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Clustered Circulating Tumor Cells in Lung Adenocarcinoma: Implications of Test Results of Continuous Variables

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Abstract. *Background/Aim: We investigated the relationship between solid component size (SS), carcinoembryonic antigen (CEA), and standardized uptake value (SUVmax) as continuous variables and postoperative clustered circulating tumor cell (C-CTC) detection in patients with pulmonary adenocarcinoma who underwent surgery. Patients and Methods: C-CTC detection was the main evaluation item, which was analyzed using the receiver operating characteristic curve to calculate areas under the curves (AUCs) for the variables. Additionally, the two-year recurrence-free survival rates (2Y-RFSRs) were analyzed. Results: Among the 84 patients examined, SS, CEA, and SUVmax had AUCs>0.7, and were independent. Their thresholds were 2.1 cm, 7.5 ng/ml, and 2.9, respectively. The 2Y-RFSR were significantly better in the non-C-CTC group (n=58) and in the group of patients without high levels of these predictors (n=32). Conclusion: SS, CEA level, and SUVmax predicted postoperative CTC detection in pulmonary adenocarcinoma patients.*

Lung cancer is a common malignancy with a poor prognosis (1), and surgical resection is one of its treatment options. Most of the cases treated by surgery are pulmonary adenocarcinomas (2). A lot of progress is being made in research on pulmonary adenocarcinomas. Associated prognostic factors include the whole tumor size (WTS) measured using computed tomography (CT), solid component size (SS), solid component ratio (SR) (3), serum carcinoembryonic antigen (CEA) level (4), and the standardized uptake value (SUV) max measured by fluorodeoxyglucose-position emission tomography (FDG-PET) (5). These are continuous variables that fluctuate and usually increase with the progression of lung cancer.

Many metastases of carcinomas occur through peripheral blood circulatory cells [circulating tumor cells (CTCs)] (6). Clustered CTCs (C-CTCs) have a high potential to form metastatic lesions (6), and their detection is a poor prognostic factor in patients undergoing lung cancer surgery (7, 8). Some studies have shown these tumor-related continuous variables to be prognostic factors (3-5), and they can be predictors of C-CTC detection, which is a precursor of metastasis. However, the relationship between C-CTC detection and these tumor-related continuous variables remains unclear. Therefore, we examined the relationship between these continuous variables and C-CTC detection in patients with lung adenocarcinoma after surgery.

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Key Words: Pulmonary adenocarcinoma, clinical stage I, surgery, circulating tumor cell, recurrence.

Patients and Methods

This study was approved by the "Medical Ethics Committee" of Nara Medical University Hospital (No. 1718) and Hoshigaoka Medical Center (No. 1412). All the study participants provided their consent.

The primary outcome was C-CTC detection performed immediately after resection of the pulmonary lesion. The data of patients with clinical stage I lung adenocarcinoma who underwent surgery between 2015 and 2018 at the study sites were examined retrospectively.

CTC extraction was performed using the micropore size-selection method (ScreenCell®, Westford, MA, USA). CTCs were observed under a microscope. Groups of at least four tumor cells were considered C-CTCs, while those occurring in groups of less than four cells were considered single cells. Patients with C-CTCs formed the C-CTC group, while patients in whom single CTCs or no CTCs were detected formed the non-C-CTC group. The CTCs were diagnosed using the CTC atlas (9).

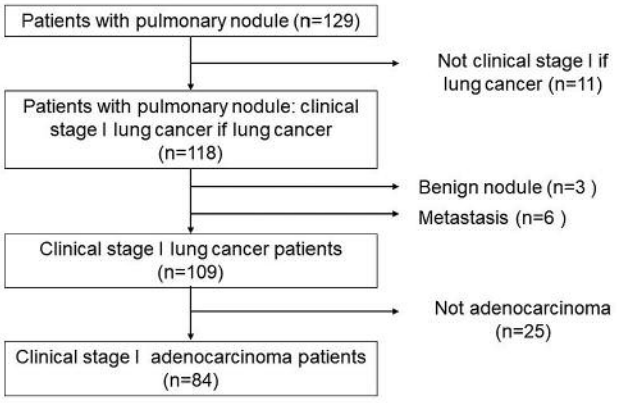

Figure 1. *Flow chart of patient selection.*

SUVmax was measured in two facilities, using the Discovery® series of devices (GE 121 Healthcare, Little Chalfont, UK) in accordance with the fluorodeoxyglucose-position emission tomography (FDG-PET) and PET/computed tomography (CT) medical guidelines of the Japanese Society of Nuclear Medicine.

The areas under the curve (AUCs) of the receiver operating characteristic curves (ROCs) of WTS on CT (cm), SS (cm), SR, CEA (ng/ml), and SUVmax were calculated in patients with C-CTC. Furthermore, the relative risk calculation was performed by multivariable logistic analysis using a continuous variable with an AUC>0.7, which was considered as the threshold value of moderate accuracy (10). Patients with values lower than the appropriate threshold for all independent factors comprised Group A. The other cases comprised Group B. Survival curves were measured by the Kaplan–Meier method, and the recurrence-free survival rate for two years (2Y-RFSR) was compared with the log rank test. Furthermore, the *t*-test was used to compare means, and the Fisher test or chi-square test was used to compare proportions. Statistical analysis was performed using R (11), and *p*-values <0.05 were considered statistically significant.

Results

A total of 129 patients with pulmonary nodules underwent surgery during the study period. Among them, 84 patients had clinical stage I lung adenocarcinoma (Figure 1). They were followed up until December 2019, and only then was the final prognosis recorded. The clinical and pathological characteristics of the patients are shown in Table I. All patients with pathological stage IV disease had associated minor malignant pleural effusion or minor dissemination lesions.

In 40 patients (47.6%), CTCs were detected immediately after surgery. C-CTCs were detected in 26 patients (31.0%). Single CTCs were detected in 14 (16.7%) patients, and CTCs were not detected in 44 (52.4%) patients.

The results of the ROC analysis are shown in Table II. At an AUC>0.7, the threshold of SS, CEA, and SUVmax was 2.1 cm, 7.5 ng/ml, and 2.9, respectively. Furthermore, in the logistic regression analysis, SS >2.1 cm, CEA >7.5 ng/ml, and SUVmax >2.9 were independent predictors of CTC detection (Table III). Group A was made of 32 patients (38.1%) and Group B was made of 52 cases (61.9%). The factors per group are shown in Table IV. In Group A, C-CTCs were detected only in one patient. However, in Group B, C-CTCs were detected in 24 patients (48.1%); one of the patients is shown in Figure 2.

The 2Y-RFSR was 88.1% and 47.5% ($p<0.01$) in the non-C-CTC group (n=58) and the C-CTC group (n=26), respectively, and 96.9% and 60.8% in Group A and Group B ($p<0.01$), respectively (Figure 3).

Table I. *Clinical stage I lung adenocarcinoma cases in which CTC was evaluated before surgery.*

Variables	n=84
Age (mean±SD)	69.4±8.8
Gender (%)	
Male	36 (42.9)
Female	48 (57.1)
Tumor size	
CT	
Whole size (cm) (mean±SD)	2.3±1.1
Solid size (cm) (mean±SD)	1.9±1.3
Solid ratio	0.8±0.3
Pathology	
Whole size (cm) (mean±SD)	2.4±1.4
Invasion size (cm) (mean±SD)	1.9±1.3
Clinical stage	
0	9 (10.7)
IA1	10 (11.9)
IA2	37 (44.0)
IA3	15 (17.9)
IB	13 (15.5)
CEA (ng/ml) (mean±SD)	9.1±16.0
SUVmax (mean±SD)	3.8±3.4
Operation	
Wedge	17 (20.2)
Segmentectomy	9 (10.7)
Lobectomy	58 (69.0)
Lymphatic invasion (%)	45 (53.6)
Vascular invasion (%)	39 (46.4)
Pleural invasion (%)	29 (34.5)
Lymph node metastasis (%)	13 (15.5)
Pathological stage (%)	
0	9 (10.7)
IA1	8 (9.5)
IA2	32 (38.1)
IA3	8 (9.5)
IB	7 (8.3)
IIA	5 (6.0)
IIB	7 (8.3)
IIIA	3 (3.6)
IV	5 (6.0)

CTC: Circulating tumor cell; SD: standard deviation; CT: computed tomography; CEA: carcinoembryonic antigen; SUV: standardized uptake value.

Table II. *ROC analysis of postoperative C-CTC detection.*

Variable	AUC	Threshold	Specificity	Sensitivity
Whole tumor size (cm)	0.67	2.1	0.67	0.56
Solid size (cm)	0.75	2.1	0.8	0.62
Solid ratio	0.68	0.87	0.41	0.96
CEA (ng/ml)	0.75	7.5	0.86	0.58
SUVmax	0.79	2.9	0.65	0.84

ROC: Receiver operating characteristic curve; C-CTC: clustered circulating tumor cell; AUC: area under the curve; CEA: carcinoembryonic antigen; SUV: standardized uptake value.

Table III. *Multivariable logistic regression analysis for immediate postoperative C-CTC detection.*

Variable	RR	95%CI		*p*-Value
CT solid size >2.1 (cm)	4.65	1.38	15.7	0.01
CEA >7.5 (ng/ml)	4.49	1.41	14.2	0.01
SUVmax >2.9	4.21	1.2	14.7	0.02

C-CTC: Clustered circulating tumor cell; CT: computed tomography; CEA: carcinoembryonic antigen; SUV: standardized uptake value.

Discussion

In this study, CTC detection immediately after lesion removal in patients with lung adenocarcinoma in relation to SS, CEA, and SUVmax was examined. We found that SS, CEA, and SUVmax were significant independent predictors. Recurrence was less likely to occur in patients who did not meet all the thresholds of these three consecutive variables, as in non-CTC cases.

In recent years, CT has become a general-purpose examination. Adenocarcinoma is mainly diagnosed with CT based on the presence of a ground grass opacity (GGO), which is a surrogate marker of lepidic lesions and suggestive of a noninvasive adenocarcinoma (12). Adenocarcinomas characterized by mixed GGO lesions (concomitant solid and GGO) on CT are considered to have less postoperative recurrence and a good prognosis. SS has been reported as one of the prognosis predictors (13, 14). In addition, CEA (4) and SUVmax measured by FDG-PET (5) are also considered prognostic predictors. Since these results continuously change with the progression of lung adenocarcinoma, their usefulness as predictors of metastasis or recurrence may be shown using thresholds. For the levels of these continuous variables measured using CT or FDG-PET, most evaluations include the presence or absence of recurrence as the main evaluation item (3-5). An

Table IV. *Characteristics of patients according to factors predicting C-CTC detection.*

Variables	Group A	Group B	*p*-Value
N	32	52	
CT solid size >2.1 (cm) (%)	0	23 (44.2)	<0.01
CEA >7.5 (ng/ml) (%)	0	22 (42.3)	<0.01
SUVmax >2.9 (%)	0	39 (75.0)	<0.01
CTC detection* (%)	7 (21.9)	33 (63.4)	<0.01
CTC count** (mean±SD)	1.0±2.7	3.3±4.0	<0.01
C-CTC (%)	1 (3.1)	25 (48.1)	<0.01
Age (mean±SD)	70.7±7.8	68.7±9.4	0.4
Gender (%)			
Male	16 (50)	20 (38.5)	0.4
Female	16 (50)	32 (61.4)	
Tumor size			
CT			
Whole size (cm) (mean±SD)	1.8±0.8	2.6±1.2	<0.01
Solid size (cm) (mean±SD)	1.0±0.7	2.5±1.3	<0.01
Solid ratio	0.6±0.4	0.9±0.2	<0.01
Pathology			
Whole size (cm) (mean±SD)	1.7±0.8	2.8±1.5	<0.01
Invasion size (cm) (mean±SD)	1.1±0.7	2.5±1.3	<0.01
Clinical stage (%)			
0	9 (28.1)	0	<0.01
IA1	10 (31.3)	0	
IA2	13 (40.6)	24 (46.1)	
IA3	0	15 (28.8)	
IB	0	13 (25.0)	
CEA (ng/ml) (mean±SD)	2.9±1.5	13.1±19.4	<0.01
SUVmax (mean±SD)	1.1±0.9	4.6±3.4	<0.01
Operation (%)			
Wedge	12 (37.5)	11 (21.2)	0.02
Segmentectomy	6 (18.9)	3 (5.8)	
Lobectomy	14 (43.8)	38 (73.1)	
Lymphatic invasion (%)	6(1.7)	39(75.0)	<0.01
Vascular invasion (%)	3(8.3)	36 (69.2)	<0.01
Pleural invasion (%)	2(6.3)	27(51.9)	<0.01
Lymph node metastasis (%)	1 (3.1)	12 (23.1)	<0.01
Pathological stage (%)			
0	9 (28.1)	0	<0.01
IA1	8 (25.0)	0	
IA2	32 (38.1)	0	
IA3	0	8 (15.4)	
IB	0	7 (13.5)	
IIA	1 (1.2)	4 (19.2)	
IIB	0	7 (13.5)	
IIIA	0	3 (5.8)	
IV	0	5 (19.2)	

CTC: Circulating tumor cell; C-: clustered; SD: standard deviation; CT: computed tomography; CEA: carcinoembryonic antigen; SUV: standardized uptake value; Group A: group of patients who did not meet any threshold value; CT solid size >2.1 cm, carcinoembryonic antigen (CEA) >7.5 ng/ml, and standardized uptake value (SUVmax) >2.9; Group B: group of patients not in Group A ; *single CTC, clustered CTC, or both; **clustered CTC was counted as one.

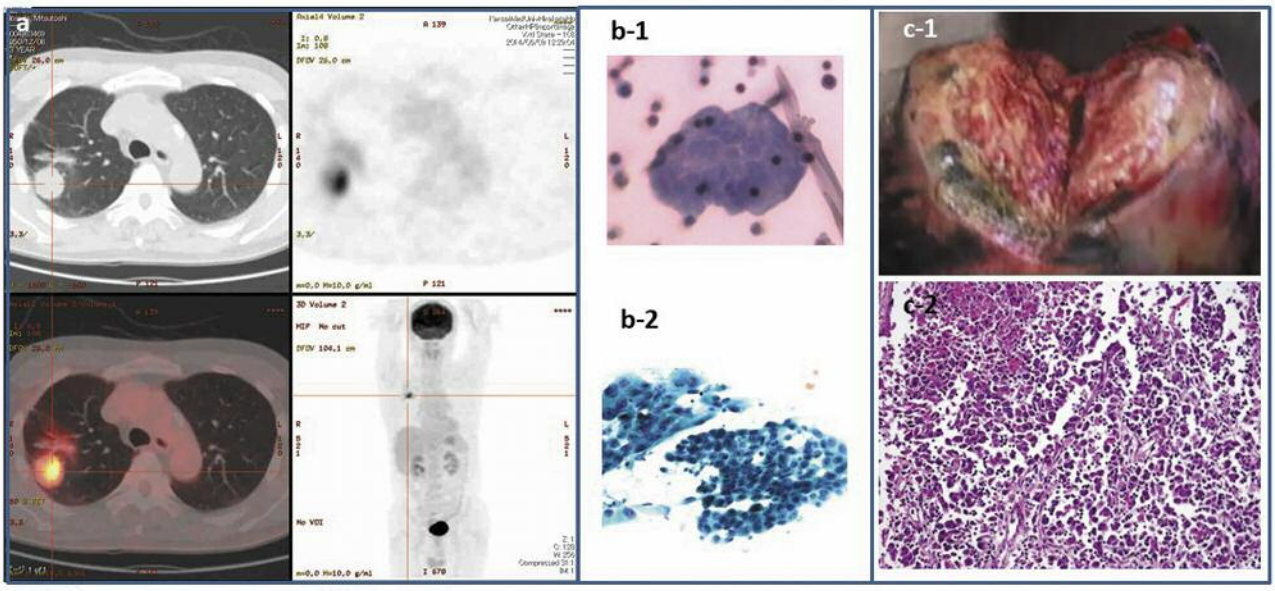

Figure 2. *Patient in Group B with clustered circulating tumor cells. In a 65-year old man with a solid tumour size of 3.2 cm, as measured by computed tomography (CT), carcinoembryonic antigen (CEA) level of 16.5 ng/ml, and SUVmax of 8.9, the lesion was a pure solid nodule diagnosed as adenocarcinoma by transbronchial biopsy (clinical stage IB) (a). Immediately after pulmonary lobectomy, clustered circulating tumor cells (b-1) were detected in the peripheral arterial blood, which was similar to clustered tumor cells extracted from the cut surface of the tumor (b-2). The gross appearance of the tumor was c-1, and pathological examination revealed poorly differentiated adenocarcinoma (c-2). The pathological stage was IIIA (T2N2M0). The patient died from multiple metastases of lung cancer 8 months after surgery. Group B: group of patients who meet any of the thresholds; CT solid size >2.1 cm, CEA >7.5 ng/ml, and standardized uptake value (SUVmax) >2.9.*

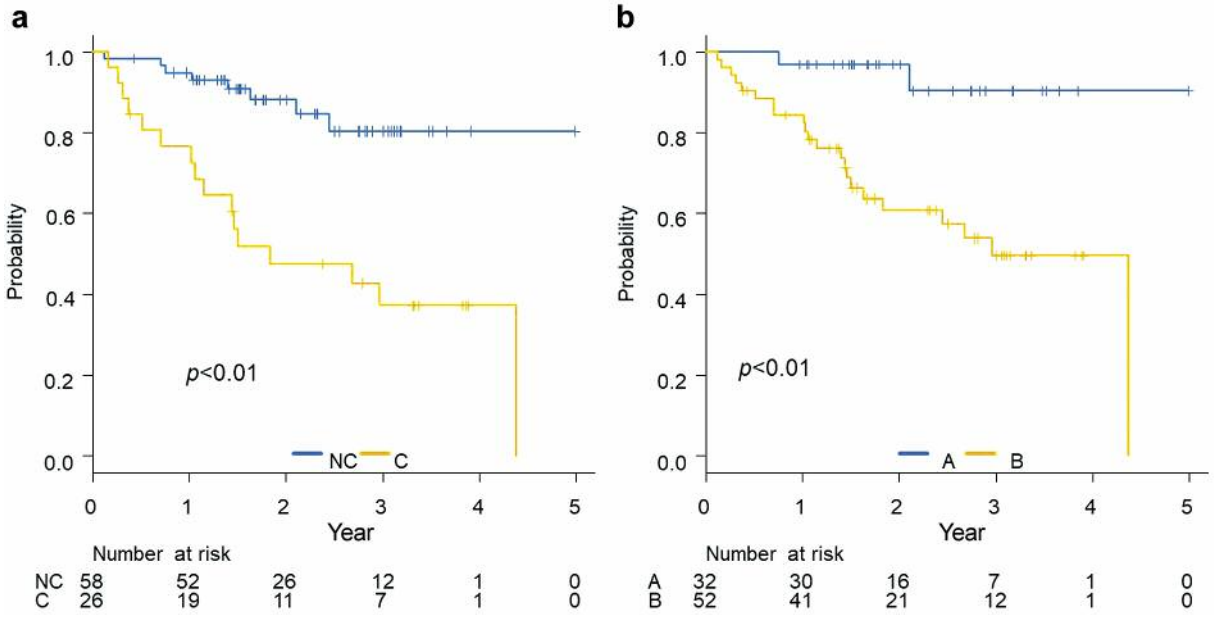

Figure 3. *Recurrence-free survival curves according to postoperative CTC status and factors predicting C-CTC detection. CTC: Circulating tumor cell; NC: not cluster; C: cluster; Group A, group of patients who did not meet any of the threshold values; computed tomography solid size >2.1 cm, carcinoembryonic antigen (CEA) >7.5 ng/ml, and standardized uptake value (SUVmax) > 2.9; Group B, group of patients not in Group A.*

evaluation of the threshold using the ROC curve, with recurrence as the endpoint, has been reported (15). However, lead-time visas occur when survival or recurrence are evaluated using the ROC curve. In this study, since the C-CTC identification immediately after pulmonary removal was the main evaluation item, there was no lead-time visas, thus it was a suitable setting to evaluate with a ROC curve.In this study, C-CTC identification immediately after surgery was the endpoint, and bias was limited in the evaluation of the test results shown by continuous variables using ROC curves.

C-CTC occurrence has decreased with the success of cancer treatment (16), the characteristics of cancer stem cells (17), and hybrid-type epithelial-mesenchymal transition (18). The perioperative identification of C-CTC has been reported to be a predictor of recurrence (7, 8, 19). Therefore, C-CTC is a precursor of distant recurrence, and postoperative C-CTC detection can serve as a surrogate marker for metastasis and recurrence.

The levels of the continuous variables measured using CT or FDG-PET in this study are regarded as prognostic factors (3-5), which may indicate the potential for distant recurrence. In a prior study wherein C-CTC detection was the main evaluation item, SS, CEA, and SUVmax were significant predictors, and the prognosis of patients with at least one of them above their threshold value was poor compared to those with values below the threshold for all the factors. Therefore, these variables may serve as indices for when to start active treatment, such as surgery, in lung adenocarcinoma cases. Besides, since CTC monitoring may be practical to monitor treatment response and recurrence, identification of predictors of C-CTC, which in turn predicts cancer recurrence, is crucial.

Our study had the following limitations: 1) The SUVmax value was not measured in a single facility. However, bias was kept to a minimum as it was measured in a similar way and with the same device; 2) CTC identification was visual; 3) CTCs could have been induced by surgery; 4) The study was a retrospective observational study. However, the certainty of CTC diagnosis has been proven in previous studies (8). In addition, C-CTC as a recurrence predictor has been proven in studies using the pulmonary vein of the excised lung as a sample (20). Since the morphological characteristics of CTCs have been reported to match the pulmonary artery and peripheral arterial blood (7), it is valid to use immediate postoperative CTC detection as a recurrence predictor. In addition, in this study, the frequency of anatomical pulmonary resection was high in Group B, which had a poor prognosis, and the negative effect on postoperative recurrence after reduced pulmonary resection was small. There is a possibility that high-precision results can be obtained by conducting prospective research; therefore, further research that takes into consideration the above is required.

In conclusion, SS on CT, CEA and SUVmax on FDG-PET as continuous variables are predictors of postoperative CTC detection, in which patients with values above the threshold value have a poor prognosis.

Conflicts of Interest

The Authors have no conflicts of interest to declare regarding this study.

Authors' Contributions

Conception and design: Takashi Watanabe, Noriyoshi Sawabata. Administrative support: Shigeru Nakane, Takeshi Kawaguchi, Noriyoshi Sawabata. Provision of study materials or patients: Takashi Watanabe, Noriyoshi Sawabata. Collection and assembly of data: Noriyoshi Sawabata. Data analysis and interpretation: Takashi Watanabe, Noriyoshi Sawabata. Article writing and final article approval: All Authors.

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