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Ⅲ. 研究成果の刊行物・別刷

研究代表者	後藤　功一	国立がん研究センター東病院
研究分担者	田村　友秀	国立がん研究センター中央病院
	森　　清志	栃木県立がんセンター
	岡本　浩明	横浜市立市民病院
	山本　信之	静岡県立静岡がんセンター
	横山　　晶	新潟県立がんセンター新潟病院
	樋田　豊明	愛知県がんセンター中央病院
	今村　文生	大阪府立成人病センター
	松井　　薫	大阪府立呼吸器･アレルギー医療センター
	平島　智徳	大阪府立呼吸器･アレルギー医療センター
	中川　和彦	近畿大学医学部
	武田　晃司	大阪市立総合医療センター
	木浦　勝行	岡山大学大学院医歯薬学総合研究科
	河原　正明	近畿中央胸部疾患センター
	安宅　信二	近畿中央胸部疾患センター
	根来　俊一	兵庫県立がんセンター
	里内美弥子	兵庫県立がんセンター
	西條　長宏	近畿大学医学部
	瀬戸　貴司	九州がんセンター
	工藤　新三	大阪市立大学大学院医学研究科
	湊　　浩一	群馬県立がんセンター
	澤　　祥幸	岐阜市民病院
	西尾　誠人	がん研究会有明病院
	野上　尚之	四国がんセンター

Prognostic Impact of Circulating Tumor Cells in Patients with Small Cell Lung Cancer

Tateaki Naito, MD, PhD, Fumihiro Tanaka, MD, PhD,† Akira Ono, MD,* Kazue Yoneda, BS,‡ Toshiaki Takahashi, MD, PhD,* Haruyasu Murakami, MD, PhD,* Yukiko Nakamura, MD,* Asuka Tsuya, MD, PhD,* Hirotsugu Kenmotsu, MD,* Takehito Shukuya, MD,* Kyoichi Kaira, MD, PhD,§ Yasuhiro Koh, MD,‖ Masahiro Endo, MD, PhD,¶ Seiki Hasegawa, MD, PhD,‡ and Nobuyuki Yamamoto, MD, PhD**

Background: Enumeration of circulating tumor cells (CTCs) may be valuable for prognostic assessment in lung cancer patients. In this study, we report the clinical significance of CTCs in small cell lung cancer (SCLC).

Methods: In total, 51 consecutive patients newly diagnosed as having SCLC and starting chemotherapy or chemoradiotherapy were prospectively enrolled. Blood samples were drawn at the baseline, after chemotherapy, and at relapse. CTCs were isolated using the CellSearch System (Veridex LLC). Thresholds of 1 to 100 cells at the baseline were systematically correlated with the overall survival. The optimal cutoff was determined by comparing the Cox proportional hazard ratios (HRs).

Results: Two or more CTCs were detected at baseline in 35 patients (68.6%; 95% confidence interval, 55.0–79.7). The HR signifying the difference between the unfavorable (more than or equal to threshold) and favorable (less than threshold) groups was maximal at the threshold of 8 CTCs (HR, 3.50; 95% confidence interval, 1.45–8.60). Patients with ≥8 CTCs had worse survival than those with <8 CTCs at baseline ($p = 0.0014$). Patients with ≥8 CTCs posttreatment or at relapse also showed worse survival than those with <8 CTCs ($p = 0.0096$ and <0.0001). Patients whose baseline and posttreatment CTC levels remained <8 tended to show better survival than those whose CTC level converted from ≥8 to <8 cells ($p = 0.0288$) or whose posttreatment CTC level was ≥8 cells ($p = 0.0047$).

Conclusions: CTCs were highly detectable in SCLC, and higher CTC levels were strongly associated with worse survival. Consistently favorable CTC levels were associated with favorable outcomes.

*Division of Thoracic Oncology, Shizuoka Cancer Center, Shizuoka Prefecture; †Second Department of Surgery, University of Occupational and Environmental Health, Fukuoka Prefecture; ‡Department of Thoracic Surgery, Hyogo College of Medicine, Hyogo Prefecture; §Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Gunma Prefecture; ‖Division of Drug Discovery and Development, Shizuoka Cancer Center Research Institute, Shizuoka Prefecture; and ¶Division of Diagnostic Radiology, Shizuoka Cancer Center, Shizuoka Prefecture, Japan.

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Address for correspondence: Nobuyuki Yamamoto, MD, PhD, Division of Thoracic Oncology, Shizuoka Cancer Center, 1007 Shimnagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka, Japan. E-mail: n.yamamoto@scchr.jp

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Small cell lung cancer (SCLC) accounts for 15% of all lung cancer diagnoses and is characterized by aggressive tumor growth, often presenting with metastases in the regional lymph nodes and distant organs. Because SCLC is highly sensitive to chemotherapy and radiotherapy, early diagnosis followed by appropriate treatment can be expected to yield favorable outcomes.[1,2] Circulating tumor cells (CTCs) are known to circulate in the peripheral blood in patients with several types of malignancies,[3–6] while rarely being detected (0.3–1.0%) in healthy control subjects or patients with non-malignant diseases.[3,7,8] The CellSearch system (Veridex LLC, Raritan, NJ) is a well-validated system for quantitative evaluation of CTCs, in which CTCs are immunomagnetically captured using an antibody against epithelial cell adhesion molecules (EpCAMs).[9,10] A growing body of evidence suggests the existence of a correlation between CTC level as measured by the CellSearch system and the progression-free survival (PFS) and overall survival (OS) in patients with metastatic breast, colorectal, castration-resistant prostate, and non-small cell lung cancers (NSCLC).[7,11–15] In SCLC, the detection rate of CTCs by the Cell Search 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.[8,16,17] However, the prognostic impact of CTCs and their relationship to the presence of metastases in patients with SCLC remain unknown. We conducted this study to evaluate the relationship of CTC levels to the disease extent and prognosis and to determine the optimal CTC level cutoff for predicting the outcomes in SCLC patients.

METHODS

Study Design

This prospective study was conducted at two institutions (Shizuoka Cancer Center and Hyogo College of Medicine) to evaluate the usefulness of measurement of the CTC

levels for predicting the OS. Patients with chemotherapy-naive, pathologically confirmed SCLC scheduled to commence first-line standard chemotherapy with or without thoracic radiotherapy were eligible. All patients were enrolled at the Shizuoka Cancer Center and had an Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0 to 2. The institutional review boards at each center approved the study protocol, and all patients provided written informed consent. Before the start of the new treatment, the patients underwent an evaluation of metastatic sites by means of standard imaging studies, including contrast-enhanced computed tomography of the chest to lower abdomen, contrast-enhanced magnetic resonance imaging of the brain, and bone scan or positron emission tomography, along with the collection of blood sampled for counting of the baseline CTCs. The post-treatment blood samples were collected 3 weeks after completion of the last chemotherapy cycle or completion of sequential thoracic radiotherapy. The samples were collected 2 weeks after relapse had been diagnosed by imaging and before administration of the second-line chemotherapy. The sampling date could be adjusted depending on the type of treatment and the visit schedule, with allowance for ±2 weeks. Reevaluations of the disease status were conducted using the same techniques as those applied at the baseline, every 8 to 12 weeks, depending on the type of treatment the patient had received and the treatment schedule. Disease status was assessed according to the RECIST[18] by examiners with no knowledge of the CTC levels. Serum lactate dehydrogenese (LDH) levels and the levels of other biomarkers, including neuron-specific enolase (NSE) and progastrin-releasing peptide (ProGRP), were measured at the same time point as the baseline CTC measurement. The blood samples for the serum biomarker measurements were obtained by venous puncture, and the sera were stored at −40°C until use. The ProGRP concentration was measured using an ELISA kit (FUJIREBIO Inc., Tokyo, Japan), and the NSE concentration was measured using the radioimmunoassay solid-phase method (SRL Inc., Tokyo, Japan).

Counting of CTCs

Blood samples were drawn into 10-ml vacuum tubes (CellSave, Immunicon, Huntingdon Valley, PA). Samples were maintained at room temperature, mailed overnight, and processed within 96 hours of collection. The results were reported quantitatively as the number of CTCs per 7.5 ml of blood. All CTC evaluations were performed without knowledge of the patient clinical status in one of two laboratories (Hyogo College of Medicine, Japan, or the laboratory of SRL Inc.). The CellSearch system was used for the CTC counting, the technical details of which, including accuracy, precision, linearity, and reproducibility, have been previously described.[3] CTCs were defined as EpCAM-isolated intact cells showing positive staining for cytokeratin and negative staining for CD45. At each time point, the favorable and unfavorable groups were defined as those with CTC levels less than or more than or equal to the selected threshold, respectively.

Statistical Analysis

The primary analysis was a comparison of the OS between the unfavorable and favorable groups stratified according to the selected threshold of CTC level. The study was designed to enroll 50 patients for a statistical power of 80% with a two-sided log-rank test at a level of 0.05 to detect an absolute difference of 40% points between the two groups in the 1-year estimates of OS (20% in the unfavorable group versus 60% in the favorable group). To select the threshold CTC level that most clearly distinguished patients with an unfavorable prognosis from those with a favorable prognosis, thresholds of 1 to 100 cells at baseline were systematically correlated with the OS. The Cox proportional hazard ratio (HR), goodness-of-fit, and discriminatory power of each threshold were compared. The Bonferroni correction was applied for multiple testing for 14 thresholds, and a p value of <0.0036 was set to obtain a statistical significance of $p < 0.05$. The goodness-of-fit of the model was assessed by the coefficient of determination (R^2) defined as $1 - \{$(log likelihood of the estimated model)/(log likelihood of the model with only the intercept)$\}$. The discriminatory power was assessed by the accuracy rate ([AR] defined as the rate of correct diagnosis among all predictions of 1-year survivors) and the area under the receiver operator characteristics curve (AUROC). The treatment-free interval (TFI) was defined as the time between the completion of first-line chemotherapy and the diagnosis of relapse. Patients with a TFI of 90 days or more were considered to have treatment-sensitive disease, and those with a TFI of less than 90 days were considered to have treatment-refractory disease. For all survival analyses, the time to death was defined as the time between the date when the blood sample was obtained and the date of death or date of the last follow-up visit. Separate Kaplan-Meier survival plots were generated based on the CTC levels at baseline and the results in the follow-up blood collections. Survival curves were compared using the log-rank test. Cox proportional hazards regression was used to determine the HRs for the OS adjusted for age, gender, pretreatment stage (extensive disease [ED] versus limited disease [LD]), and ECOG-PS at the time of blood collection. The discriminatory power of the baseline CTC, LDH, NSE, and ProGRP for predicting 1-year survivors was compared by AUROC. The χ^2 test or Fisher exact test was used to compare categorical variables. For comparison of the means, the nonparametric Wilcoxon's test or analysis of variance was used. We tested the correlations between variables by calculating the Spearman's rank correlation coefficients. Calculations were carried out using the statistical program, JMP version 9.0 for Windows (SAS Institute Inc., Cary, NC).

RESULTS

Patient Characteristics

In total, 51 consecutive patients met the inclusion criteria and were prospectively enrolled between July 2009 and September 2010. The cutoff date for analysis was August 31, 2011. The median age of the patients was 67 years, and 44 of the patients (86.3%) were men (Table 1). Nineteen of the

TABLE 1. Baseline Characteristics

Characteristics	All (n = 51)	Extensive Disease (n = 24)	Limited Disease (n = 27)
Age, median (range)	67 (34–92)	66.5 (57–80)	68 (34–92)
Gender (female:male)	7:44	3:21	4:23
ECOG-PS, n (%)			
0	21 (41.2)	6 (25.0)	15 (55.6)
1	21 (41.2)	10 (41.7)	11 (40.7)
2	9 (17.6)	8 (33.3)	1 (3.7)
No. of organs with metastasis, median (range)	0.5 (0–3)	1 (0–2)	None
Brain metastasis, n (%)	7 (13.7)	7 (29.2)	None
Liver metastasis	8 (15.7)	8 (33.3)	None
Bone metastasis	3 (5.9)	3 (12.5)	None
Malignant effusion	12 (23.5)	11 (45.8)	1 (3.7)
Serum biomarkers (mean ± SE)			
NSE (ng/ml)	75.7 ± 24.3	131.2 ± 49.5	26.4 ± 5.5
ProGRP (pg/ml)	657.2 ± 205.7	1071.3 ± 419.1	289.0 ± 66.7
LDH (IU/L)	360.5 ± 79.9	529.7 ± 164.4	210.1 ± 8.8
Treatments, n (%)			
Chemotherapy alone	32 (62.7)	24 (100.0)	8 (29.6)
Chemoradiotherapy	19 (37.3)	None	19 (70.4)
Regimens, median cycle (range)	4 (1–6)	4 (1–6)	4 (1–5)
Cisplatin + etoposide, n (%)	23 (45.1)	16 (66.7)	7 (25.9)
Carboplatin + etoposide	21 (41.2)	2 (8.3)	19 (76.4)
Cisplatin + irinotecan	7 (13.7)	6 (25.0)	1 (3.7)

ECOG-PS, Eastern Cooperative Oncology Group performance status; SE, standard error; NSE, neuron-specific enolase; ProGRP, progastrin-releasing peptide; LDH, lactate dehydrogenese.

27 patients with LD had received chemoradiotherapy, while the remaining 8 patients could not receive radiotherapy for the following reasons and were treated by chemotherapy alone. The first patient was a 73-year-old man with a treatment history of thoracic chemoradiotherapy for esophageal cancer 6 years before the current treatment. Reirradiation was avoided because of the potential late adverse effects of radiotherapy. The second patient was a 79-year-old man with poor pulmonary functions who was scheduled for sequential radiotherapy after chemotherapy. However, his tumor progressed, with the development of contralateral pulmonary metastases after the first course of chemotherapy, and radiotherapy could not be administered. The remaining six patients had interstitial lung disease before the start of the treatment. Thoracic radiotherapy was withheld because of the potential risk of severe radiation pneumonitis. Twenty-four patients (47.1%) were still alive at the time of analysis. The median follow-up period for determining the survival was 13.0 months after the baseline blood sample collection. All 51 patients were evaluable for the baseline CTC level. Blood samples were not obtained during follow-up from two patients who died of interstitial lung disease and cancer progression. The remaining 49 patients were evaluable for the posttreatment CTC levels. The median time between the baseline and posttreatment blood collections was 3.4 months. Thirty-eight patients (74.5%) exhibited tumor progression; 37 were evaluable for the CTC level at the time of relapse, and 1 woman refused to provide blood samples.

Circulating Tumor Cells

Two or more CTCs were detected in 68.6% of the patients (95% confidence interval [CI], 55.0–79.7) at baseline, in 26.5% of the patients (95% CI, 16.2–40.3) posttreatment, and in 67.6% of the patients (95% CI, 51.5–80.4) at the time of relapse (Table 2). The CTC counts at baseline were higher in patients with ED, who showed a median of 9.5 cells (range, 0–5648), than in those with LD, who showed a median of 1 cell (range, 0–58; $p = 0.0001$, Figure 1*A*). Fourteen of the 16 patients (87.5%) who had a baseline CTC level of ≤1 had LD. The median CTC levels at baseline in patients with 0, 1, and ≥2 organs showing metastases were 2.0 (range, 0–58), 7.5 (1–799), and 21.0 (0–5648), respectively, showing a statistically significant correlation of the CTC count with the number of organs showing metastases (Spearman's rho, 0.72, $p < 0.0001$, Figure 1*B*). Patients with liver metastasis had higher CTC levels than those without liver metastasis (64 [range, 5–5648] versus 3 [range, 0–799]; $p = 0.0007$). There was no association between brain or bone metastasis and the CTC levels (data not shown).

Stratification According to Levels of Circulating Tumor Cells

The baseline CTC level was predictive of the OS when it was stratified by the threshold of 8 cells ($p = 0.0029$; Table 3). The Cox proportional HR signifying the difference between the unfavorable (more than or equal to threshold) and favorable (less than threshold) groups showed a waxing and waning pattern with the peak at the level of 8 CTCs. The HR associated with a CTC level of 8 cells was 3.50 (95% CI, 1.45–8.60) after adjustment for stage (ED or LD), age, gender, and ECOG-PS at the baseline. The Cox proportional hazard model at this level also showed a favorable goodness-of-fit and discriminatory power with the highest R^2, AR, and AUROC among all the thresholds examined. Thus, a cutoff level of 8 CTCs was chosen for the subsequent analyses. Analyses based on the stage (ED or LD) and therapy type

TABLE 2. CTC Levels at the Baseline, Posttreatment, and at the Time of Relapse

	Baseline	Posttreatment	At Relapse
Total[a]	51	49	38
Evaluable[b]	51	49	37
CTC, median (range)	4 (0–5648)	0 (0–253)	1 (0–510)
CTC, mean ± SE	203.2 ± 118.5	10.2 ± 5.9	44.6 ± 16.8
CTC ≥ 2, % (95% CI)	68.6 (55.0–79.7)	26.5 (16.2–40.3)	67.6 (51.5–80.4)

[a] Number of patients alive and evaluable.
[b] Number of patients with nonmissing data for CTCs at the time-point indicated.
CTC, circulating tumor cell; SE, standard error; CI, confidence interval.

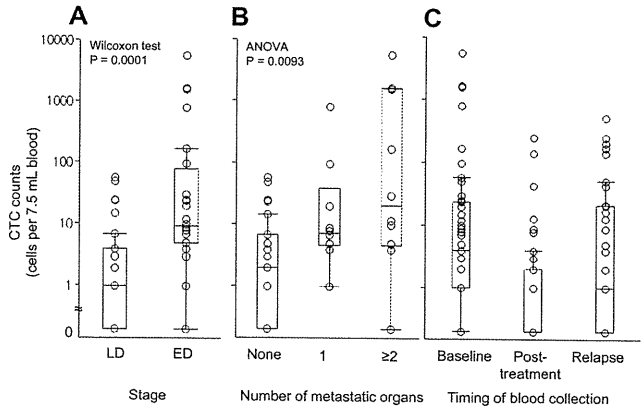

FIGURE 1. Box plots were drawn using the minimum and maximum values and the 25th, 50th, and 75th percentiles. *A*, Circulating tumor cell (CTC) levels at the baseline and the disease stage. ED, extensive disease; LD, limited disease. *B*, CTC levels at the baseline and number of metastatic organs. *C*, CTC levels at the baseline and the timing of blood sampling. *p* values calculated by Wilcoxon's test and analysis of variance (ANOVA) are presented.

TABLE 3. Baseline CTC and Prognosis

CTC Level[a]	Adjusted HR (95% CI)[b]	p^c	R^2	AR	AUROC (95% CI)
1	0.74 (0.26–2.40)	0.0604	0.06	0.49	0.55 (0.43–0.65)
2	0.67 (0.25–1.87)	0.0532	0.06	0.51	0.55 (0.42–0.67)
3	0.76 (0.27–2.11)	0.0606	0.06	0.55	0.58 (0.45–0.71)
4	0.85 (0.25–2.79)	0.0656	0.05	0.61	0.63 (0.48–0.75)
5	1.59 (0.61–4.29)	0.0481	0.06	0.67	0.68 (0.53–0.80)
6	2.97 (1.24–7.31)	0.0063	0.08	0.73	0.73 (0.58–0.84)
7	2.97 (1.24–7.31)	0.0063	0.08	0.73	0.73 (0.58–0.84)
8	3.50 (1.45–8.60)	0.0029	0.09	0.76	0.74 (0.59–0.85)
9	2.90 (1.20–7.04)	0.0072	0.08	0.73	0.71 (0.57–0.83)
10	2.41 (0.99–5.81)	0.0151	0.07	0.71	0.69 (0.54–0.80)
15	3.00 (1.19–7.40)	0.0079	0.08	0.71	0.68 (0.54–0.79)
25	2.02 (0.74–5.04)	0.0318	0.06	0.67	0.62 (0.50–0.73)
50	3.49 (1.23–9.79)	0.0107	0.08	0.67	0.62 (0.50–0.72)
100	3.97 (0.90–15.59)	0.0181	0.07	0.65	0.58 (0.48–0.67)

[a] CTC levels are expressed as the number of cells per 7.5 ml of blood.
[b] The Cox proportional hazard ratios were adjusted for stage, age, gender, and ECOG-PS at the baseline.
[c] The level of significance calculated by the Bonferroni method was $p < 0.0036$.
HR, hazard ratio; CTC, circulating tumor cell; CI, confidence interval; AR, accuracy rate for predicting 1-year survivors; AUROC, area under the receiver operator characteristics curve for predicting 1-year survivors.

(chemotherapy alone or chemoradiotherapy) showed that the prognostic significance of the CTC level was significant only in the ED subset and in the patients treated by chemotherapy alone (Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/JTO/A204).

Baseline CTC and Prognosis

Figure 2*A* shows the Kaplan-Meier curves for the OS according to the baseline CTC levels. Patients in the unfavorable group had significantly shorter survival than those in the favorable group ($p = 0.0014$). The 1-year survival rates and the median OS in the unfavorable and favorable groups were 31.6% versus 78.0% and 8.5 versus 17.2 months, respectively. The sensitivity, specificity, AR, and AUROC for predicting 1-year survivors using the cutoff level of 8 CTCs were 0.81, 0.65, 0.75, and 0.73 (95% CI, 0.58–0.84), respectively. The 1-year survival rates in the unfavorable and favorable groups were 21.4 and 70.0% ($p = 0.0282$), respectively, in the ED subset, and 60.0 and 81.6% ($p = 0.4387$), respectively, in the LD subset (Figures 2*B*, *C*).

Posttreatment CTC and Prognosis

During the posttreatment period, the CTC levels were measured in the 49 patients who were available for the evaluation. Of these 49 patients, 7 (14.3%) with ≥8 CTCs had a significantly shorter posttreatment survival than the remaining 42 (85.7%) with <8 CTCs ($p = 0.0096$, Figure 2*D*). The HR of the threshold CTC count adjusted for stage, age, and posttreatment PS was 2.76 (95% CI, 0.97–6.92, $p = 0.0562$). The median posttreatment survivals in the unfavorable and favorable groups were 4.1 and 13.9 months, respectively. At the time of relapse, CTC levels were measured in 37 patients. Of these 37 patients, the 13 (35.1%) with ≥8 CTCs had a significantly shorter postrelapse survival than the remaining 24 (64.9%) with <8 CTCs ($p < 0.0001$, Figure 2*E*). The HR of the threshold CTC adjusted for stage, age, TFI (<90 versus ≥90 days), and PS at the time of relapse was 6.20 (95% CI, 2.39–17.52, $p = 0.0002$). The median postrelapse survivals in the unfavorable and favorable groups were 4.0 and 11.8 months, respectively.

Posttreatment CTC Status and Prognosis

Among the 42 patients with posttreatment CTC levels of <8, 29 had a baseline CTC level also of <8 (group A), and in the remaining 13, the baseline CTC level was ≥8 (group B). Among the seven patients with posttreatment CTC levels of ≥8 (group C), four had a baseline CTC level also of ≥8, and the remaining three had a baseline CTC level of <8. As shown in Figure 3, the survival impact of conversion from an unfavorable to favorable CTC level was assessed by using the Kaplan-Meier curve for posttreatment survival according to the posttreatment CTC status. The median posttreatment survival was >18.8 months in group A, 7.2 months in group B, and 4.1 months in group C ($p = 0.0066$). The difference in the survival between group A and group C was significant ($p = 0.0047$ by log-rank test; level of significance calculated by the Bonferroni method, $p = 0.0166$). Conversely, there was no significant difference between group A and group B ($p = 0.0288$), or group B and group C ($p = 0.2489$). The HR adjusted for the pretreatment stage, posttreatment ECOG-PS, and TFI was 3.08 (95% CI, 1.03–8.90; $p = 0.0450$) in group B and 3.29 (95% CI, 1.01–10.07; $p = 0.0479$) in group C, both calculated using group A as the reference (Table 4).

Discriminatory Power of CTCs and Serum Biomarkers for Predicting the Prognosis

Figure 4 shows the receiver operator characteristics curves for CTCs, and the serum levels of LDH, NSE, and ProGRP measured at the baseline. Data on survival at 1 year

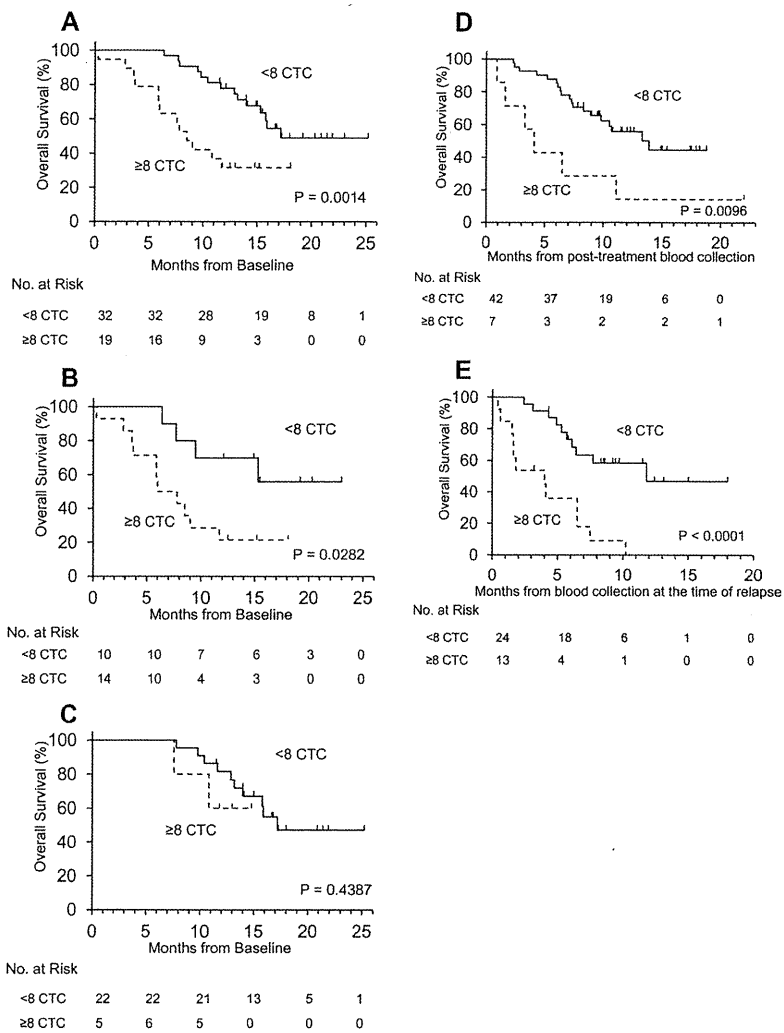

FIGURE 2. Kaplan-Meier curves for overall survival in patients with <8 and ≥8 circulating tumor cells (CTCs) at the baseline in the full set of data (*A*), extensive disease subset (*B*), and limited disease subset (*C*). Kaplan-Meier curves for posttreatment survival and postrelapse survival in patients with <8 and ≥8 CTCs posttreatment and at relapse (*D* and *E*). *p* values calculated by the log-rank test are presented.

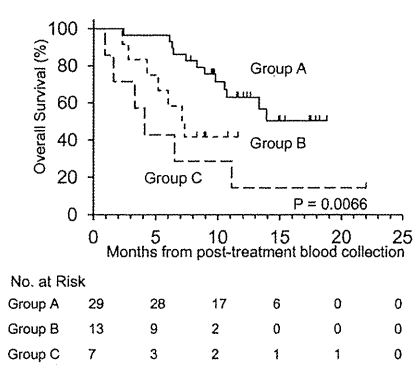

FIGURE 3. Kaplan-Meier curves for posttreatment survival in three groups, including patients in whom the baseline and posttreatment circulating tumor cell (CTC) levels remained at <8 (group A), patients in whom the CTC level converted from ≥8 to <8 cells (group B), and patients in whom the posttreatment CTC level was ≥8 cells (group C). *p* values calculated by the log-rank test are presented.

TABLE 4. Hazard Ratios of the Posttreatment Status of CTC Level

Posttreatment CTC Status	CTC Level[a]	*n*	MST (mo)	Adjusted HR (95% CI)[b]	*p*
Group A	<8–<8	29	NR	Reference	
Group B	≥8–<8	13	7.2	3.08 (1.03–8.90)	0.0450
Group C	≥8–≥8 or <8–≥8	7	4.1	3.29 (1.01–10.07)	0.0479

Group A: patients whose baseline and posttreatment CTC levels remained <8 cells; group B: patients whose CTC level converted from ≥8 to <8 cells; and group C: patients whose posttreatment CTC level was ≥8 cells.

[a] CTC levels are expressed as the number of cells per 7.5 ml of blood.

[b] The Cox proportional hazard ratios were adjusted for the pretreatment stage, posttreatment ECOG-PS, and treatment-free interval.

CTC, circulating tumor cell; HR, hazard ratio; MST, median survival time; CI, confidence interval; NR, not reached.

were available for all 51 patients. The baseline CTC level showed a favorable discriminatory profile, showing an AUROC of 0.70 (95% CI, 0.52–0.83), as compared with that of 0.67 (0.49–0.82) for LDH, 0.68 (0.52–0.82) for NSE, and 0.46

(0.29–0.64) for ProGRP. The differences in the AUROC among the parameters were not significant ($p = 0.1044$).

Radiologic Response and Changes in the CTC Levels

Assessment of the best radiologic response to the first-line treatment was performed using the RECIST criteria in 50 patients. One man died of interstitial lung disease before the follow-up imaging study. Figure 5 shows the baseline and posttreatment CTC levels in patients showing complete response (CR, $n = 6$), partial response (PR, $n = 27$), stable disease (SD, $n = 5$), and progressive disease (PD, $n = 12$). There was no significant difference between the CR/PR subsets and SD/PD subsets in the baseline CTC (median, 4 [range, 0–1683] versus 4 [range, 0–5648]; $p = 0.7337$ by the Wilcoxon's test) or posttreatment CTC (0 [0–44] versus 0.5 [0–253]; $p = 0.3370$) level. The numbers of patients with undetectable posttreatment CTCs or patients with lower posttreatment CTC levels than the baseline CTC levels were 4 (66.7%) in the CR group, 24 (88.9%) in the PR group, 4 (80.0%) in the SD group, and 7 (58.3%) in the PD group, with no significant differences among the groups showing the various treatment responses ($p = 0.2878$ by the χ^2 test).

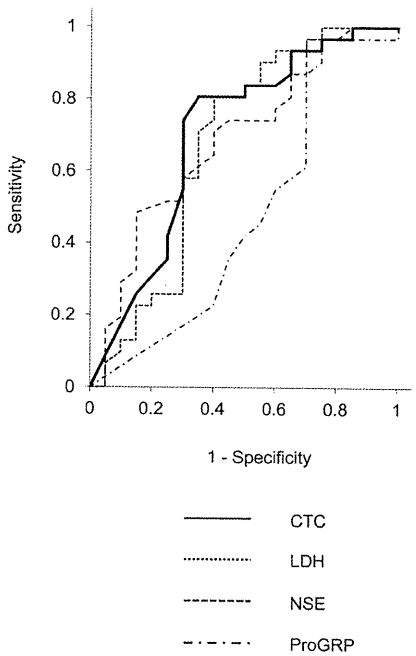

FIGURE 4. Receiver operator characteristics curve analysis for predicting 1-year survivors. The area under the curve is 0.70 (95% confidence interval [CI], 0.52–0.83) for the circulating tumor cell (CTC) level at baseline, 0.67 (95% CI 0.49–0.82) for serum lactate dehydrogenese (LDH) at baseline, 0.68 (95% CI 0.52–0.82) for serum neuron-specific enolase (NSE) at baseline, and 0.46 (95% CI 0.29–0.64) for serum progastrin-releasing peptide (ProGRP) at baseline.

DISCUSSION

This study is the first prospective evaluation of the optimal CTC cutoff to predict the OS in patients with chemotherapy-naive SCLC. First, we showed that the CTC level was strongly predictive of the OS, especially in the ED subset. Then, an optimal cutoff level, CTC count of ≥8 cells per 7.5 ml of blood was identified by comparing the Cox proportional HRs of various CTC levels for the OS. This cutoff level was also found to be valid for predicting the posttreatment survival and postrelapse survival in the same cohort. We also showed that the baseline CTC level had a high discriminatory power, similar to the serum NSE and LDH.

Circulating SCLC cells have been reported to show high expression levels of EpCAM,[19] which has been used as a key marker to isolate CTCs using the CellSearch system. The appropriateness of using the CellSearch system for detecting circulating SCLC cells was previously assessed by Hou et al.[16] They showed that 15 CTC samples obtained from patients with SCLC by the CellSearch system were neuroendocrine in nature (CD56 positive) and confirmed their neoplastic origin by immunohistochemical comparison of these cells with the cells obtained from matched tumor biopsy specimens. The detection rate (≥2 CTCs per 7.5 ml blood) of circulating SCLC cells by the CellSearch system in cases of SCLC is reportedly quite high, being 67 to 86%,[8,16] as compared with that in cases with other tumors with metasta-

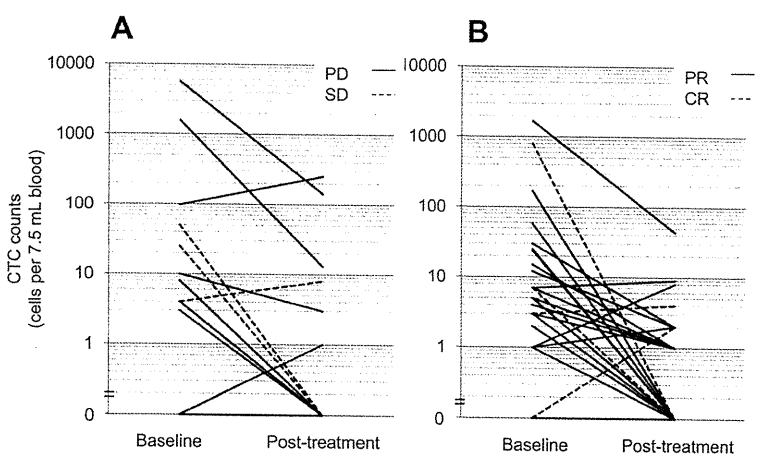

FIGURE 5. Relationship between radiologic response and the changes in the circulating tumor cell (CTC) level. *A*, Baseline and posttreatment CTC levels in patients showing PD (solid line) and SD (dotted line). *B*, Baseline and posttreatment CTC levels in patients showing PR (solid line) and CR (dotted line). PD, progressive disease; SD, stable disease; PR, partial response; CR, complete response.

ses.[3,7,11,15] Consistent with these reports, the detection rate in the SCLC patients in our study was 68.6%. Given that approximately half of our patients had nonmetastatic disease, we consider that CTCs are detected in a high percentage of cases of SCLC. Higher CTC counts have been reported as an indicator of the presence of distant metastases, such as bone metastasis in prostate cancer[20] and liver metastasis in colorectal cancer.[11] In patients with NSCLC, the CTC levels reportedly correlated with the number of organs showing metastatic involvement, and higher CTC levels are predictive of liver and bone metastasis.[15] Our results also showed an association between the CTC levels and the presence of metastasis, especially to the liver.

The CTC cutoff level (8 CTCs per 7.5 ml of blood) in our study to discriminate between groups with a favorable and unfavorable prognosis was higher than that reported for other tumors. In metastatic breast cancer, the cutoff level of 5 was chosen by comparing the median PFS and the Cox proportional HR for each threshold from 1 to 10,000 CTCs. The same cutoff was also shown to be correlated with the OS.[7] The cutoff of five cells was then applied to metastatic castration-resistant prostate cancer and was well validated to be predictive of the OS.[14] In metastatic colorectal cancer, the cutoff level of three cells was chosen by correlating the baseline CTC level with the response at the first follow-up imaging study. The cutoff level was well validated to be predictive of both the OS and PFS in a subsequent validation cohort.[11] Our cutoff level was based on a comparison of the Cox proportional HR for OS. The differences in the cutoff levels may be attributable to the statistical method used for choosing the optimal cutoff level or might reflect the highly metastatic potential of SCLC itself. In addition, we observed the prognostic significance of the baseline CTC only in the ED subset or patients treated by only chemotherapy in the subset analyses. As the previous studies in other malignancies have been conducted only in patients with metastatic disease, another study for ED-SCLC will be required to validate our results.

Conversion from an unfavorable baseline CTC level to a favorable follow-up CTC level reportedly has a strong impact on the survival. Patients with such conversion showed a favorable OS, statistically similar to that in patients with a persistent favorable CTC level in breast, prostate, and colorectal cancers.[7,11,14] In contrast, our study showed a relatively small impact of such conversion on the survival in SCLC patients. This difference might reflect the nature of SCLC itself, known to be aggressive and to rarely be in a dormant state.[2,3] A lower CTC level might be an appropriate treatment goal if minimal residual cancer cells after treatment had a larger impact on the survival in SCLC patients. Chemotherapeutic agents active against SCLC are as yet limited, and the classic platinum doublet with etoposide or irinotecan remains the standard first-line treatment regimen. Treatment options for relapsed SCLC are further limited to several cytotoxic agents,[21,22] and no molecular-targeted agents have yet been approved.[23] These limitations in treatment modalities might be related to the small impact of conversion after first-line treatment. NSE and ProGRP are commercially available serum biomarkers and are used as markers for monitoring of SCLC patients. They have been reported to be highly sensitive and specific for the diagnosis of SCLC, and elevated levels of these markers at baseline have been shown to be associated with poor prognosis.[24–26] LDH has also been reported to have prognostic significance in patients of SCLC.[27] We showed that the baseline CTC level showed a good discriminatory power for predicting the prognosis in SCLC patients, similar to serum NSE and LDH, and furthermore, that the baseline CTC level was probably a better predictor of survival than the serum ProGRP, by receiver operator characteristics curve analysis.

The treatment response was reported to be associated with the CTC level at the time of imaging in breast cancer.[28] In colorectal cancer, the CTC level measured 3 to 5 weeks after the initiation of therapy had a relatively low sensitivity (27%) for predicting PD.[11] In our study, we found no correlation between the results of the response assessment using the RECIST criteria and the baseline CTC level, posttreatment CTC level, or change in the CTC level associated with treatment. The changes in the tumor size might not always be related to the changes in the outflow of tumor cells from the tumors.

The major limitation of this study was that the study population was small. The threshold value was derived from a cohort at a single institution and not validated in an independent validation cohort. In addition, our study included not only patients receiving chemotherapy alone but also patients treated by chemoradiotherapy. Because the treatment goals are different for chemotherapy and chemoradiotherapy, that is, palliation versus cure, separate derivation studies will be required to choose the optimal CTC cutoff level.

There has been an increasing interest in several aspects of CTCs. First, measurement of the CTC levels has been expected to guide decision making, such as determining the timing of changing, continuing, or discontinuing the current treatment, or identifying appropriate candidates for adjuvant chemotherapy.[29–31] Second, CTC analysis is anticipated to provide samples for biomarker analysis. Monitoring of human EGFR-related 2-positive CTCs in breast cancer patients during human EGFR-related 2-targeted therapy[32–34] and analysis of androgen receptor gene alterations in the CTCs of prostate cancer patients[35,36] have been reported. In addition, the newly developed CTC analyzer shows a high detection power for CTCs and was used for the analysis of *EGFR*-gene alterations in the CTCs from patients with NSCLC.[37,38] These studies have established a new role for CTC analysis as a noninvasive method of tumor profiling or target monitoring during treatment with molecular-targeted agents. Although few molecular-targeted agents currently available are active against SCLC, the high detection rate of CTCs in cases of SCLC might provide an opportunity for the screening of active drugs and accelerate the development of new therapeutic strategies.

In conclusion, this study showed that CTCs are readily detectable by the CellSearch system in patients with SCLC and that the CTC levels before and after treatment had strong