individuals, and a remarkable decline in CTCs after systemic treatment occurred in some patients. However, a lower CTC count after treatment did not necessarily lead to a better prognosis. One patient with Stage IIIB disease had the highest level of CTCs at baseline (13,786), which decreased to 79 after the fourth cycle of chemotherapy and concurrent radiation; nonetheless, the OS of this patient was only 8 months.

A contrary result was previously reported, in which the absolute CTC count after the first cycle of chemotherapy was the strongest predictor for response to chemotherapy and survival (15). Differences in TNM staging may explain the contradictory results of these two works. Our study enrolled only patients with Stage III disease, while Hiltermann et al. (15)
included tumors from Stages I to III. This may have caused a significant difference in CTCs at baseline, and thus the conclusions drawn may not be accurate enough.

In the present study, the CTC count decreased remarkably after the first cycle of chemotherapy, and this finding significantly correlated with tumor response after the fourth cycle of chemotherapy. Nonetheless, CTCs after the first cycle of chemotherapy was not a prognostic factor after considering TNM stage, CTCs at baseline, CTCs after four cycles of chemotherapy, tumor response, gender, age and performance status. We suggest that the factors analyzed in this study may not be independent from each other, especially as they influence the CTC count after the first and fourth cycles of chemotherapy and tumor response. Thus the predictive value of CTCs at these timepoints may weaken in multivariate regression analysis.

Tai et al. (1) reported a recurrence rate of 18.75% (24/128) in patients who experienced brain metastasis as the first site of relapse after receiving PCI; however, we observed a 2-fold higher incidence (38%) in our work. One explanation for this specific finding could be the difference in eligible criteria, as only Stage III diseases were included in our study, while limited-stage SCLC was the targeted subject in the former. Another study which focused on extensive-stage SCLC also showed a lower rate of symptomatic brain metastasis (16.8%) after PCI, with 24 months of follow-up (17). However, different chemotherapy plans and subsequent tumor response in the previous study might contribute to the disparity in brain metastasis rate. Also, in our cohort the administered thoracic radiation dose (in total, 54–60 Gy) may have been slightly insufficient; 60–70 Gy is now recommended with conventional fractions in the NCCN Guideline for Small-Cell Lung Cancer (18). Our investigation was initiated in 2006, during which a total dose of 50–60 Gy for SCLC was widely accepted as the standard regimen (11), and the treatment plan was considered appropriate at that time.

Several recent studies (19–21) investigating the reasons for failure in SCLC indicated that the most crucial factors included hematogenous distant metastases and locoregional recurrence after PCI. However, these studies focused on the effect of the failure pattern on outcome. In our cohort, the 105 patients were divided into four groups according to the pattern of failure. Significant differences were detected in PFS and OS among the four groups. Yet, no differences in PFS or OS were found between Groups A and B. This suggests that brain metastasis as the first site of recurrence indicates poor prognosis, whether or not further metastases to other organs occur.

### Table 2. Unit- and multivariate analyses of predictive factors for specific progression-free survival (SPFS) in patients with small-cell lung cancer

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<th>Univariate</th>
<th>Multivariatea</th>
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<tr>
<td></td>
<td>P value</td>
<td>Relative risk</td>
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<tr>
<td>Age (58.5 years)</td>
<td>0.115</td>
<td>0.983</td>
</tr>
<tr>
<td>Gender (male cf. female)</td>
<td>0.417</td>
<td>1.502</td>
</tr>
<tr>
<td>Performance score (≤3)</td>
<td>0.444</td>
<td>0.397</td>
</tr>
<tr>
<td>Disease stage (IIA–IIIB)</td>
<td>0.138</td>
<td>1.601</td>
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<tr>
<td>Circulating tumor cell (CTC)</td>
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<tr>
<td>Baseline</td>
<td>&lt;0.001</td>
<td>5.243</td>
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<tr>
<td>Post-first cycle</td>
<td>0.393</td>
<td>1.066</td>
</tr>
<tr>
<td>Post-fourth cycle</td>
<td>0.857</td>
<td>1.002</td>
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<tr>
<td>Tumor response</td>
<td>0.136</td>
<td>1.727</td>
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*aCox regression analysis (the enter method). bCox regression analysis (stepwise method). CTCs at baseline is the only predictor in the final Cox multivariate proportional-hazards model with stepwise method.

### Table 3. Median progression-free survival (PFS), SPFS and overall survival (OS) in patients divided by CTCs at baselinea

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<th>PFS</th>
<th>SPFS</th>
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<tr>
<td>CTC</td>
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<tr>
<td>≤218</td>
<td>21.0</td>
<td>11.6</td>
<td>28.0</td>
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<tr>
<td>&gt;218</td>
<td>12</td>
<td>7.3</td>
<td>19.0</td>
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| P                   | 0.007 | 0.001 | 0.001 |

*aMonths, 95% CI.

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**Figure 4.** The sensitivity curve. Receiver operating characteristic analysis reveals that the area under the curve = 0.688 (95% confidence interval, 0.568–0.807).
Drug-induced differentiation to a more complex, secondary chemoresistant SCLC phenotype after cytotoxic therapy has proved to be a leading cause of failure in primary treatment (22). Therefore, we assumed that the SCLC phenotype could become radio-resistant after chemotherapy in some patients, among whom the conventional PCI dose would be insufficient. To explore for a predictive factor we analyzed SPFS, defined as the period from the initiation of chemotherapy to brain metastasis as the first site of recurrence. Multivariate Cox proportional hazards regression analyses indicated that the CTC count at baseline was the only independent prognostic factor for SPFS. Consequently, an ROC curve was applied to determine the CTC cutoff value for achieving a compromise between sensitivity and specificity. Priority was given to specificity, since the cutoff would be chosen for indicating the suitable dose of PCI, and radiation dose should be carefully controlled to avoid ironizing toxicity in the brain. The median CTC count at baseline of 218 had the best area under the curve, with a specificity of 81.5% and a sensitivity of 40%.

Thus a CTC count of 218 was chosen as the cutoff point for subsequent analyses. However, this threshold may be dissatisfactory, given the low sensitivity. One factor contributing to this result is that CTC detection based on immunocytochemistry has the potential to miss cells that do not express the intended target antigens, such as EpCAM (23). In addition, real-time continuous surveillance of CTCs cannot be conducted in clinical practice since the release of CTCs from the tumor is intermittent. This may affect the CTC count throughout the course of the disease and limit its accuracy (24).

PFS, SPFS and OS were significantly higher for patients below the CTC cutoff of 218 at baseline. It was previously shown that higher CTC levels were associated with worse survival in SCLC, although various cutoffs were determined for different purposes in these studies (6, 15). Stott et al. (25) reported that increasing CTCs released from primary cancer could cause CTC aggregation and the hematogenous dissemination of malignancy in remote sites including the brain, which thus worsened survival. Based on our present

![Figure 5. Kaplan–Meier analyses of PFS, specific progression-free survival and OS in patients with circulating tumor cells ≤ 218 and patients with circulating tumor cells >218. Patients presenting CTCs ≤ 218 at baseline show better PFS, specific progression-free survival and OS (P = 0.003, 0.001, 0.001, respectively).](image-url)
observations regarding the significant differences in survival for patients with different CTCs at baseline, we suggest that for patients with CTCs > 218 at baseline, the standard PCI dose may be insufficient, and a trial of dose escalation is reasonable and logical. Currently, 25 Gy in 10 daily fractions of 2.5 Gy, or 30 Gy in 15 daily fractions of 2 Gy, is considered standard practice for SCLC patients undergoing PCI (NCCN) (11). A large randomized clinical trial concluded that a higher PCI total dose conferred no advantage over the standard dose (26). However, the authors had not investigated factors correlated with metastasis to the brain to allow for customizing the total PCI dose to the individual patient, and acknowledged they could not exclude the possibility of a false-positive result, despite the large number of randomized patients. Thus, further study on the rationale and necessity of escalating the total PCI dose is necessary.

Acknowledgements
The authors thank Mr Weijiang Fu, Guanzhong Gong, Lisheng Liu and Yongsheng Gao for their assistance with this manuscript.

Conflict of interest statement
None declared.

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