Surg Today
DOI 10.1007/s00595-016-1318-4

ORIGINAL ARTICLE

Perioperative circulating tumor cells in surgical patients with non-small cell lung cancer: does surgical manipulation dislodge cancer cells thus allowing them to pass into the peripheral blood?

Noriyoshi Sawabata[1,2] · Soichiro Funaki[3] · Takeru Hyakutake[1] · Yasushi Shintani[3] · Ayako Fujiwara[3] · Meinoshin Okumura[3]

Received: 28 September 2015 / Accepted: 16 February 2016
© Springer Japan 2016

Abstract
Purpose We herein evaluated the status of circulating tumor cells (CTC) dislodged from the tumor during surgery in patients who underwent pulmonary resection for non-small cell lung cancer (NSCLC) to assess the clinical implications.
Methods Tumor cells in the peripheral arterial blood before surgery (Before) and immediately after lung resection (After) and in the blood from the pulmonary vein of the resected lung were detected using a size selective method. The clinicopathological characteristics and the prognosis were then analyzed according to the CTC status: no tumor cells detected (*N*), single tumor cell or total number less than 4 cells (*S*), and existence of clustered cells (*C*).
Results According to the CTC status, the patients were classified into the following three groups: Before-*C* and After-*C*, Group I ($n = 6$); Before-*S* or *N* and After-*C*, Group II ($n = 9$); and Before-*S* or *N* and After-*S* or *N*, Group III ($n = 8$). Group III showed a high rate of p-stage IA, smaller tumor size, lower CEA level, lower SUVmax level, and a higher relapse-free survival rate than the other groups.
Conclusions CTCs were detected in patients after undergoing lung resection, some of which may have been dislodged by the surgical procedure. The presence of clustered CTCs after the operation indicated an unfavorable outcome.

Keywords Lung cancer · Circulating tumor cells · Surgery

Introduction

Relapse of non-small cell lung cancer (NSCLC) is not uncommon, even when the cancer lesion is completely resected. According to a nationwide registry conducted in Japan, approximately 15 % of pathological stage IA patients suffer from cancer recurrence within 5 years of the initial operation [1]. Such recurrence after complete resection of pulmonary carcinoma is speculated to arise from residual isolated tumor cells (ITCs) in the body. Some ITCs may exist prior to surgery, while others may be derived from circulating tumor cells (CTCs), some of which may be dislodged from the tumor into the peripheral blood during the operation. We herein evaluated the CTC status in patients who underwent pulmonary resection in order to assess the clinical implications.

Methods

Background of patients

In the setting of a referral community healthcare hospital, 23 patients with NSCLC who underwent an anatomical pulmonary resection procedure (1 segmentectomy, 21 lobectomies, and 1 pneumonectomy) (10 males, 13 females; median age 68 years) from June 2014 to October

✉ Noriyoshi Sawabata
nsawabata@hotmail.com

[1] Division of General Thoracic Surgery, Hoshigaoka Medical Center, Japan Community Healthcare Organization (JCHO), Hirakata, Japan

[2] Division of Health Science, Osaka University Graduate School of Medicine, Suita, Japan

[3] Department of General Thoracic Surgery, Osaka University Graduate School of Medicine, Suita, Japan

Published online: 07 March 2016

Table 1 Clinical-pathological characteristics

No.	Age	Sex	c-stage	p-stage	TNM			Tumor size			Tumor marker		PET-scan	Pre-OP	Operation		His	Gene mutation	
					p-T	p-N	p-M	CT		Gross	CEA (ng/mL)	CYFRA (ng/mL)	SUVmax	CTx	Approach	Resection		EGFR	ALK
								Whole	Solid										
1	75	F	IA	IV	1a	0	1a	1.2	1.2	1.2	7.8	1.9	4.5	No	Th	R-LML	AD	Yes	NA
2	46	F	IA	IA	1a	0	0	1.8	1.8	2.0	25.9	0.5	7.9	No	Th	L-UL	AD	No	No
3	77	M	IIIA	IIIA	3	2	0	7.5	7.5	8.0	1.9	1.9	10.8	Yes	Th	L-Pn	SQ	NA	NA
4	53	M	IIB	IIB	3	0	0	9.0	9.0	10.5	6.7	17.4	21.8	No	Th	R-UML, cost III, IV, V	SQ	No	NA
5	80	F	IB	IB	2a	0	0	3.0	3.0	3.0	1.3	1.8	6.8	No	Th	L-UL	AD	Yes	No
6	68	F	IIA	IIA	2b	0	0	5.5	5.5	5.0	55.0	7.7	13.8	No	VATS	L-UL	AD	Yes	NA
7	64	F	IIB	IIB	3	0	0	3.7	3.7	3.5	13.0	1.9	6.4	No	Th	R-UL, cost IV, V	AD	No	No
8	63	M	IB	IIIA	2a	2	0	3.4	3.4	3.0	1.7	1.2	9.7	No	Th	R-UL	Pleo	No	No
9	63	M	IA	IA	1b	0	0	1.8	1.8	2.5	1.9	0.7	10.6	No	Th	R-UL	Pleo	Yes	No
10	64	M	IA	IA	1b	0	0	2.7	0.5	2.5	2.7	1.7	1.6	No	Th	R-LL	AD	Yes	NA
11	71	F	IB	IB	2a	0	0	4.6	4.0	3.8	0.9	1.0	2.4	No	Th	R-UL	AD	Yes	No
12	72	F	IB	IB	2a	0	0	3.5	3.5	3.4	11.7	1.9	5.4	No	Th	R-UL	AD	Yes	NA
13	76	F	IA	IA	1b	0	0	2.6	2.6	1.5	3.9	0.8	2.3	No	VATS	R-LL	AD	No	No
14	66	F	IIA	IIA	2a	1	0	4.1	4.1	4.0	2.6	1.3	4.3	No	VATS	R-UL	AD	No	Yes
15	72	M	IA	IA	1b	0	0	2.8	2.8	2.5	4.2	1.6	13.8	No	VATS	R-UL	SQ	NA	NA
16	66	F	IA	IA	1b	0	0	2.8	1.2	2.1	1.8	1.2	1.8	No	VATS	R-LL	AD	Yes	NA
17	71	F	IA	IA	1a	0	0	3.8	2.1	3.0	0.7	0.8	1.8	No	VATS	R-UL	AD	Yes	NA
18	74	F	IA	IA	1a	0	0	2.8	1.0	2.5	1.2	1.0	6.4	No	VATS	R-UL	AD	Yes	NA
19	57	M	IA	IA	1b	0	0	2.2	2.0	2.1	2.0	0.7	6.9	No	Th	R-LL	AD	No	Yes
20	76	M	IA	IA	1a	0	0	1.2	0.0	1.1	1.2	0.7	0.0	No	Th	L-S123	AD	Yes	NA
21	76	M	IA	IA	1a	0	0	1.5	1.5	1.5	1.2	1.0	0.8	No	Th	R-LL	SQ	NA	NA
22	66	M	IA	IA	1b	0	0	2.6	2.6	2.6	3.2	1.9	3.1	No	Th	R-UL	AD	No	No
23	64	F	IA	IA	1b	0	0	1.9	1.9	1.6	1.5	1.0	1.8	No	VATS	L-UL	AD	Yes	No
Mean	67.8							3.1	2.9	3.2	6.7	2.2	6.2						
SD	8.2							1.9	2.1	2.2	12.0	3.6	5.3						

C Clinical, *p* pathological, *PET* positron emission tomography, *OP* operation, *His* histology, *CT* computed tomography, *CEA* carcinoembryonic antigen, *CYFRA* cytokeratin fragment A, *SUV* standard uptake value, *CTx* chemotherapy, *F* female, *M* male, *Th* thoracotomy, *VATS* video-assessed thoracic surgery, *R* right, *L* left, *MLL* middle and lower lobectomy, *LL* lower lobectomy, *UL* upper lobectomy, *seg* segmentectomy, *AD* adenocarcinoma, *SQ* squamous cell carcinoma, *Pleo* pleomorphic carcinoma, *NA* not assessed, *SD* standard deviation

2014 were enrolled in this clinical study (Table 1). The histological diagnosis was adenocarcinoma in 17 cases, squamous cell carcinoma in 4, and pleomorphic carcinoma in 2; the clinical stage was IA in 15 cases, IB in 3, IIA in 2, IIB in 2, and IIIA in 1; and the pathological stage was IA in 14 cases, IB in 2, IIA in 2, IIB in 2, IIIA in 2, and IV in 1. The patient with clinical IIIA lung cancer underwent induction chemotherapy with 4 cycles of carboplatin and taxotel. Patient characteristics, tumor size [whole size shown by computed tomography (CT), size of the solid component shown by CT, gross size], tumor markers [carcinoembryonic antigen (CEA), cytokeratin fragment A (CYFRA)], maximum standardized uptake value (SUVmax) in the tumor shown by fluorodeoxyglucose positron emission tomography (FDG-PET), type of operation, tumor histology findings, and genetic mutation results are shown in Table 1.

Follow-up examinations

The median follow-up period was 12 months (range 5–15 months) and the last follow-up examination was in August 2015. All patients were evaluated at 1-month intervals, which included physical and chest X-ray examinations, and blood tests including tumor markers, with additional thoraco-abdominal CT scans generally performed at 6-month intervals. Mortality and recurrence data were collected by the primary physician (NS).

Detection of CTCs

The detection of CTCs was performed during the operation and postoperative period. Peripheral atrial blood (3 mL) was collected into an EDTA tube before surgery (Before), immediately after resection of the lung (After), and 6 h after the end of surgery (6 h). In addition, pulmonary vein (PV) blood (3 mL) from the resected lung was collected and stored in the same fashion. Tumor cells in the 4 blood samples from each patient were simultaneously extracted using a ScreenCell® CTC selection kit with the size selection method using a micro-pore film that extracts formalin-fixed tumor cells. The extracted cells were stained using a hematoxylin–eosin method and observed with a light microscope, using a previously described method [2]. Tumor cells were classified into 3 categories: no tumor cells detected (*N*), single cell or less than 4 cells (*S*), and clustered cells (*C*).

Statistical analyses

To analyze the relationships between the clinicopathological findings and the CTC status, we examined the following parameters: pathological stage (p-IA vs. greater), whole tumor size (cm) on CT images, solid component size (cm) on CT images, pattern of CT appearance (pure solid vs. glass or ground-glass opacity + solid and solid), serum CEA and CYFRA levels [mean ± standard deviation (SD)], and SUVmax values on fluorodeoxyglucose positron emission tomography (FDG-PET) images (mean ± SD). Patients were classified into 3 categories according to their CTC status: Before-*C* and After-*C*, Group I; Before *S* or *N* and After *C*, Group II; and Before *S* or *N* and After *S* or *N*, Group III. This classification was used because clustered cancer cells in the blood have been reported to be an indicator of a poor prognosis [2, 4]. Statistical analyses were performed using a commercially available statistics software program (StatView 5.0, Abacus Computer, Berkeley, CA). Fisher's exact test was used to compare two dichotomous variables and the *t* test to compare mean values. For analyses among multiple groups, a Chi-square or Kruskal–Wallis test was used as appropriate. As for the follow-up data, the relapse-free survival curves were calculated using the Kaplan–Meier method and the log-rank test was utilized to compare two groups, when possible.

Ethical approval

This investigation was approved by the institutional review board of the Hoshigaoka Medical Center following discussions and all patients provided their informed consent to participate in this study.

Results

Detection of CTCs

CTCs were detected in both peripheral and PV samples (Figs. 1, 2). Morphological and quantitative findings of tumor cells detected before surgery, immediately after resection of the lung, at 6 h after the end of surgery, and in the PV blood from the resected lung are shown in Table 2. Seven cases were positive (30.4 %; *S*, 1; *C*, 6) among samples collected before surgery, 17 (73.9 %; *S*, 2; *C*, 15) were positive among those collected immediately after resection of the lung, 1 (4.3 %; *S*, 1) was positive among those collected 6 h from the end of surgery, and 19 (82.6 %; *S*, 3; *C*, 16) were positive among those collected from PV blood. All of the 17 cases with positive samples obtained immediately after resection of the lung also had positive PV samples, while 10 of those (58.8 %) were negative prior to surgery. According to the CTC status, there were 6 patients in Group I, 9 in Group II, and 8 in Group III. Regarding the status of tumor cells in the PV blood according to our classification (*N*, *S*, *C*), all patients with C status were C in the

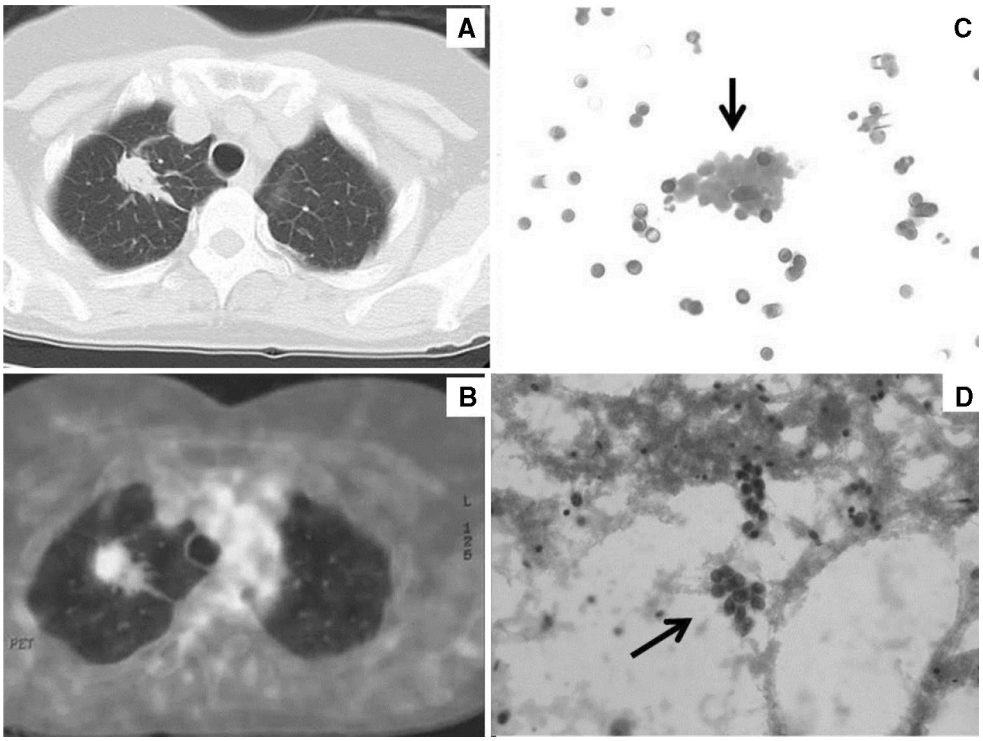

Fig. 1 Representative case of invasive non-small cell lung cancer (case no. 11). A tumor located in the right upper lobe (**a**) showed a high standardized uptake value ((SUV)max = 5.4) in fluorodeoxyglucose positron emission tomography (FDG-PET) findings (**b**). Clustered tumor cells were observed in the peripheral arterial blood immediately after lung resection (**c**, hematoxylin–eosin stain) and in the pulmonary vein blood from the resected lung. Stump smear cytology reveals the tumor is adenocarcinoma (**d**, Papanicolaou stain). *Bold arrow* tumor cells

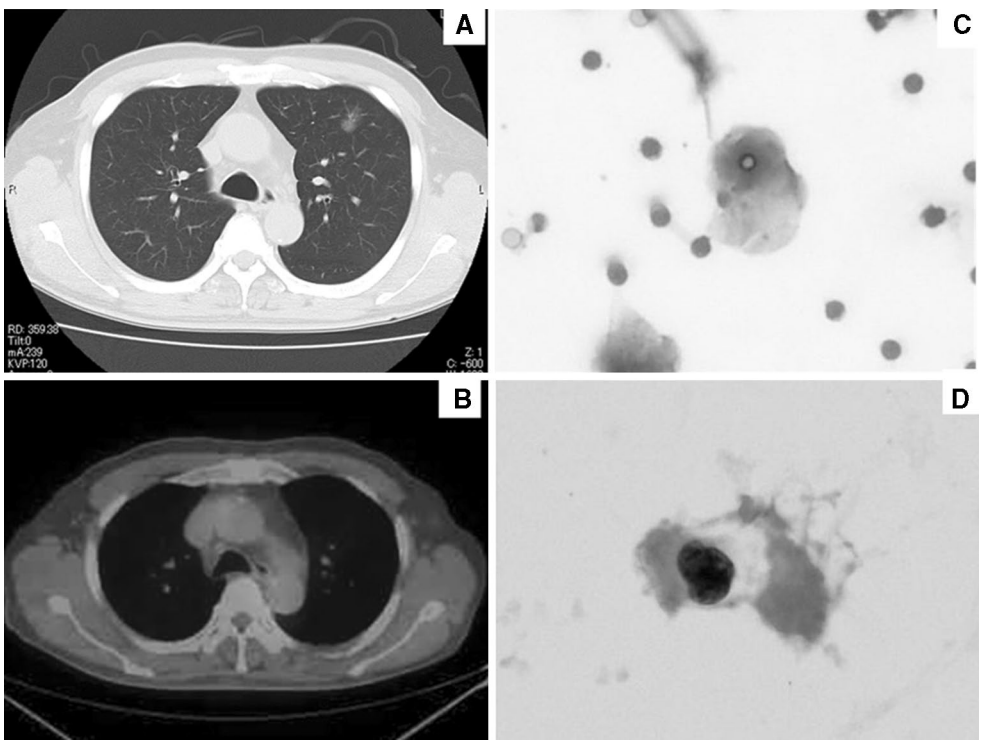

Fig. 2 Representative case of non-invasive adenocarcinoma (case no. 20). A tumor located in the left upper lobe showed a ground-glass opacity with no consolidation and was diagnosed as non-invasive adenocarcinoma (**a**) with a standardized uptake value [(SUV)max = 0] in fluorodeoxyglucose positron emission tomography (FDG-PET) findings (**b**). Abnormal alveolar cells were observed in the pulmonary vein blood from the resected lung (**c**, hematoxylin–eosin stain). Stump smear cytology revealed the tumor to be adenocarcinoma (**d**, Papanicolaou stain)

Table 2 Morphological and quantitative findings of circulating tumor cells

Case no.	Group	Before			After			6 h			PV		
		Morphology	Amount		Morphology	Amount		Morphology	Amount		Morphology	Amount	
			C	*S*		*C*	*S*		*C*	*S*		*C*	*S*
1	I	*C*	2	4	*C*	3	5	*N*	0	1	*C*	6	10
2	I	*C*	3	24	*C*	6	8	*N*	0	0	*C*	6	9
3	I	*C*	3	2	*C*	2	1	*N*	0	0	*C*	5	12
4	I	*C*	1	3	*C*	2	1	*N*	0	0	*C*	1	1
5	I	*C*	4	0	*C*	1	2	*N*	0	0	*C*	1	3
6	I	*C*	8	4	*C*	12	2	*N*	0	0	*C*	6	3
7	II	*S*	0	1	*C*	1	1	*N*	0	0	*C*	1	0
8	II	*N*	0	0	*C*	1	2	*S*	0	0	*C*	1	4
9	II	*N*	0	0	*C*	2	0	*N*	0	0	*C*	1	1
10	II	*N*	0	0	*C*	1	1	*N*	0	0	*C*	2	2
11	II	*N*	0	0	*C*	1	3	*N*	0	0	*C*	1	5
12	II	*N*	0	0	*C*	2	2	*N*	0	0	*C*	1	2
13	II	*N*	0	0	*C*	1	1	*N*	0	0	*C*	1	2
14	II	*N*	0	0	*C*	2	2	*N*	0	0	*C*	2	4
15	II	*N*	0	0	*C*	2	1	*N*	0	0	*C*	1	6
16	III	*N*	0	0	*S*	0	3	*N*	0	0	*S*	0	1
17	III	*N*	0	0	*S*	0	3	*N*	0	0	*S*	0	2
18	III	*N*	0	0	*N*	0	0	*N*	0	0	*S*	0	3
19	III	*N*	0	0	*N*	0	0	*N*	0	0	*S*	0	2
20	III	*N*	0	0	*N*	0	0	*N*	0	0	*N*	0	0
21	III	*N*	0	0	*N*	0	0	*N*	0	0	*N*	0	0
22	III	*N*	0	0	*N*	0	0	*N*	0	0	*N*	0	0
23	III	*N*	0	0	*N*	0	0	*N*	0	0	*N*	0	0
Total	I, 6; II, 9; III, 8	*C*, 6; *S*, 1; *N*, 16			*C*, 15; *S*, 2; *N*, 6			*C*, 0; *S*, 1; *N*, 22			*C*, 15; *S*, 4; *N*, 4		

Before Before surgery, *After* immediately after lung resection, *6 h* 6 h after surgery, *PV* pulmonary vein, *N* tumor cell not detected, *S* single tumor cell or less than 4 cells, *C* clustered cells

Table 3 Comparison of clinical parameters according to change in circulating tumor cells during surgery

	Group			*p*-value	
	I	II	III		
p-IA	2 (33.3 %)	4 (44.4 %)	8 (100 %)	0.01	I vs. II, 0.6; I vs. III, 0.02; II vs. III, 0.2
Pure solid	6 (100 %)	7 (77.8 %)	2 (25.0 %)	0.01	I vs. II, 0.5; I vs. III, 0.01; II vs. III, 0.06
Tumor size (cm)					
CT, whole	4.7 ± 3.2	3.2 ± 0.9	2.4 ± 0.8	0.2	I vs. II, 0.2; I vs. III, 0.07; II vs. III, 0.05
CT, solid	4.7 ± 3.2	2.9 ± 1.2	1.5 ± 0.8	0.04	I vs. II, 0.2; I vs. III, 0.02; II vs. III, 0.01
Gross	4.6 ± 3.7	3.0 ± 0.8	2.1 ± 0.6	0.1	I vs. II, 0.1; I vs. III, 0.05; II vs. III, 0.02
CEA (ng/mL)	16.4 ± 20.1	14.7 ± 4.5	1.6 ± 0.8	0.03	I vs. II, 0.1; I vs. III, 0.06; II vs. III, 0.07
CYFRA (ng/mL)	5.2 ± 6.5	1.3 ± 0.5	1.0 ± 0.4	0.1	I vs. II, 0.1; I vs. III, 0.1; II vs. III, 0.2
SUVmax	11.0 ± 6.1	6.3 ± 4.2	2.8 ± 2.5	0.02	I vs. II, 0.1; I vs. III, 0.005 II vs. III, 0.06

p Pathological, *CEA* carcinoembryonic antigen, *CYFRA* cytokeratin fragment A, *SUV* standard uptake value, *CT* computed tomography

Table 4 Relapse and survival

No.	Adj	Relapse					Prognosis	
	CTx		Period (months)	Pattern	Organ	Therapy	Period (months)	Status
1	No	Yes	4	*L*	Pleura	TKI	13	Alive
2	PO	No	14				14	Alive
3	No	No	11				11	Alive
4	No	Yes	11	*L*	Med. LN	CTx	12	Alive
5	PO	No	8				8	Alive
6	IV	No	10				10	Alive
7	IV	No	8				8	Alive
8	No	Yes	2	*L* + *D*	Med. LN, bone, spinal cord	CTx	5	Death
9	No	Yes	7	*D*	Bone	RTx	8	Alive
10	No	No	15				15	Alive
11	PO	No	15				15	Alive
12	PO	No	14				14	Alive
13	No	Yes	9	*L*	Med. LN	CTx	14	Alive
14	IV	No	10				10	Alive
15	No	No	10				10	Alive
16	No	No	10				10	Alive
17	No	No	10				10	Alive
18	No	No	8				8	Alive
19	PO	No	15				15	Alive
20	No	No	12				12	Alive
21	No	No	13				13	Alive
22	PO	No	12				12	Alive
23	No	No	10				10	Alive

Adj Adjuvant, *CTx* chemotherapy, *PO* Per Os (tegafur + uracil), *IV* intravenous injection (carboplatin + docetaxel), *Med* mediastinum, *LN* lymph node, *TKI* tyrosine kinase inhibitor, *CTx* chemotherapy, *RTx* radiotherapy

peripheral arterial blood immediately after resection of the lung (Table 2).

Clinical pathological results

The tumor characteristics according to the CTC status are shown in Table 3. The ratio of p-IA was greatest in Group III (100 %), followed by Group II and Group I, which was in contrast to the ratio of pure solid tumors, tumor size, CEA level, and SUVmax value. As shown in Table 4, there were 5 cases of cancer recurrence and 1 cancer death. Survival curves for all groups are shown in Fig. 3. The 1-year relapse-free survival rate was 55.6 % in Group I, 64.8 % in Group II, and 100 % in Group III, indicating no significant difference between Group I and Group II ($p = 0.9$).

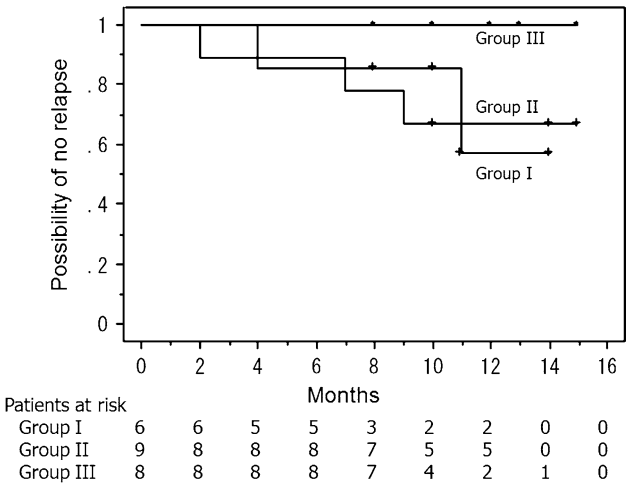

Fig. 3 Relapse-free survival curves. Patients were classified into the following three categories according to the CTC status: Before-*C* and After-*C*, Group I; Before *S* or *N* and After *C*, Group II; and Before *S* or *N* and After *S* or *N*, Group III. The 1-year relapse free survival rates for the groups were 55.6, 64.8, and 100 %, respectively. The difference between Group I and Group II was not significant ($p = 0.9$)

Discussion

The detection of CTCs during surgery was initially performed using a cytological technique [5], after which

polymerase chain reaction (PCR) [6, 7] and flow cytometry [8] methods were introduced to improve the sensitivity and specificity. In recent years, advancements in commercially available equipment and methods focusing on CTC detection have been developed and reports of CTC detection using clinical specimens have also been presented [2, 9].

Among the modern available CTC detection systems, the EpCAM positive selection method using magnified antibodies (CellSearch® system) was the first to show morphological evidence of CTCs during lung cancer surgical procedures [10–12]. However, the sensitivity for the detection of CTCs in peripheral blood samples obtained during lung cancer surgery is not high [11] and clustered cells might be missed by the CellSearch® system. To compensate for the low sensitivity in peripheral blood samples and for clustered cells, the ISET® system was developed [13], although the equipment is costly. In contrast, the CD45 negative selection method (RosetteSep™) does not require a specialized machine and can detect clustered CTCs perioperatively among lung cancer tumor cells [3, 4], although its sensitivity is lower than other CTC detection methods. As a result, tests for perioperative CTCs in lung cancer cases have been performed using pulmonary vein blood from resected lungs with RosetteSep™ [3, 4]. In a recently published report on the CTC status in lung cancer surgery patients that utilized an exhaustive gene analysis, however, cells were collected from the pulmonary vein blood using a flow cytometry method for EpCAM [14].

The ScreenCell® CTC selection kit is a highly sensitive size selection method that collects nearly 100 % of spiked tumor cells in the blood without the use of a specialized machine and can also detect clustered cells [2]. It is considered to be a useful method for revealing CTCs in clinical settings, although its use is limited because the detection method is subjective and overestimation of non-cancer cells that might be defined as cancerous is possible. However, because the method to determine the cell status is the same as that of clinically used cytology techniques, there is a slight possibility of overestimation.

The results of the present investigation showed that patients without clustered CTCs at the time of surgery had a higher rate of p-stage IA, lower tumor size and CEA level, and a higher relapse-free survival rate compared with those with clustered CTCs revealed during surgery. This finding may be attributed to the potential malignancy of the original cancer lesion [15, 16] as well as of clustered CTCs, which are reported to have a high ratio of epithelial–mesenchymal transition markers, such as N-cadherin and vimentin, and a low ratio of Ki-67 antigen [17]. Therefore, clustered CTCs may have the potential to become a lesion associated with recurrence.

In the present cohort, 16 patients did not have CTCs detected in the peripheral blood prior to surgery, of whom 10 (62.5 %) became CTC positive after lung resection. This finding indicates that surgical manipulation for the treatment of lung cancer can dislodge cancer cells thus allowing them to pass into the circulating blood. The presence of CTCs has been reported to be a prognostic indicator in cases of lung cancer [18], with clustered CTCs an indicator of high malignancy [17, 19, 20] and early recurrence [3, 4]. Our study results also showed that the detection of clustered CTCs during surgery for NSCLC was a marker of high malignancy and indicated early recurrence. However, our findings are limited by the small number of patients, short follow-up period, and retrospective setting, and a prospective study with a large number of patients is needed. Nevertheless, if it is confirmed that CTCs caused by surgical manipulation during lung cancer surgery are related to cancer recurrence, such knowledge may contribute to improvements in the outcome of affected patients by greater control of CTC development and dislodgement.

Conclusion

CTCs detected during surgery for NSCLC may become dislodged by surgical manipulation, and clustered CTCs found during the operation may be related to tumor malignancy, thus indicating an unfavorable outcome.

Acknowledgments The authors thank Dr. Hiroshi Maruyama and Dr. Minako Torii (Department of Pathology, Hoshigaoka Medical Center) for their contributions to the pathological diagnoses. This research was supported by a Grant-in-Aid for Scientific Research [(B) 25293301] from the Japan Ministry of Education, Science, Sports and Culture.

References

1. Sawabata N, Miyaoka E, Asamura H, Nakanishi Y, Eguchi K, Mori M, et al. Japanese lung cancer registry study of 11,663 surgical cases in 2004: demographic and prognosis changes over decade. J Thorac Oncol. 2011;6:1229–35.
2. Desitter I, Guerrouahen BS, Benali-Furet N, Wechsler J, Jänne PA, Kuang Y, et al. A new device for rapid isolation by size and characterization of rare circulating tumor cells. Anticancer Res. 2011;31:427–41.
3. Funaki S, Sawabata N, Nakagiri T, Shintani Y, Inoue M, Kadota Y, et al. Novel approach for detection of isolated tumor cells in pulmonary vein using negative selection method: morphological classification and clinical implications. Eur J Cardiothorac Surg. 2011;40:322–7.
4. Funaki S, Sawabata N, Abulaiti A, Nakagiri T, Shintani Y, Inoue M, et al. Significance of tumour vessel invasion in determining the morphology of isolated tumour cells in the pulmonary vein in non-small-cell lung cancer. Eur J Cardiothorac Surg. 2013;43:1126–30.
5. Hansen E, Wolff N, Knuechel R, Ruschoff J, Hofstaedter F, Taeger K. Tumor cells in blood shed from the surgical field. Arch Surg. 1995;130:387–93.

6. Yamashita J, Matsuo A, Kurusu Y, Saishoji T, Hayashi N, Ogawa M. Preoperative evidence of circulating tumor cells by means of reverse transcriptase-polymerase chain reaction for carcinoembryonic antigen messenger RNA is an independent predictor of survival in non-small cell lung cancer: a prospective study. J Thorac Cardiovasc Surg. 2002;124:299–305.
7. Brown DC, Purushotham AD, Birnie GD, George WD. Detection of intraoperative tumor cell dissemination in patients with breast cancer by use of reverse transcription and polymerase chain reaction. Surgery. 1995;117:95–101.
8. Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW, Terstappen LW. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res. 2004;10:6897–904.
9. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumor cells. Nat Rev Cancer. 2008;8:329–40.
10. Sawabata N, Okumura M, Utsumi T, Inoue M, Shiono H, Minami M, et al. Circulating tumor cells in peripheral blood caused by surgical manipulation of non-small-cell lung cancer: pilot study using an immunocytology method. Gen Thorac Cardiovasc Surg. 2007;55:189–92.
11. Okumura Y, Tanaka F, Yoneda K, Hashimoto M, Takuwa T, Kondo N, et al. Circulating tumor cell in pulmonary venous blood of primary lung cancer patients. Ann Thorac Surg. 2009;87:1669–75.
12. Tanaka F, Yoneda K, Kondo N, Hashimoto M, Takuwa T, Matsumoto S, et al. Circulating tumor cell as a diagnostic marker in primary lung cancer. Clin Cancer Res. 2009;15:6980–6.
13. Hofman V, Bonnetaud C, Ilie MI, Vielh P, Vignaud JM, Fléjou JF, et al. Preoperative circulating tumor cell detection using the isolation by size of epithelial tumor cell method for patients with lung cancer is a new prognostic biomarker. Clin Cancer Res. 2011;17:827–35.
14. Yao X, Williamson C, Adalsteinsson VA, D'Agostino RS, Fitton T, Smaroff GG, et al. Tumor cells are dislodged into the pulmonary vein during lobectomy. J Thorac Cardiovasc Surg. 2014;148:3224–31.
15. Nakamura H, Takagi M. Clinical impact of the new IASLC/ATS/ERS lung adenocarcinoma classification for chest surgeons. Surg Today. 2015;45:1341–51.
16. Fukumoto K, Taniguchi T, Usami N, Kawaguchi K, Fukui T, Ishiguro F, Nakamura S, Yokoi K. Preoperative plasma D-dimer level is an independent prognostic factor in patients with completely resected non-small cell lung cancer. Surg Today. 2015;45:63–7.
17. Hou JM, Krebs M, Ward T, Sloane R, Priest L, Hughes A, et al. Circulating tumor cells as a window on metastasis biology in lung cancer. Am J Pathol. 2011;178:989–96.
18. Wang J, Wang K, Xu J, Huang J, Zhang T. Prognostic significance of circulating tumor cells in non-small-cell lung cancer patients: a meta-analysis. PLoS One. 2013;8:e78070.
19. Yu M, Bardia A, Wittner BS, Stott SL, Smas ME, Ting DT, et al. Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition. Science. 2013;339:580–4.
20. Aceto N, Bardia A, Miyamoto DT, Donaldson MC, Wittner BS, Spencer JA, et al. Circulating tumor cell clusters are oligoclonal precursors of breast cancer metastasis. Cell. 2014;158:1110–22.