and the MCA system, 1 was identified as CTC positive by the CellSearch system only, and 9 were identified as CTC positive by the MCA system only. Considering the results obtained by both systems together, 18 (82%) of the NSCLC patients were identified as CTC positive. Analysis of these findings revealed that a significantly greater number of NSCLC patients were identified as CTC positive by the MCA system (median cell count 13, range 0–291 cells/7.5 mL; Figure 2) than by the CellSearch system (median cell count 0, range 0–37 cells/7.5 mL), demonstrating the statistical superiority of the MCA system in CTC enumeration ($p = 0.0015$, Wilcoxon test; Table 3).

In contrast, 20 of the 20 (100%) SCLC patients were identified as CTC positive using the MCA system versus 12 of the 21 (57%) patients using the CellSearch system. The median CTC count was found to be 2 cells/7.5 mL (range 0–325) using the CellSearch system and 23 cells/7.5 mL (range 2–2329) using the MCA system (Figure 2). Although not reaching a level of statistical significance, the detection sensitivity of the MCA system in CTC enumeration showed a trend toward being greater than that of the CellSearch system ($p = 0.2888$, Wilcoxon test; Table 3). For each outcome, agreement between the test results of the systems was assessed by Bland–Altman plots [33]. In the analysis of agreement regarding CTC enumeration in NSCLC patients, the mean difference was 50.1 (95% CI, range 11.1–89.1), with the limits of agreement ranging from −125.8 to 226.0. The MCA system yielded disproportionally higher CTC counts at higher mean values compared to The CellSearch system (Figure S2a). In contrast, in the analysis of agreement regarding CTC enumeration in SCLC patients, the mean difference was 202.6 (95% CI, range −116.7–521.9), with the limits of agreement ranging from −1162.0 to 1567.2. Unlike with the analysis of NSCLC blood samples, no bias was observed between the systems in the analysis of SCLC samples except for subjects with extremely high CTC titer (Figure S2b). Statistical analysis also revealed no association between site of metastasis and the CTC count of lung cancer patients using either system (data not shown).

Table 2. Patient characteristics.

		NSCLC	SCLC
No. of patients		**22**	**21**
Gender	Male	10	18
	Female	12	3
Median age		68	73
	(Range)	(36–77)	(53–83)
Smoking	Smoker	16	21
	Never-smoker	6	-
ECOG-PS	0–1	16	13
	2–4	6	8
No. of organs with metastasis	Median	2	2
	(Range)	(1–6)	(1–5)
Metastasis	Brain	9	10
	Bone	8	4
	Liver	6	6
Histology	Adenocarcinoma	14	–
	Squamous	3	–
	Others	5	–
	SCLC	–	21

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Table 3. Comparison of CTC enumeration by the CellSearch system and the MCA system.

Sample ID		CellSearch CTC (cells/7.5 mL)	MCA CTC (cells/7.5 mL)
NSCLC	1	0	0
	2	0	0
	3	9	0
	4	0	0
	5	0	5
	6	0	8
	7	2	90
	8	0	13
	9	0	13
	10	0	3
	11	1	35
	12	37	20
	13	2	246
	14	18	108
	15	0	73
	16	10	231
	17	19	20
	18	1	4
	19	0	0
	20	0	4
	21	0	291
	22	0	38
SCLC	23	200	20
	24	189	30
	25	0	13
	26	0	9
	27	0	40
	28	0	7
	29	33	23
	30	2	14
	31	3	122
	32	18	2
	33	1	2329
	34	1	2021
	35	4	13
	36	15	5
	37	325	40
	38	2	–
	39	13	36
	40	110	110
	41	0	3
	42	0	23
	43	0	109

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Morphologic Features of CTCs Isolated using the MCA System

CTCs were counted, identified as being cytokeratin positive and CD45 negative, and as having a visible nucleus on the basis of analysis of fluorescent images. As can be observed in Figure 3, which shows a representative gallery of CTCs identified by image analysis, CTCs are larger than the surrounding leukocytes and often appear in clusters, defined here as contiguous groupings of cells containing 3 or more nuclei. Figure 4 shows a solitary CTC and a CTC cluster detected in one SCLC patient using the MCA system. Using the MCA system, CTC clusters were observed in 2 of the 22 NSCLC patients (Patient No. 13 and 21) and 4 of the 21 SCLC patients (Patient No. 31, 33, 34, and 43). May-Grünwald–Giemsa staining of the CTCs isolated using the MCA system revealed that they are characterized by a high N/C ratio, nuclear molding, and morphological similarity to primary tumor cells.

Discussion

ISET systems have been found to have higher CTC detection sensitivity than the CellSearch system in several cancers, including NSCLC [17,22] However, the pores of ISET filters, which are made of polycarbonate by track etching, are randomly placed within the systems at a nonuniform density. Unlike such track-etched polycarbonate filters, the size, geometry, and density of the microcavities in the MCA system assessed in the current study are precisely controlled to achieve specific cell separation according to differences in cellular size and deformability. Aligning cells on the MCA not only eases cell imaging by allowing for the scanning of specified areas with an automated fluorescence microscope but also enables reduction in the labor required for CTC counting [29,31]. As such, the MCA system provides a platform for the use of high-throughput imaging technologies that provide more rapid and less expensive data collection as well as CTC enumeration and advanced analysis of molecular phenomena, including fluorescence in situ hybridization for detection of tumor-specific genomic changes. Furthermore, the MCA is integrated with a miniaturized device so that enrichment of CTCs from blood, as well as staining and washing in the microfluidic assay, can be performed within one integrated device.

In the present study, CTCs isolated on the MCA were successfully stained with fluorescent-labeled antibodies that target tumor cell markers, and staining and washing were found to have little or no effect on the retention of tumor cells on the microcavities. Due to its very small size, the MCA system is portable, which, by enabling point-of-care CTC counting, eliminates the need to ship blood for testing under unfavorable shipment conditions and expedites clinical decision-making. These features, in addition to our recently developed procedure for isolating single cells from the MCA using microcapillaries, allow tumor cells to be recovered from the MCA for subsequent molecular analysis of CTCs [29].

In this blind comparison of use of the MCA system to that of the conventional CellSearch System for CTC enumeration in lung cancer patients, the MCA system was found capable of isolating various lung cancer cell lines spiked within whole blood at high levels of efficiency. However, the MCA system performed isolation of SCLC cell lines slightly less efficiently compared to that of NSCLC cell lines, indicating that small (<8 μm in diameter) cells of the SCLC cell lines might pass through the microcavities during blood filtration. In a previous study [31], we found that breast (MCF-7 and Hs578T), gastric (AGS and SNU-1), and colon (SW620) tumor cells lines that include EpCAM-negative tumor cells could be successfully recovered using the MCA system with greater than 80% efficiency. However, we also found that the efficiency of recovery of small cells (average diameter 11.6 μm) of the tumor cell line SW620 to be slightly less than that of other cell lines, as we did of the SCLC cell lines examined in this study.

The MCA system assessed in the present study was found to possess a higher detection sensitivity than the CellSearch system in NSCLC CTC enumeration, suggesting the superiority of size- and deformability-based isolation techniques compared to immuno-magnetic-based techniques. The poor sensitivity of CellSearch has been attributed to the low EpCAM expression in advanced NSCLC. However, one of the NSCLC patients assessed in the present study was found to be CTC positive using the CellSearch system but CTC negative using the MCA system, indicating that

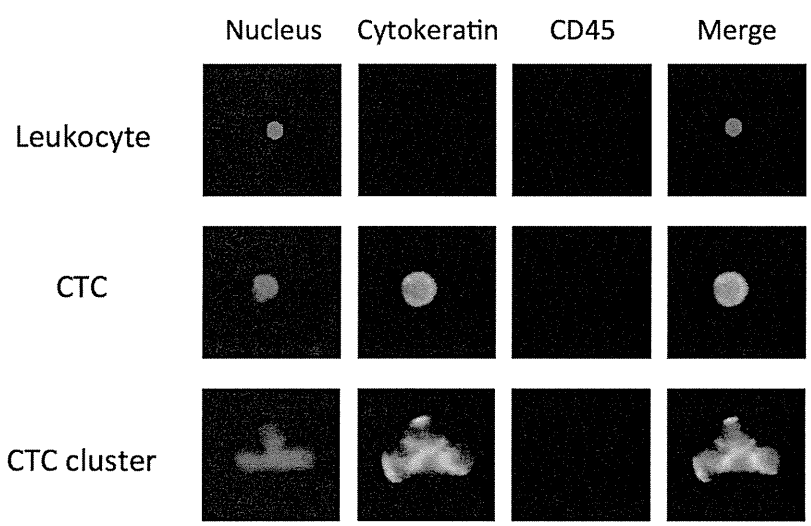

Figure 3. Gallery of cells captured on the MCA from blood of advanced lung cancer patients. Cells were stained with Hoechst 33342, FITC-labeled anti-cytokeratin antibody, and PE-labeled anti-CD45 antibody.
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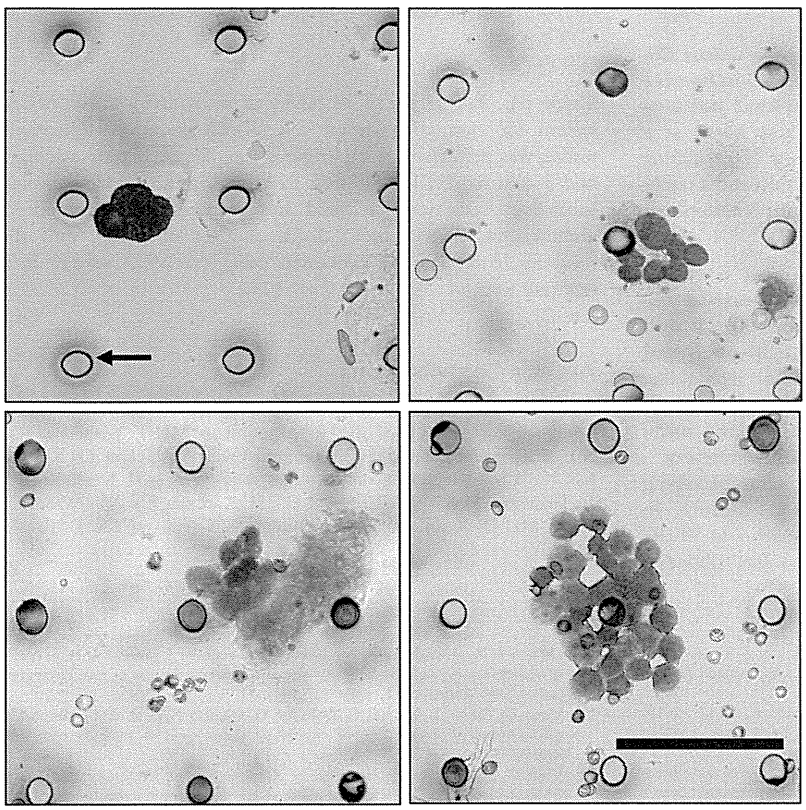

Figure 4. Gallery of CTCs captured on a transparent MCA from SCLC patient blood. May-Grünwald–Giemsa-stained cells showed a high nucleus–cytoplasm ratio and nuclear molding (×40). Black arrow indicates 9-μm microcavity. Scale bar = 60 μm.
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changes in EpCAM expression cannot solely account for the differences found between the two systems in NSCLC enumeration.

The CTC detection rate using the CellSearch system in SCLC patients was 67%, considerably higher than that in NSCLC patients and consistent with that found in previous studies [7,34–36]. Although the MCA system does not rely on EpCAM expression, which circulating SCLC cells have been reported to show high levels [37], in performing CTC isolation, its use was found to yield a high detection rate, indicating that it could be utilized for CTC detection in not only NSCLC but also SCLC patients. Nevertheless, the CTC counts of several patients were higher when analyzed using the CellSearch System compared to the MCA system, indicating that some small tumor cells in patient blood might flow through the microcavities, as described above. Previous research has suggested that immunomagnetic separation techniques lack the capacity to isolate large clusters, whereas use of size-based separation techniques leads to loss of small CTCs [17]. To address these problems, the shape of the microcavities in the MCA was modified to improve their efficiency in isolating small cells from tumor cells in whole blood in our recent study [38].

Observation of CTC clusters has been reported in various cancers, including lung cancer [23,24,39–42]. It is hypothesized that forming in clusters provides CTC cells with advantages over remaining solitary in terms of survival, proliferative capacity, and ability to form micrometastases. In this study, CTC clusters were isolated from both NSCLC and SCLC patients using the MCA system. Interestingly, the CTC-positive clusters were identified as having a small number of CTC cells by the CellSearch system but a large number by the MCA system. One reason why several SCLC patients were found to have a large CTC count when assessed by the MCA system may be that this system enables isolation of larger CTC clusters that cannot be isolated by immunomagnetic separation. Examination of this hypothesis requires further detailed analysis of the characteristics of CTC clusters, such as expression of epithelial markers and the presence of apoptotic cells within CTC clusters, which could be performed using the MCA system.

In conclusion, our results suggest that the MCA system is potentially superior to the CellSearch system in the CTC detection of lung cancer patients, with the former found capable of isolating significantly more CTCs and CTC clusters than the latter. The major limitation of this study was its examination of a small sample of patients with only one type of cancer. Further studies should thus examine larger cohorts of patients with various types of cancers to assess whether the MCA system is a more appropriate tool for CTC enumeration and characterization of metastatic tumors in patients with cancers other than lung cancer compared to other systems. We are currently planning the development of an automated MCA system that achieves robust, reliable, and reproducible sample processing for validation study using large cohorts of patients presenting at multiple institutes to assess the prognostic utility of CTC count in cancer patients.

Supporting Information

Figure S1 Comparison of cell recovery rate using the microcavity array (MCA) system and an isolation by size of epithelial tumor cell (ISET) filter. Non-small cell lung cancer cell line NCI-H358 was spiked into whole blood at a volume of 100 cells/mL to perform 3 separate tests of circulating cancer cell recovery using an MCA (pore size = 8 μm) and a track-etched polycarbonate ISET filter (pore size = 8 μm; Nucleopore).
(TIFF)

Figure S2 Bland–Altman plots of agreement between circulating tumor cell (CTC) test results obtained for non-small cell lung cancer (NSCLC; a) and small cell lung cancer (SCLC; b) patients using the CellSearch and microcavity array (MCA) systems. The solid horizontal line represents the mean difference and the dashed lines the limits of agreement (mean difference +/−2SD). In NSCLC, the mean difference was 50.1 (95%CI, 11.1 to 89.1), limits of agreement (-125.8 to 226.0) with the difference between systems becoming disproportionately greater with higher average CTC-count. In SCLC, the mean difference was 202.6 (95%CI, −116.7 to 521.9), limits of agreement (-1162.0 to 1567.2) with no bias observed between systems except for subjects with extremely high titer of CTCs.
(TIFF)

Table S1 Evaluation of sensitivity of microcavity array (MCA) system for circulating tumor cell (CTC) detection. Sensitivity testing was performed using artificial samples created by adding 1 and 3 cultured NCI-H358 cells to healthy donor blood samples. Individual cells were selected by micropipette under direct visualization, spiked into 7.5 mL aliquots of blood, and the resulting blood samples processed using the MCA system in 3 separate tests.
(DOC)

Author Contributions

Conceived and designed the experiments: MH H. Kenmotsu YK T. Yoshino TN TM NY. Performed the experiments: MH T. Yoshikawa RW SO. Analyzed the data: MH H. Kenmotsu YK T. Yoshikawa TN RW SO KM NY. Contributed reagents/materials/analysis tools: H. Kenmotsu TN T. Takahashi HM YN AT TS AO HA H. Kanbara KY NY. Wrote the paper: MH H. Kenmotsu YK T. Yoshino T. Yoshikawa TN T. Tanaka TM NY.

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Outcome of platinum-based chemotherapy for non-small-cell lung cancer patients with pleural dissemination detected during surgery

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Abstract. Pleural dissemination detected by computed tomography (CT) is considered to be unfavorable for patients with non-small-cell lung cancer (NSCLC). However, the prognosis of NSCLC patients who are diagnosed with pleural dissemination at the time of surgery has yet to be adequately elucidated. To assess the outcomes of platinum-based chemotherapy in NSCLC patients in whom pleural dissemination was detected during exploratory thoracotomy with or without a videoscope, the clinical records of NSCLC patients who were admitted to Shizuoka Cancer Center between September, 2002 and April, 2009 were reviewed. A total of 19 patients were included in this study, 12 males and 7 females, with a median age of 65 years. All patients were diagnosed with adenocarcinoma and 6 were epidermal growth factor receptor (EGFR) gene mutation-positive. The median number of treatment cycles of first-line platinum-based chemotherapy was 4 (range, 1-6 cycles) and the objective response rate was 21% [95% confidence interval (CI): 8.5-43]. The median progression-free and overall survival were 10.4 (95% CI: 6.3-18.4) and 50.5 months (95% CI: 32.5-98.0), respectively. Of the 18 patients with reported disease progression, 9 (50%) developed locoregional tumor progression. In conclusion, NSCLC patients in whom pleural dissemination is detected during surgery tend to have a favorable prognosis for survival. Systemic chemotherapy and additional local treatment may improve their clinical outcomes.

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Key words: non-small-cell lung cancer, pleural dissemination, surgery, unresectable, chemotherapy

Introduction

In early-stage non-small-cell lung cancer (NSCLC), surgical resection is the core of curative treatment. However, unexpected pleural dissemination is occasionally detected during surgery. According to TNM revisions (7th edition) and new stage groupings proposed by the International Association for the Study of Lung Cancer (IASLC), patients with pleural dissemination are classified as stage IV (1,2). Therefore, when malignant pleuritis is identified during thoracotomy, resection of the primary tumor is considered to be a contraindication and the thorax is closed without performing resection (1-5). In the majority of cases, these patients are administered systemic chemotherapy. Previous studies on platinum-based chemotherapy in patients with advanced NSCLC reported that the median progression-free survival (PFS) and overall survival (OS) were 3.1-4.8 and 11.4-15.2 months, respectively (6-8). However, there is little available data on systemic chemotherapy and prognosis for NSCLC patients with pleural dissemination detected during surgery, compared to patients who are preoperatively diagnosed as stage IV by thorough examination. The aim of this study was to assess the outcome of platinum-based chemotherapy in NSCLC patients with pleural dissemination detected during surgery who did not undergo any form of resection.

Patients and methods

Study population. The subjects of this study were non-resected NSCLC patients with pleural dissemination detected during thoracotomy. Patient selection was performed based on the clinical records of NSCLC patients who underwent thoracotomy with or without videoscope at Shizuoka Cancer Center between September, 2002 and April, 2009. Ethics approval for this study was obtained by the Institutional Review Board. Written informed consent was obtained from the patients.

Of the 681 patients who underwent thoracotomy, pleural dissemination was identified in 31 patients during exploratory thoracotomy. Of these 31 patients who did not undergo any form of resection, 19 received platinum-doublet chemotherapy. The

19 patients underwent preoperative workup, including chest X-ray, bronchoscopy, computed tomography (CT) scans of the chest and spirometry. A thorough search for distant metastases was also conducted, including magnetic resonance imaging of the brain in 17 patients (89%) and positron emission tomography scans in 18 patients (95%), prior to exploratory thoracotomy. The epidermal growth factor receptor (EGFR) gene status was assessed by the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp-based method (9).

Statistical analysis. Survival was defined as the time from surgery until death from any cause. The survival curves of the patients were calculated using the Kaplan-Meier method and statistical evaluation was performed by means of a log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Patients. A total of 19 patients whose pleural disseminated nodules were identified during surgery were included in this study. The patient characteristics are listed in Table I. The patients comprised 12 men and 7 women with a median age of 65 years (range, 48-80 years). All patients had histologically confirmed adenocarcinoma. The EGFR gene status was evaluated in 10 patients (53%), of whom 6 were mutant, 5 had exon 19 deletions and 1 had an exon 21 point mutation (L858R). Reclassification was performed according to the TNM revisions (7th edition) and the new stage groupings proposed by IASLC (1). No lymph node metastasis (N0) was diagnosed in 18 patients (95%) and N1 disease was diagnosed in 1 patient (5%). Pleural dissemination was detected in all the included patients and was confirmed by pleural biopsy during surgery. Positive pleural lavage cytology was diagnosed in 16 patients (84%). All patients were diagnosed as stage IV and did not undergo any form of resection.

Systemic chemotherapy. Patients were administered systemic chemotherapy following exploratory thoracotomy. The median time between surgery and systemic chemotherapy initiation was 17 days (range, 7-50 days). The chemotherapy regimens were as follows: 13 patients received carboplatin and paclitaxel, 2 received carboplatin and nab-paclitaxel, 1 received carboplatin and gemcitabine, 1 received carboplatin and paclitaxel plus bevacizumab, 1 received cisplatin and pemetrexed plus axitinib and 1 received cisplatin and gemcitabine. The median number of treatment cycles of first-line platinum-based chemotherapy was 4 (range, 1-6 cycles). The chemotherapy response was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (10). The overall response rate (ORR) was 21% (95% CI: 8.5-43), stable disease (SD) was observed in 69% of the patients, progressive disease (PD) in 5% and the remaining 5% of patients were not evaluable. There were no treatment-related mortalities.

Survival. The median PFS and OS were 10.4 (95% CI: 6.3-18.4) and 50.5 months (95% CI: 32.5-98.0), respectively (Fig. 1), and the 2- and 5-year survival rates were 84 and 37%, respectively. Following first-line platinum-based chemotherapy, disease progression was observed in 18 of the 19 patients. One patient has remained alive without disease progression for ~56 months; this patient is continuing bevacizumab as maintenance therapy following 6 cycles of carboplatin and paclitaxel plus bevacizumab as first-line chemotherapy. The sites of initial failure are shown in Table II. Of the 18 patients with disease progression, 9 (50%) developed locoregional tumor progression. The remaining 9 patients developed distant tumor progression and the majority had pleural disease progression.

In the EGFR-mutant group, the median PFS was 19.6 months (95% CI: 6.3-60.2). The median OS was not reached due to the limited number of mortalities. The median follow-up time was 55.3 months (range, 40.2-73.6 months) and the 2- and 5-year survival rates were 100 and 80%, respectively. In the EGFR wild-type or unknown group, the median PFS and OS were 9.8 (95% CI: 4.2-14.9) and 33.9 months (95% CI: 17.3-55.1), respectively, and the 2- and 5-year survival

Table I. Patient characteristics.

Characteristics	Values
Age, years [median (range)]	65 (48-80)
Gender	
Male	12 (63%)
Female	7 (37%)
ECOG performance status	
0	17 (89%)
1	2 (11%)
Histology	
Adenocarcinoma	19 (100%)
EGFR gene status	
Mutant	
Exon 19 deletions	5 (27%)
Exon 21 L858R	1 (5%)
Wild-type	4 (21%)
Unknown	9 (47%)
Smoking history	
Yes	11 (58%)
No	8 (42%)
Clinical T factor	
T1a	1 (5%)
T1b	7 (37%)
T2a	8 (42%)
T3	3 (16%)
Clinical N factor	
N0	18 (95%)
N1	1 (5%)
Pleural lavage cytology	
Positive	16 (84%)
Negative	1 (5%)
Not estimated	2 (11%)

ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor.

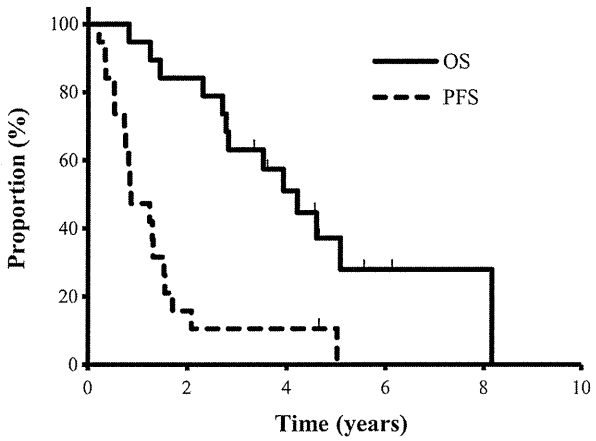

Figure 1. Kaplan-Meier curves of progression-free survival (PFS) and overall survival (OS). The median PFS and OS were 10.4 and 50.5 months, respectively.

rates were 84 and 37%, respectively. The patients positive for EGFR mutations appeared to exhibit a significantly prolonged PFS (P=0.02; HR=0.27; 95% CI: 0.07-0.81; 18 events) and OS (P=0.02; HR=0.13; 95% CI: 0.01-0.68; 13 events) compared to the patients with EGFR wild-type or unknown gene status.

Subsequent chemotherapy. The 18 patients who exhibited disease progression following first-line chemotherapy were administered systemic chemotherapy. Second-line chemotherapy regimens are listed in Table III. The 5 patients with EGFR mutations were administered anti-EGFR therapy as second-line chemotherapy. The EGFR gene status was wild-type in 5 patients and unknown in the remaining 8 patients. The second-line chemotherapy regimens were EGFR-tyrosine kinase inhibitors (EGFR-TKIs) in 5, single-agent chemotherapy in 6 and doublet chemotherapy in 2 patients.

Discussion

In this study, the prognosis for NSCLC patients who received platinum-based chemotherapy following detection of pleural dissemination during surgery was significantly better compared to that of general stage IV NSCLC patients. Previous studies reviewed the N status and histological characteristics as prognostic indicators for patients with pleural dissemination identified during surgery and suggested that N0 status was a good prognostic indicator, reporting a 5-year survival rate of 24-35% (11-14). In this study, 18 of the 19 patients (95%) were classified as N0. Thus, the N factor may be an indicator of prolonged survival. As regards histological characteristics, Mordant *et al* (11) analyzed 27 NSCLC patients with pleural malignant disease detected during thoracotomy (21 adenocarcinomas and 6 other types of NSCLC) and reported that the histological characteristics were not significantly associated with overall survival (P=0.40). All patients included in the present study had adenocarcinomas. Therefore, whether histological characteristics had a tendency towards improved survival could not be determined.

Pleural lavage cytology is also considered to be an important prognostic factor. According to the International Pleural Lavage Cytology Collaborators, a positive pleural lavage cytology result is an independent predictor of poor survival (15). It was stated that the effect on the survival of patients with positive pleural lavage cytology justified upstaging patients by one T category. In this study, pleural lavage cytology was performed in 17 patients (89%) and although only 1 patient was negative and the remaining 16 patients were positive for malignancy, the prognosis was better compared to the M1a designation of the IASLC proposals for stage grouping in the 7th edition of TNM on lung cancer (1).

With regards to the EGFR gene status, the consensus is that EGFR gene mutation is a strong predictor of a better outcome with EGFR-TKIs (16-19). Previous studies on NSCLC patients harboring EGFR mutations reported that the median PFS and OS were 9.6-10.8 and 30.5-35.5 months, respectively, with an ORR of 62.1-73.7% in patients receiving gefitinib as first-line chemotherapy, whereas the median PFS and OS were 5.4-6.6 and 23.6-38.8 months, respectively, with an ORR of 30.7-32.2%, in patients receiving platinum-based chemotherapy as first-line chemotherapy (16,17). In this study, in the EGFR mutant group, platinum-based chemotherapy was administered rather than EGFR-TKIs and the median PFS was 19.6 months. The median OS was not achieved (the median follow-up time was 55.3 months) and the ORR was 33%. The EGFR wild-type or gene status-unknown patients in this study also exhibited a longer OS compared to that previously reported (6).

Pleural dissemination indicates the systemic spread of cancer. Systemic chemotherapy is indicated for patients

Table II. Sites of initial failure.

Sites	No. (%)
Local	9 (50)
Distant	9 (50)
Pleural dissemination	7 (39)
Lung	4 (22)
Bone	2 (11)

Table III. Second-line chemotherapy.

	EGFR gene status	
	Mutant (n=5)	Wild-type or unknown (n=13)
Anti-EGFR therapy		
Gefitinib	1	5
Erlotinib	1	
Other	3	
Docetaxel		4
Pemetrexed		2
Cisplatin + Pemetrexed		1
Docetaxel + Aflibercept		1

EGFR, epidermal growth factor receptor.

with pleural dissemination detected during surgery, which is classified as stage IV with M1a disease. Chemotherapy may suppress tumor progression or micrometastasis and contribute to the improvement of survival rates. As regards local treatment, previous studies supported surgery as an option for NSCLC patients with malignant pleuritis detected during thoracotomy, suggesting that it was beneficial to survival (11,12,14,20-23). Furthermore, Mordant *et al* (11) reported a long-term survival of 16% and suggested that identification of clinical T1-2N0 NSCLC with previously undiagnosed pleural malignant metastatic disease may justify surgery as part of a multimodality treatment. In this study, all the patients were administered systemic chemotherapy, but none underwent resection. One patient underwent thoracic radiotherapy [stereotactic radiotherapy (SRT)] following platinum-doublet chemotherapy. That patient, whose EGFR gene status was mutant, was administered carboplatin and paclitaxel as first-line chemotherapy and the best overall response was SD. The tumor size remained unchanged for 8 months after the last administration of chemotherapy and SRT were performed as a curative attempt. The PFS of the patient was 60.2 months, whereas the median PFS was 10.1 months (95% CI: 6.3-15.7) in the remaining 17 patients. Of the 18 patients, 9 developed initial failure as local progression. A multimodality treatment centered on systemic chemotherapy following local treatment, such as limited resection or radiation, may improve the prognosis of such patients.

In conclusion, platinum-based chemotherapy for NSCLC patients with pleural dissemination detected during surgery demonstrated a favorable prognosis for survival. It is recommended that NSCLC patients with previously undiagnosed pleural dissemination are classified as a special group, for whom systemic chemotherapy followed by local treatment may improve clinical outcomes. Additional multimodality treatment trials are required in this population.

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RESEARCH ARTICLE Open Access

Prognostic impact of serum CYFRA 21–1 in patients with advanced lung adenocarcinoma: a retrospective study

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Abstract

Background: Serum CYFRA 21–1 is one of the most important serum markers in the diagnosis of non-small cell lung cancer (NSCLC), especially squamous-cell carcinoma. However, it remains unknown whether pretreatment serum CYFRA 21–1 values (PCV) may also have prognostic implications in patients with advanced lung adenocarcinoma.

Methods: We retrospectively reviewed the data of 284 patients (pts) who were diagnosed as having advanced lung adenocarcinoma and had received initial therapy.

Results: Of the study subjects, 121 pts (43%) had activating epidermal growth factor receptor (EGFR) mutations (Mt+), while the remaining 163 pts (57%) had wild-type EGFR (Mt-). Univariate analysis identified gender (male/ female), ECOG performance status (PS) (0-1/ ≥2), PCV (<2.2 ng/ml/ ≥2.2 ng/ml), EGFR mutation status (Mt+/ Mt-), pretreatment serum CEA values (<5.0 ng/ml/ ≥5.0 ng/ml), smoking history (yes/ no) and EGFR-TKI treatment (yes/ no) as prognostic factors ($p = .008$, $p < .0001$, $p < .0001$, $p < .0001$, $p = .036$, $p = .0012$, $p < .0001$ respectively). Cox's multivariate regression analysis identified PCV < 2.2ng/ml as the only factor significantly associated with prolonged survival ($p < .0001$, hazard ratio: 0.43, 95% CI 0.31-0.59), after adjustments for PS ($p < .0001$), EGFR mutation status ($p = .0069$), date of start of initial therapy ($p = .07$), gender ($p = .75$), serum CEA level ($p = .63$), smoking history ($p = .39$) and EGFR-TKI treatment ($p = .20$). Furthermore, pts with Mt+ and PCV of <2.2 ng/ml had a more favorable prognosis than those with Mt+ and PCV of ≥2.2 ng/ml (MST: 67.0 vs. 21.0 months, $p < .0001$), and patients with Mt- and PCV of <2.2 ng/ml had a more favorable prognosis than those with Mt- and PCV of ≥2.2 ng/ml (MST: 24.1 vs. 10.2 months, $p < .0001$).

Conclusion: PCV may be a potential independent prognostic factor in both Mt+ and Mt- patients with advanced lung adenocarcinoma.

Keywords: Lung adenocarcinoma, Prognostic factor, CYFRA 21–1, CEA, EGFR mutation, Tumor heterogeneity, EGFR-TKI, Chemotherapy

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Background

Lung cancer is the leading cause of cancer death, and at present, there exists no cure of stage IV non-small cell lung cancer (NSCLC) [1]. Adenocarcinoma and squamous cell carcinoma are the most common histological subtypes of lung cancer and account for about 70% of all lung cancers [2]. The folate antagonist pemetrexed has been shown to exhibit efficacy against non-squamous cell lung cancers [3], and is currently used in combination with cisplatin as a standard treatment regimen for patients with non-squamous cell lung carcinoma. Chemotherapy with the angiogenesis inhibitor bevacizumab administered in combination with platinum agents has also been shown to exhibit favorable efficacy against non-squamous cell lung carcinoma [4,5]. Somatic gain-of-function mutations in exons encoding the EGFR tyrosine kinase domain have been identified in NSCLC [6,7]. Several previous studies have reported prolongation of the survival time in patients with EGFR-mutation-positive lung carcinomas treated with EGFR-tyrosine kinase inhibitors (TKIs) [8-11], therefore, EGFR-TKIs are widely used in medical practice. EGFR mutations occur more frequently in lung cancer patients who are Asians, females and non-smokers with the histological subtype of adenocarcinoma [12-14]. On the other hand, while there have also been scattered reports of EGFR mutations among cases of lung squamous-cell carcinoma [15-17], a recent report showed that there were no EGFR mutation-positive cases among lung cancer patients with pure squamous cell carcinoma [18,19].

CYFRA 21–1 is a fragment of cytokeratin (CK) 19. CKs, which are now called keratins, are the principal structural elements of the cytoskeleton (keratin filaments) of epithelial cells, including bronchial epithelial cells, and have been classified into 20 subtypes based on differences in the molecular mass and isoelectric point as determined by 2-dimensional electrophoresis [20,21]. CK types 1–8 are categorized as type I CKs, and CKs 9–20 as type II CKs. Microfilaments are heteropolymers formed from type I and type II keratins, and constitute the cytoskeleton [22]. CK19 is a soluble type I CK (acidic type), and has the lowest molecular mass (40 kDa) among the CKs. It is expressed in the unstratified or pseudostratified epithelium lining the bronchial tree [23], and been reported to be overexpressed in many lung cancer tissue specimens [24]. The CK expression patterns in tissues are well-maintained even during the process of transformation of the tissue from normal to tumor tissue [25]. Accelerated CK19 degradation occurs in neoplastically transformed epithelial cells as a result of increased protease activity of caspase 3, a regulator of the apoptosis cascade, and fragments are released into the blood. This results in an increase of the blood CYFRA 21–1 values, because CK19 fragments are recognized by two monoclonal antibodies [26].

Measurement of serum CYFRA 21–1 level is a useful auxiliary test in the diagnosis of NSCLC, and particularly high specificity of this test has been reported for the diagnosis of squamous cell carcinoma of the lung [27,28]. On the other hand, a meta-analysis also revealed that serum CYFRA 21–1 may be a useful prognostic factor in NSCLC patients [29]; analysis of the histological background in the aforementioned meta-analysis showed that non-adenocarcinoma accounted for the majority of cases of NSCLC (65%). There has also been a report suggesting that serum CYFRA 21–1 levels might serve as a prognostic factor in patients with recurrent NSCLC receiving 3rd-line or later gefitinib therapy [30]. Some studies have suggested the possible prognostic value of pretreatment serum CYFRA 21–1 values (PCV) in patients with surgically treated lung adenocarcinoma [31] and advanced NSCLC [32-34]. However, none of the studies suggesting serum CYFRA 21–1 as a prognostic factor in patients with untreated advanced lung adenocarcinoma has included the EGFR mutation status as a variable. Therefore, in the present study, we investigated the impact of serum CYFRA 21–1 on the prognosis of untreated advanced lung adenocarcinoma patients.

Methods

Patients

Of patients diagnosed as having primary lung carcinoma between January 2003 and June 2010 at the Shizuoka Cancer Center, EGFR mutation analysis was performed on 424 patients from April 2008 to June 2010. Of these, 284 lung adenocarcinoma patients had received initial therapy, and we retrospectively reviewed the data of the 163 patients who were found to harbor wild-type EGFR and 121 patients who were found to harbor activating EGFR mutations (Figure 1). The following inclusion criteria were set for this study; patients with pathologically proven adenocarcinoma who had received initial therapy (including chemotherapy or chemoradiotherapy) and survived for more than one month; Eastern Cooperative Oncology Group performance status (ECOG PS) of 3 or less. The histological and cytological diagnoses were performed according to the WHO classification criteria [35]. The study was conducted with the approval of the Shizuoka cancer center Institutional Review Board #1 (HHS IRB registration number; IRB00006744).

We outsourced some of the clinical laboratory tests, such as measurement of the tumor markers and EGFR mutation analysis. Serum CYFRA 21–1 and serum CEA concentrations were measured at the baseline, before the initial therapy. The serum CYFRA 21–1 concentration was measured using a Lumipulse Presto® kit (FUJIREBIO Inc, Tokyo, Japan), based on a CLEIA (chemiluminescent enzyme immunoassay) method, while the serum CEA concentrations were measured using an ARCHITECT® kit

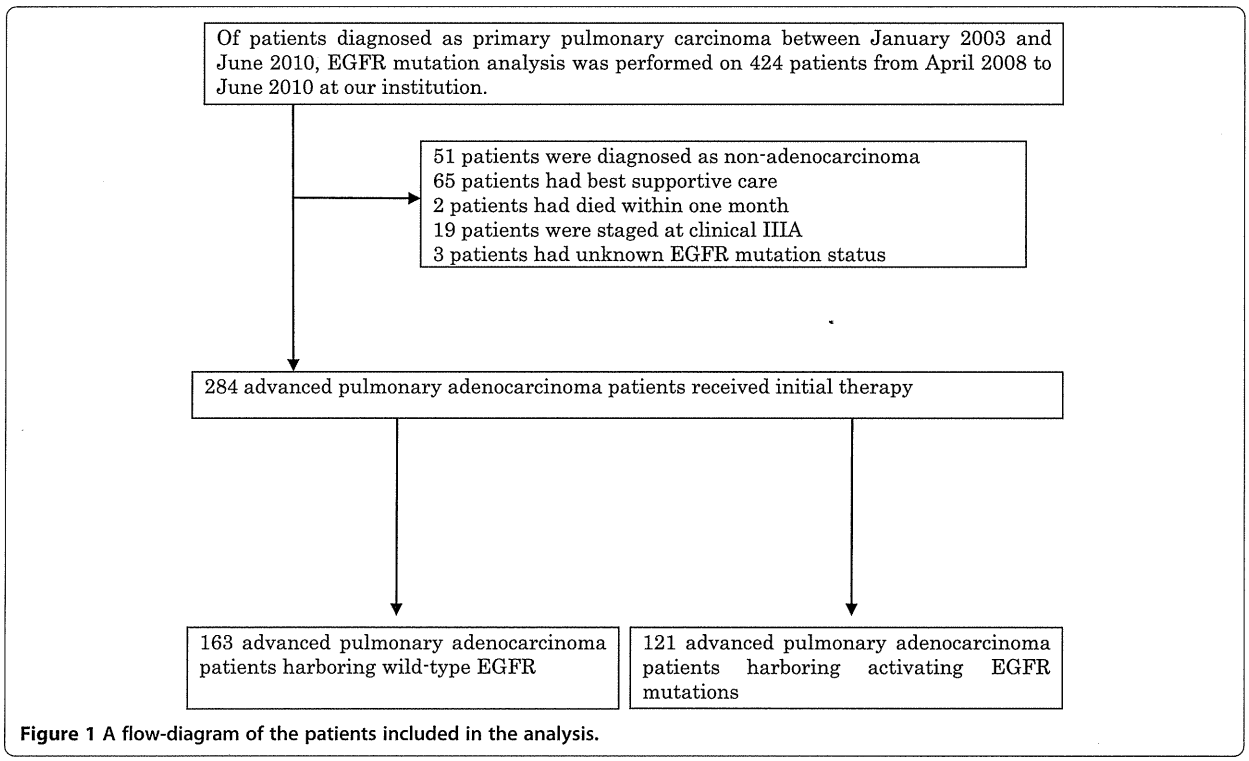

Figure 1 A flow-diagram of the patients included in the analysis.

(Abbott Japan, Tokyo, Japan). EGFR mutation analysis was performed by fragment analysis using polymerase chain reaction (PCR) and the cycleave real-time quantitative PCR technique (SRL Inc, Tokyo, Japan).

The reported upper limit of normal for the diagnosis of NSCLC and upper limit of the percentiles for healthy individuals of serum CYFRA 21–1 as measured by EIA are 3.5 ng/ml and 2.8 ng/ml, respectively [36]. In contrast, the reported upper limit of the percentiles for healthy individuals of serum CYFRA 21–1 measured by the CLEIA method is 1.6 ng/ml [37], a lower value as compared to that set for measurement by the EIA method. Therefore, for our study, we set the cutoff value for CYFRA 21–1 at 2.2 ng/ml, based on the mean value for healthy subjects + 3SD [37], a lower value as compared to that set for measurement by the EIA method. The cutoff value for serum CEA was set at 5.0 ng/ml, which is the upper limit of normal.

A standard evaluation of the patients, including assessment of the medical history, physical examination and routine laboratory tests, was performed before each treatment. All patients were staged based on the International Association for the Study of Lung Cancer (IASLC) TNM (tumor-node-metastasis) classification, 7th edition [38].

Statistical methods

There were no missing data in our study. Survival was estimated using the Kaplan-Meier method. Overall survival was measured from the date of the first course of the initial therapy to the date of death or that of the last follow-up examination. A log-rank test was performed to evaluate the significance of differences in the overall survival among the groups. P values < 0.05 were considered to be indicative of statistical significance. A multivariate analysis using the Cox proportional hazards model was used to establish the association between the clinical variables and survival. All statistical analyses were carried out using SPSS, version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). To reduce the potential bias arising from some patients dying too early to receive initial therapy, the two patients who died within a month (30 days) of the start of initial therapy were excluded from the analysis.

Results

The cohort consisted of 284 patients who were diagnosed as having stage IIIB or IV lung adenocarcinoma and had received initial therapy.

The clinical characteristics of the patients are summarized in Table 1. The median patient age prior to the start of initial therapy was 65 years (range, 23 to 87 years). The patients were predominantly younger than 70 years of age (81%), the ECOG PS was 0–2 in 93% of patients, and 91% of the patients had stage IV disease. While the lung adenocarcinoma patients with EGFR mutations were predominantly female (64%) and non-smokers

Table 1 Patient characteristics

Characteristic	Mt + (n= 121)		Mt – (n= 163)		All (n= 284)	
	No.	%	No.	%	No.	%
Age, years						
Median (range)	66 (32–87)		65 (23–83)		65 (23–87)	
< 70	97	80	134	82	231	81
≥ 70						
	24	20	29	18	53	19
Gender						
Male	43	36	125	77	168	59
Female	78	64	38	23	116	41
ECOG PS						
0-1	103	85	135	83	238	84
> 2	18	15	28	17	46	16
Smoking status						
Yes	50	41	124	76	174	61
No	71	59	39	24	110	39
Stage						
IIIB	6	5	19	12	25	9
IV	115	95	144	88	259	91
EGFR mutation						
Exon 19 deletion	59	49			59	21
Exon 21 L858R	57	47			57	20
Exon 18 G719X	5	4			5	2
Wild type			163	100	163	57
PCV						
Median (range)	1.6 (0.1-110.0)		2.3 (0.1-80.0)		2.0 (0.1-110.0)	
< 2.2 ng/ml	72	60	78	48	150	53
≥ 2.2 ng/ml	49	40	85	52	134	47
CEA						
Median (range)	8 (0.7-11942)		7 (0.5-14985)		7.4 (0.5-14985)	
< 5.0 ng/ml	45	37	63	39	108	38
≥ 5.0 ng/ml	76	63	100	61	176	62

EGFR: epidermal growth factor receptor, Mt+: mutant EGFR, Mt-: wild-type EGFR, PCV: pretreatment CYFRA 21–1 value.

(71%), those with wild-type EGFR were predominantly male (77%) and smokers (76%).

Details about the first-line chemotherapy were available for 284 patients including both patient groups with wild-type (Mt-) and mutant EGFR (Mt+) groups (Table 2). About 40% of the EGFR mutation-positive patients received EGFR-TKIs as the initial treatment.

Carboplatin-paclitaxel, the treatment of choice across both groups, was administered to half of the platinum doublet cohort in the Mt- patient group. Meanwhile, docetaxel was administered to half of the monotherapy cohort in the same patient group. However, cisplatin-pemetrexed was the most common regimen of second choice across both the Mt+ and Mt- groups.

The EGFR-TKI used for each treatment line in the Mt+ group is shown in Table 3. Forty-one (58%) patients received gefitinib, while 16 (22%) received erlotinib as first- or second-line treatment in the Mt+ group with PCV (<2.2 ng/ml). Thirty-seven (73%) patients received gefitinib, and 10 (20%) patients received erlotinib as first- or second-line treatment in the Mt+ group with PCV (≥2.2 ng/ml). Of the 121 patients in the Mt+ group, 27 did not receive gefitinib at any treatment-line stage of treatment; among these 27 patients, 19 received erlotinib (6 as first-line, 10 as second-line, 1 as third-line and 2 as further-line treatment). In the Mt+ group, a total of 113 patients (93%) received EGFR-TKIs, while 8 patients did not receive EGFR-TKIs at any stage of treatment.

Table 2 Summary of initial treatment delivered among 284 patients

EGFR mutation	Mt – (n= 163)				Mt + (n= 121)			
	IIIB		IV		IIIB		IV	
	(n= 19)		(n= 144)		(n= 6)		(n= 115)	
	No.	%	No.	%	No.	%	No.	%
Treatment								
Platinum doublet	4	3	114	70	2	2	54	45
Monotherapy	0		30	18	0		11	9
EGFR-TKI	0		0		0		50	41
Chemoradiotherapy	15	9	0		4	3	0	
Specific regimens								
Cisplatin-pemetrexed	1		24	15	1		9	7
Carboplatin-paclitaxel	3		52	32	0		27	22
Carboplatin-paclitaxel+ bev	0		2		0		2	
Other platinum doublets	0		36	22	1		12	10
Gefitinib	0		0		0		41	34
Erlotinib	0		0		0		7	6
Docetaxel	0		16	10	0		3	
Vinorelbine	0		5		0		2	
Others	0		24	15	0		6	

Mt+: mutant EGFR, Mt-: wild-type EGFR, bev: bevacizumab.

Furthermore, of the 160 patients in the Mt- group, 30 patients received EGFR-TKIs (11 as second-line, 7 as third-line, 6 as fourth-line, 3 as fifth-line, 1 as sixth-line, 1 as seventh-line, and 1 as eighth-line treatment). Fifty-three patients (18%) were still alive at the time of the analysis. The median follow-up period for determining the survival was 39.3 (range; 11.8-84.9) months after the start of initial therapy. The clinical variables identified by univariate analysis to be associated with significantly better survival (Table 4) included female gender (MST 32.4 months versus 20.1 months in males: p = .0086), no smoking history (33.4 months versus 20.1 months in smokers, p = .0012), ECOG PS (0–1) (29.5 months versus 7.9 months in those with a PS of 2–3, p < .0001), presence of EGFR mutation (39.2 months versus 17.8 months in patients without EGFR mutations, p < .0001), PCV < 2.2 ng/ml (38.6 months versus 15.0 months in those with PCV ≥ 2.2 ng/ml, p < .0001), serum CEA < 5.0 ng/ml (32.6 months versus 21.0 months in those with serum CEA ≥ 5.0 ng/ml, p = .036), start date of initial therapy before April 1, 2008 (34.1 months versus 19.3 months in the group that received the initial therapy after April 1, 2008, p = .003) and EGFR-TKI treatment (33.7 months versus 15.3 months in the group not treated with EGFR-TKIs, p < .0001). Multivariate analysis identified EGFR mutation positivity (HR 0.53; 95% CI: 0.34-0.84, p = .0069) and PCV < 2.2 ng/ml (HR 0.43; 95% CI: 0.31-0.59, p < .0001) as independent favorable prognostic factors. Another factor that was found to be an independent prognostic indicator of overall survival was the PS (Table 4). The overall survival rates of patients with advanced lung adenocarcinoma with/ without EGFR mutation are shown in Figure 2. Among the Mt+ patients, the prognosis was more favorable in the group with PCV < 2.2 ng/ml (n = 70) than in the group with PCV > 2.2 ng/ml (n = 48) (median survival time [MST]: 67.0 vs. 21.0 months, p < 0.0001). Among the patients with Mt- also, the prognosis was more favorable in the group with PCV < 2.2 ng/ml (n = 78) than in the group with PCV ≥ 2.2 ng/ml (n = 86) (MST: 24.1 vs. 10.2 months, p < 0.0001).

Table 3 Summary of EGFR-TKI delivered among EGFR mutation positive patients

	EGFR mutation positive							
	Low PCV				High PCV			
	(< 2.2 ng/ml) (n= 72)				(≥ 2.2 ng/ml) (n= 49)			
	Gefitinib		Erlotinib		Gefitinib		Erlotinib	
	No.	%	No.	%	No.	%	No.	%
First-line	20	28	5	7	23	47	2	4
Second-line	21	29	11	15	14	29	8	16
Third-line	9	12	6	8	3	6	4	8
Further-line	2	3	14	20	2	4	4	8
Unadministered	20	28	36	50	7	14	31	63

PCV: pretreatment CYFRA 21–1 value.

Discussion

In the present study, we demonstrated PCV and EGFR mutation status as independent prognostic factors in untreated advanced lung adenocarcinoma patients. We also showed that PCV < 2.2 ng/ml was a predictor of a favorable outcome in both advanced lung adenocarcinoma patients with wild-type and mutant EGFR.

Serum CYFRA 21–1 has been reported as a prognostic factor in patients with a variety of cancer types, including resectable NSCLC [39,40], biliary tract cancer [41], urothelial cancer [42], head and neck cancer [43], esophageal cancer [44], and cervical cancer [45].

A meta-analysis of CYFRA 21–1 as a prognostic indicator in advanced NSCLC patients showed that the PCV may be a reliable prognostic factor [29], however, since non-adenocarcinoma accounted for 65% of the cases and squamous cell carcinoma for 50%, the role of serum CYFRA 21–1 as a prognostic indicator in the lung adenocarcinoma population remained unclear. Moreover, in a study of PCV as a prognostic indicator in advanced NSCLC patients in whom gefitinib was used as 3rd-line or later therapy, adenocarcinoma accounted for fewer than a half of the cases (47%) [30]. The EGFR mutation status was not included as a variable in the analysis, and the test population was small, consisting of only 50 patients.

Table 4 Variables associated with overall survival among 284 patients

Co-variable	No.	Univariate analysis		Multivariate analysis			
		MST (months)	P	Variate	OR	95% CI	P
Age							
< 70	231	22.8					
> 70	53	24.3	0.625				
Gender							
Male	168	20.1					
Female	116	32.4	0.0086	Female	1.06	0.75-1.58	0.75
Smoking status							
Yes	174	20.1					
No	110	33.4	0.0012	No smoking status	0.84	0.52-1.24	0.39
ECOG PS							
0-1	238	29.5					
>2	46	7.9	<.0001	PS 0-1	0.34	0.24-0.50	<.0001
Stage							
IIIB	25	30.2					
IV	259	22.5	0.269				
EGFR mutation							
Mt (+)	121	39.2					
Mt (–)	163	17.8	<.0001	Mutant EGFR	0.53	0.34-0.84	0.0069
PCV							
< 2.2 ng/ml	150	38.6					
≥ 2.2 ng/ml	134	15.0	<.0001	< 2.2 ng/ml	0.43	0.31-0.59	<.0001
CEA							
< 5.0 ng/ml	108	32.6					
≥ 5.0 ng/ml	176	21.0	0.036	< 5.0 ng/ml	0.93	0.67-1.26	0.63
Start dates of IT							
Before 1/ 4/ 2008	79	34.1		After 1/ 4/ 2008			
After 1/ 4/ 2008	205	19.3	0.0030		0.73	0.50-1.15	0.07
EGFR-TKI treatment							
Yes	143	33.7					
No	141	15.3	<.0001	Yes	0.76	0.50-1.15	0.20

IT: initial therapy, PCV: pretreatment CYFRA 21–1 value, Mt(+): mutant EGFR, M(–): wild-type EGFR.

Several factors may have contributed to identification of serum CYFRA 21–1 as a prognostic indicator in the advanced lung adenocarcinoma population in the present study. First, there could be a relationship between the serum levels of CYFRA 21–1 and the microfilament formation trend in the tumor cells [22]. CKs are the principal structural elements of intracellular microfilaments. Microfilaments have been shown to be heteropolymers formed from type I and type II keratins which form the cytoskeleton. Moreover, while the CKs (CKs 1, 2, 10/11), on which the degree of keratinization within tumors depends, are strongly expressed in well-differentiated squamous cell carcinomas, they are not detected in the serum. The possibility that they are preferentially removed by macrophages because of their poor solubility has been suggested as the reason for the failure to detect them in the serum [46]. By contrast, soluble CK19 is degraded by tumor lysis and tumor necrosis and released into the blood. Therefore, serum levels of CK19 may indicate the degree of cytoskeleton formation by microfilaments within the tumor cells. Second, there may also be a relationship between serum CYFRA 21–1 levels and the degree of tumor differentiation towards squamous epithelium. CKs with a relatively high molecular mass tend to be associated with differentiation into squamous cell carcinoma, while CKs with a relatively low molecular mass tend to be associated with differentiation into adenocarcinoma [47]. In a study in

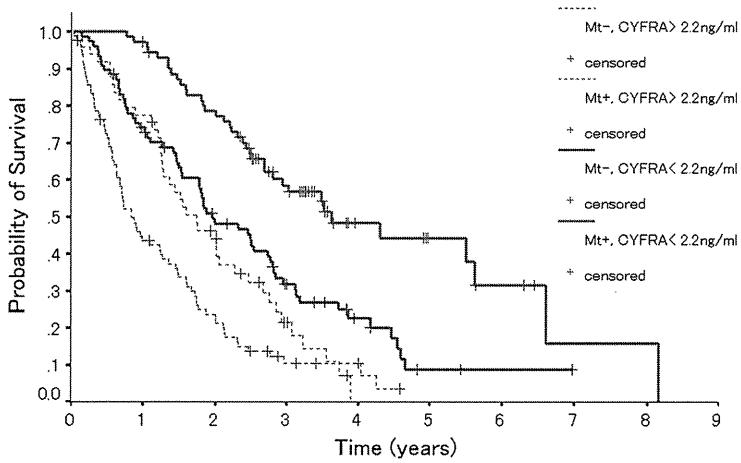

Figure 2 Kaplan-Meier curves for overall survival in four groups, EGFR mutation status- stratified by PCV. M+: mutant EGFR, M-: wild-type EGFR, PCV: pretreatment CYFRA 21–1 value.

which monoclonal antibodies were used, the number of cells containing CK19 increased with decreasing degree of differentiation into squamous cell carcinoma, and the presence of intracellular CK19 was consistently demonstrated in pure lung adenocarcinomas [25]. On the other hand, a negative correlation between intracellular CK19 expression and serum CYFRA 21–1 levels has also been shown [24]. Increase in the serum level of CYFRA 21–1 may also be the result of a greater degree of degradation and release of intracellular CK19 into the serum with an increasing tendency towards differentiation into squamous cell carcinoma.

Because identical EGFR mutations have been seen in both the adenocarcinoma component and squamous cell carcinoma component in resected cases of adenosquamous carcinoma [48], it has been suggested that the two components may arise from a single clone [48,49]. Resected cases of adenosquamous carcinoma have been reported to account for 3% of all cases of NSCLC [50], and adenosquamous carcinoma patients have also been reported to have a poor prognosis [51]. The prognosis of patients in whom the tumor tissue consists of a mixture of mutant EGFR cells and wild-type EGFR cells has been reported to be inferior to that of patients with tumors consisting of only mutant EGFR cells, and intratumor heterogeneity has also been investigated [52]. On the other hand, there is a report suggesting that no intratumor heterogeneity of EGFR expression is found in mutant EGFR lung adenocarcinomas, and also that no disparity is found between the EGFR mutation status of the primary tumor and lymph node metastasis [53].

There are several limitations of the present study. The first is that it was a retrospective study conducted at a single institution, and the possibility of a selection bias is undeniable. The prognosis of patients who received initial therapy before April 1, 2008 was significantly superior to that of those who received their initial therapy after 2008. Because we started to perform EGFR mutation analysis in routine clinical practice from April 1, 2008, there is the possibility of a selection bias towards patients who received the initial therapy before April 1, 2008. This is one of the major limitations of our retrospective study. Some studies have reported that EGFR mutations may be a positive prognostic factor for survival in advanced NSCLC patients, regardless of EGFR-TKI therapy [54,55]. Also in the BR.21 trial, the median survival time was reported to be longer in patients with mutant EGFR as compared to that in patients with wild-type EGFR [56]. Although mutant EGFR patients not treated with EGFR-TKIs were found to be a confounding factor, we performed adjustment for the confounding factor using a Cox proportional hazards model. According to the univariate analysis, the date of start of the initial therapy (before April 1, 2008) was a favorable prognostic factor. However, PCV < 2.2 ng/ml, EGFR mutation positivity and PS 0–1 were found to be independent favorable prognostic factors after adjustment for the date of start of the initial therapy. In this study, while the MST (39.2 months) in the mutant EGFR group was not favorable as compared to previous reports [57], the mutant EGFR group with PCV < 2.2 ng/ml had a more favorable prognosis than that of the mutant EGFR group with PCV ≥ 2.2 ng/ml. The proportion of patients who received erlotinib was less in the group with PCV ≥ 2.2 ng/ml than in the group with PCV < 2.2 ng/ml, which could have influenced the more favorable prognosis in the group with PCV < 2.2 ng/ml than in the group with PCV ≥ 2.2 ng/ml. All of the patients with advanced lung adenocarcinoma in whom the diagnosis was made after April 1, 2008 were tested for EGFR mutations at

the time of the diagnosis, whereas in the patients with other histological types of lung cancer, the testing was performed at the discretion of the attending physician. Second, the follow-up period was inadequate, especially in the mutant EGFR group with PCV < 2.2 ng/ml, and the censored cases were conspicuous. There was also a problem with the stage distribution (there were relatively few stage IIIB cases). Distant metastasis occurred in all of the stage IIIB cases in which local treatment had been performed, and all of the patients with disease recurrence were tested for EGFR mutations. Moreover, significant survival differences in stage IIIB/ IV were not found in the univariate analysis. Furthermore, the treatment regimens used in the stage IV cases were not standardized, with each of the attending physicians administering any of the various standard treatments used in routine clinical practice recommended by the guidelines of the Japan Lung Cancer Society.

In advanced lung adenocarcinoma, which may be considered as a generalized systemic disease, it may be particularly difficult to determine the characteristics of an entire heterogeneous tumor by tissue diagnosis alone based on examining just one part of the tumor. Based on the results of the present study, we propose that mutant EGFR patients with serum PCV < 2.2 ng/ml have a better prognosis than the mutant EGFR patients with higher PCV.

Conclusions

The potential applications of PCV measurements might include identification of candidates in whom it might have some prognostic value. Furthermore, PCV might be regarded as a routine demographic variable having prognostic value in patients with advanced lung adenocarcinoma.

Abbreviations

NSCLC: Non-small cell lung cancer; PCV: Pretreatment serum CYFRA 21–1 levels; pts: patients; EGFR: Epidermal growth factor receptor; Mt+: Mutant EGFR; Mt-: Wild-type; TKI: Tyrosine kinase inhibitor; CK: Cytokeratin; ECOG PS: Eastern Cooperative Oncology Group performance status; CLEIA: Chemiluminescence enzyme immunoassay; PCR: Polymerase chain reaction; IASLC: International Association of the Study of Lung Cancer; TNM: Tumor-node-metastasis.

Competing interests

The authors have no competing interests to declare.

Authors' contributions

AO contributed to the drafting of this manuscript and data collection, and KM contributed to the study design and statistical analysis. TT, HA, TS, TT, HK, TN, HM, TN, ME, NY contributed to analysis of the data and interpretation of the findings. All authors have read and approved of the submission of the final manuscript.

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BRIEF ARTICLE

Long-term survival of more than 3 years among patients with advanced non-small cell lung cancer treated with chemotherapy

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Abstract

AIM: To evaluate the prognostic factors of long-term survival of more than 3 years in patients with advanced non-small cell lung cancer (NSCLC).

METHODS: We retrospectively analyzed the records of 474 patients with advanced ⅢB/Ⅳ NSCLC who received chemotherapy as initial treatment between September 2002 and March 2007.

RESULTS: The median survival time (MST) was 12.5 mo and the 3 year and 5 year survival rates were 14.6% and 5.3%, respectively. Long-term survival of more than 3 and 5 years was observed in 65 and 16 patients, respectively. The MST for the 65 patients was 61.5 mo (range, 60.1-81.0 mo). In the 474 patients, a good performance status (PS), female sex, non-smoking status and adenocarcinoma histology were significantly associated with a favorable outcome. Furthermore, female sex, a good PS, non-smoking status and adenocarcinoma histology were significantly correlated with long-term survival of more than 3 years and most of these patients (89.2%, 58/65) received epidermal growth factor receptor-tyrosine kinase inhibitors as any line treatment. Survival analysis of long-term survivors showed that a PS of 0 was an independent prognostic factor for predicting favorable outcomes.

CONCLUSION: Our results suggest that a good PS and adenocarcinoma histology play an important role in long-term survival of more than 3 years. A PS of 0 was an independent prognostic factor for predicting favorable outcomes in patients with advanced NSCLC who survived for more than 3 years.

Key words: Non-small cell lung cancer; Long-term survivor; Chemotherapy; Performance status; Epidermal growth factor receptor-tyrosine kinase inhibitors

Core tip: The aim of this study is to evaluate the prognostic factors of long-term survival of more than 3 years in advanced non-small cell lung cancer. Female sex, good performance status (PS), non-smoker and adenocarcinoma were significantly associated with long-term survivors of more than 3 years and most patients received epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) at any line treatment. PS of 0 was an independent prognostic factor for predicting favorable prognosis in the long-term survivors of more than 3 years. PS of 0, adenocarcinoma and EGFR-TKI therapy play an important role in the