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ORIGINAL ARTICLE

Pleural lavage cytology after lung resection in patients with non-small cell lung cancer and the feasibility of 20 mL saline solution

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Summary *Background:* There are two issues to be discussed in pleural lavage cytology (PLC) for resected non-small cell lung cancer (NSCLC) whether it should be performed before (pre-PLC) or after (post-PLC) the lung resection and the dose of saline varies widely among the institutions.
Methods: We retrospectively reviewed the clinical records of 466 consecutive patients who underwent a curative resection for NSCLC and received both a pre- and post- PLC using 20 mL of saline from January 2001 to December 2011.
Results: There were 24/28 of positive pre- and post-PLC and 442/438 negative pre- and post-PLCs, respectively. Patients with a positive pre- or post-PLCs had significantly worse 5-year survival rates than those with negative results (pre-PLC positive/negative; 32.6%/69.9%, $p = 0.001$, post-PLC positive/negative; 21.4%/71.1%, $p < 0.001$, respectively). The post-PLC ($p = 0.01$) was an independent prognostic factor for the overall survival by a multivariate analysis, whereas the pre-PLC was not ($p = 0.79$).
Conclusions: The post-PLC was a more significant prognostic factor than the pre-PLC. Further, 20 mL of saline seemed feasible because of the consistent results compared to the past reports using a greater dose of saline for regarding the positive rates of the PLC and its prognostic significance.

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1. Introduction

Pleural lavage cytology (PLC) is an independent prognostic factor for resected non-small cell lung cancer (NSCLC) and its clinical significance has been confirmed by a meta-analysis.[1–3] However, the optimal timing of the test and difference in the clinical significance of the PLC determined immediately after a thoracotomy (pre-PLC) and after a lung resection (post-PLC) remain controversial and the optimal dose of the saline as a solution has not been established. We retrospectively reviewed our experience with both pre- and post- PLCs by using 20 mL of saline and investigated the clinical significance of each PLC and the feasibility of using 20 mL of saline for the test.

2. Methods

This study was approved by the Institutional review board of Seirei Hamamatsu General Hospital (Study number 2205). From January 2001 to December 2011, consecutive patients who underwent an anatomical complete resection for NSCLC with a curative intent determining both the pre- and post- PLC were enrolled into this retrospective study. A complete resection was defined as a macroscopic resection without any residual disease accompanied by a hilar and mediastinal lymph node dissection. As the clinical staging, we routinely performed computed tomography (CT) or positron emission tomography (PET)/CT of the chest and abdomen and magnetic resonance imaging of the brain. The mediastinal lymph nodes were examined by an endobronchial ultrasound-guided transbronchial needle aspiration or mediastinoscopy only when indicated. Before any surgical manipulation immediately after completion of the thoracotomy incision, 20 mL of a physiological saline solution was injected into the thoracic cavity and retrieved for cytological evaluation (pre-PLC). Further, after a lung resection with a lymph node dissection, another cytological examination was performed in the same manner (post-PLC). The fluids were centrifuged at 1500 rpm for 5 min and stained by the Papanicolau, Gimza, and PAS methods.

We reviewed their clinical records according to the PLC status and other clinical data and investigated their impact on the survival. Pathological staging was determined based on the seventh edition of the TNM Classification of Malignant Tumors (TNM).[4] Pleural invasion was defined as tumor invading beyond the visceral pleura elastic layer. After surgery, the patients were periodically observed at the outpatient clinic every 3–4 months with a physical examination and chest X-ray. A bone scan or PET/CT was done annually or when indicated. Comparisons of the categorical variables were analyzed by a Fisher's exact test or Chi-square test and continuous variables were analyzed by a t test. The overall survival was calculated using the Kaplan–Meier method and comparisons of the survival curves were performed using the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model. A p value of less than 0.05 was considered to be significant. All statistical analyses were performed with EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) which was a modified version of R software (The R Foundation for Statistical Computing, Vienna, Austria).[5]

3. Results

A total of 466 patients were enrolled in this study and the median follow up period was 60.7 months. Positive pre- and post- PLC results were obtained in 24 (5.15%) and 28 (6.0%) cases respectively. The patient characteristics such as the age, gender, type of surgery, smoking habit, and histology were comparable between the two groups (Table 1). Both the pre- and post-PLCs had a significant association with an advanced pathological stage, nodal involvement, vascular invasion, lymphatic permeation, and pleural invasions (Table 2). The number of patients with each PLC status is shown in Table 3. The overall 5-year survival rates with a 95% confidence interval in the negative pre-or post-PLC patients were 69.9 (65.1–74.1) % and 71.1 (66.4–75.3) %, and 32.6 (14.0–54.8) % and 21.4 (8.2–38.7) % in the positive patients ($p = 0.001$, $p < 0.001$; Fig. 1a and b), respectively. However, the survival difference was significant in the patients with stage I disease (Fig. 2), and not in the patients with stage II and III diseases ($p = 0.23$, $p = 0.096$; Fig. 3). The post-PLC status was found to be a significant prognostic factor ($p = 0.013$; Fig. 4a) in the patients with stage IB disease, but the pre-PLC and pleural invasion were not ($p = 0.442$, $p = 0.413$; Fig. 4b and c).

A multivariate analysis of the overall survival showed that a positive post-PLC ($p = 0.01$) and the pathologic staging ($p < 0.001$) were independent prognostic factors, whereas the pre-PLC was not ($p = 0.79$) (Table 4). In stage I disease, the patients with a positive post-PLC had a significantly worse 5-year survival rate regardless of the pre-PLC status (Fig. 5).

4. Discussion

Pleural lavage cytology (PLC) is an established independent prognostic factor for a resected NSCLC. However, the optimal timing of the test is still controversial as to whether it should be performed before (pre-PLC) or after (post-PLC) the lung resection,[6–12] and the dose of the saline solution varies from 50 to 1000 mL in the literature.[8–15]

Our study revealed that the overall survival was significantly worse in patients with a positive result of each PLC, and a multivariate analysis confirmed that the post-PLC was

Table 1 Patient characteristics and relationship between the pre- and post-PLC status.

	pre-PLC		total	*P*	post-PLC		total	*P*
	Positive	Negative			Positive	Negative		
Total	24	442	466		28	438	466	
Gender				0.14				0.43
Male	18	259	277		19	259	277	
Female	6	183	189		9	180	189	
Age (year; mean)	61.9	65.1		0.14	66.0	64.9		0.60
Type of Surgery				0.76				0.78
Lobectomy	22	410	432		26	406	432	
Segmentectomy	2	26	28		2	26	28	
Pneumonectomy	0	6	6		0	6	6	
Smoking status				0.28				0.42
Never	6	167			8	165		
Former or current	18	275			20	273		
Histology				0.78				0.60
Adenocarcinoma	22	327	349		25	324	349	
Squamous cell carcinoma	2	72	74		2	72	74	
Large cell carcinoma	0	17	17		0	17	17	
Ad-Sq	0	13	13		1	12	13	
LCNEC	0	4	4		0	4	4	
Others	0	9	9		0	9	9	

(Ad-Sq = adenosquamous cell carcinoma, LCNEC = large cell neuroendocrine carcinoma, PLC = pleural lavage cytology).

Table 2 Relationship between the pathological factors and pre- and post-PLC status.

	pre-PLC		total	*P*	post-PLC		total	*P*
	Positive	Negative			Positive	Negative		
Total	24	442	466		28	438	466	
Pathological stage				<0.001				<0.001
IA	2	213	215		0	215	215	
IB	9	101	110		10	100	110	
IIA	0	24	24		0	24	24	
IIB	2	31	33		3	30	33	
IIIA	6	48	54		11	43	54	
IIIB	5	25	30		4	26	30	
Nodal involvement				0.014				<0.001
Positive	11	100			15	96		
Negative	13	342			13	342		
Vascular invasion				0.029				0.009
Positive	20	247			23	244		
Negative	4	188			4	188		
NA	0	7			1	6		
Lymphatic permeation				<0.001				0.002
Positive	18	133			17	134		
Negative	6	302			10	298		
NA	0	7			1	6		
Pleural invasion				0.001				0.005
Positive	17	151			18	150		
Negative	7	284			10	281		
NA	0	7			0	7		

(NA = not assessed).

Table 3 The number of patients with each PLC status.

		Pre-PLC			
		Positive		Negative	
Post-PLC	Positive	11	(2.36%)	17	(3.64%)
	Negative	13	(2.79%)	425	(91.2%)

an independent prognostic factor whereas the pre-PLC status was not. These results were consistent with the previous reports[11–13] and suggested that the PLC testing should be done after the lung resection with or without being performed before the resection. In addition, the positive rates of pre- and post- PLCs in our study (5.15%,6.0%, respectively) were also consistent with those of the past reports using a greater dose of saline ranging from 3 to 10%.[6,9–16] These results suggested that 20 mL of saline was feasible for the PLC. A smaller dose of saline would be beneficial to simplify the procedures both in retrieving the solution during surgery and in the cytological evaluation after surgery. Further, it would also facilitate to generalize the routine testing of the post-PLC.

Although the reason why the post-PLC had a stronger impact on the survival than the pre-PLC is unclear, recent studies of circulating tumor cells (CTCs) might answer the question. CTCs in patients with non-small cell lung cancer have been reported to be a worse prognostic factor.[17,18] Furthermore, several studies have revealed that the more CTCs are detected after lung cancer surgery than before any surgical manipulation.[19,20] These results suggest that the surgical procedure itself could facilitate the spread of the tumor cells out of the lung parenchyma and also lead to a worse prognosis. It is likely that surgical manipulation could spread the tumor cells not only into the blood stream, presenting as CTCs, but also to the pleural cavity and also might result in a stronger impact of the post-PLC for a worse prognosis. We surgeons should pay much attention to avoid the direct spillages of tumor cells by lymph node dissection or palpating the primary lesions during surgery.

Regarding the treatment in patients with a positive PLC, local intrapleural therapy did not improve the survival[10,21] and therefore adjuvant chemotherapy might be a treatment option.[10,13,22] The current indication for an adjuvant therapy is determined only by the TNM staging system. Although a positive result of the PLC is defined as R1 (residual microscopic disease),[23] it does not directly affect the final pathological staging. Hence, upstaging of the T category based on a positive PLC status has been proposed.[2,9,14,16,24] With this incorporation of the PLC status into the TNM, we could detect more high-risk patients who

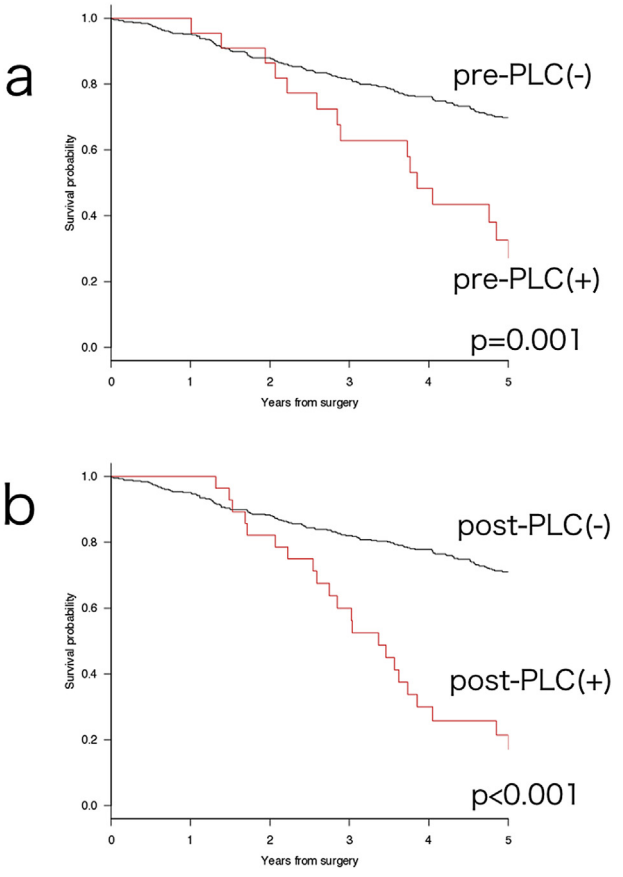

Figure 1 The overall survival was significantly worse in the patients with a positive pre- (a) and post- (b) PLC status ($p = 0.001$, $p < 0.001$, respectively).

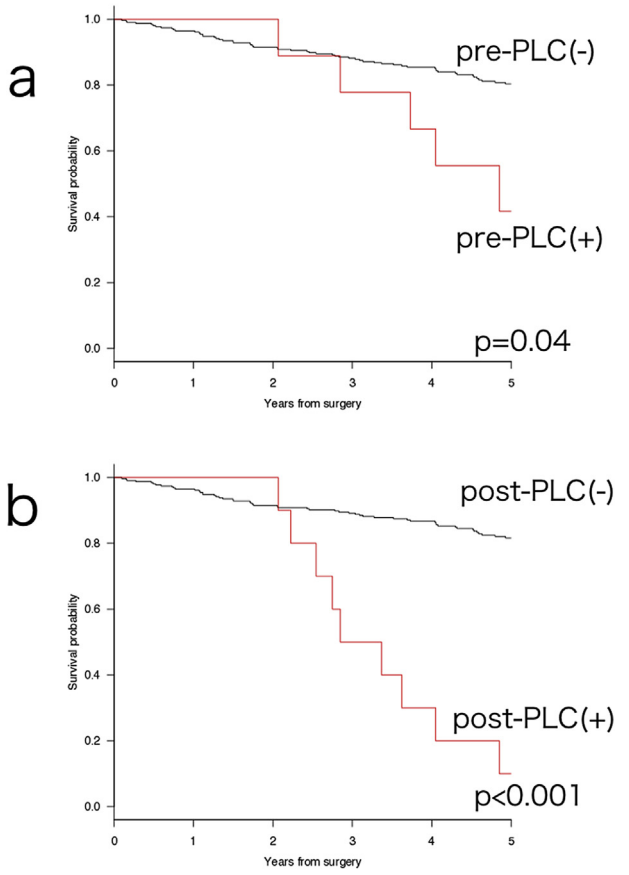

Figure 2 The overall survival was significantly worse in the patients with a positive pre- (a) and post- (b) PLC status in the patients with stage I disease ($p = 0.04$, $p < 0.001$, respectively).

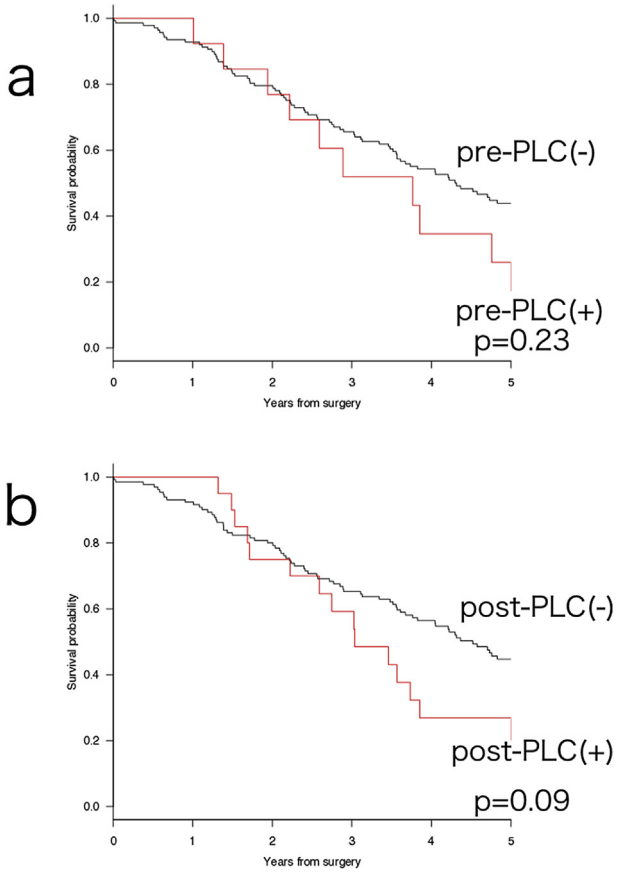

Figure 3 The overall survival was comparable regardless of the pre- (a) and post- (b) PLC status in the patients with stage II and III diseases ($p = 0.23$, $p = 0.096$, respectively).

were currently not candidates for adjuvant chemotherapy among the patients with otherwise stage I disease. Thus, the PLC, especially the post-PLC, would identify those new candidates for adjuvant therapy and therefore should be tested routinely especially in early stage disease.

One major limitation of this study was the retrospective setting and its long study period between 2001 and 2011. The therapeutic modality including molecular targeting agent have drastically changed during this period and therefore other confounding factors might have affected the results of this study other than the PLC.

Another limitation was that this study was based on the seventh edition of the TNM Classification of Malignant Tumors and the applicability of our result for the current eighth edition is unclear. However, one of the aims of this study was to investigate the feasibility of our method comparing to the past reports mostly based on the former editions of the staging system. Therefore, we consider that our results have a clinical value and their validity for the current staging system is to be investigated in the future.

The post-PLC was a more significant prognostic factor than the pre-PLC and should be tested routinely especially in stage I disease. Because this was consistent with the past reports using a greater dose of saline, 20 mL of saline would be feasible for the test. A smaller dose of saline may

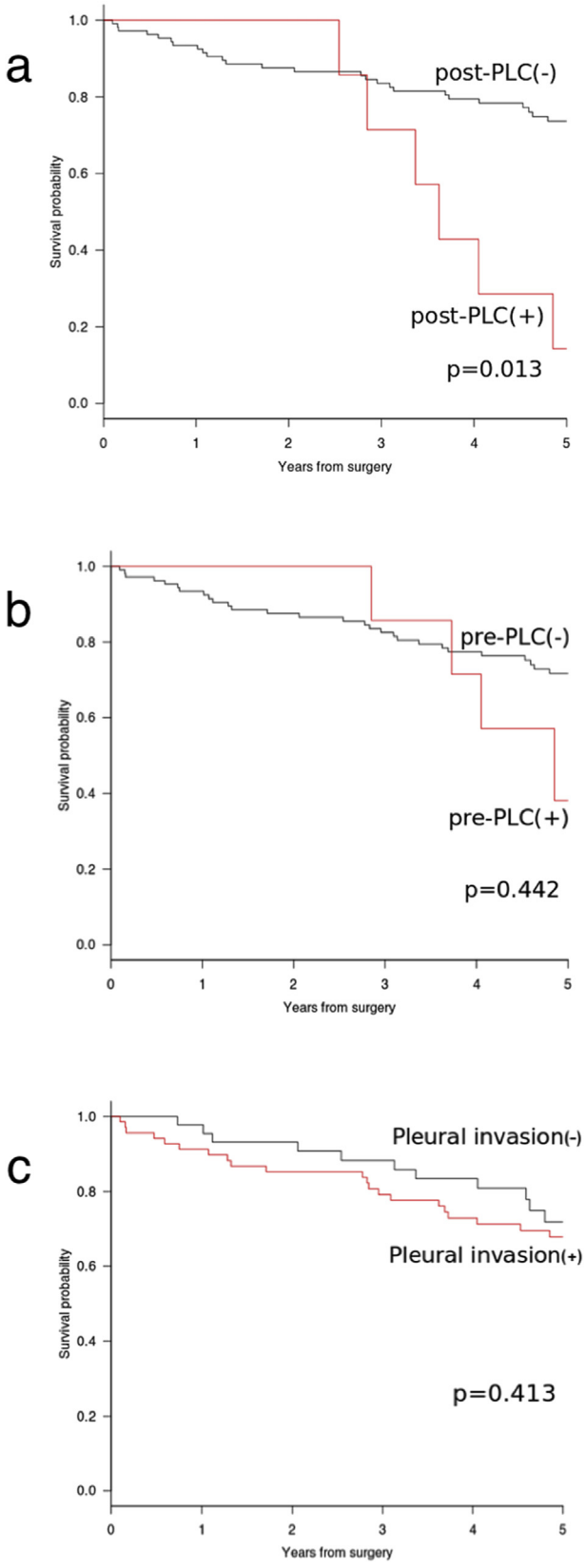

Figure 4 The overall survival was significantly worse in the patients with a positive post-PLC (a) status ($p = 0.013$) but was comparable regardless of the pre-PLC (b) and pleural invasion (c) status ($p = 0.442$, $p = 0.413$, respectively) in the patients with stage IB disease.

Table 4 Univariate and multivariate analyses for the overall survival in the eligible patients.

Parameters		Univariate Analysis			P	Multivariate Analysis			P
		HR	95% CI for HR			HR	95% CI for HR		
			Lower	Upper			Lower	Upper	
Vascular invasion	Negative/positive	1.66	0.53	5.27	0.38	—	—	—	—
Lymphatic permeation	Negative/positive	1.46	0.47	4.59	0.51	—	—	—	—
Pleural invasion	Negative/positive	1.95	0.62	6.18	0.25	—	—	—	—
Histology	Ad/Non-Ad	1.72	1.26	2.35	<0.001	1.29	0.91	1.83	0.14
Gender	Female/male	1.88	1.36	2.59	<0.001	1.19	0.74	1.89	0.48
Smoking habit	Negative/positive	1.94	1.38	2.72	<0.001	1.34	0.82	2.19	0.24
Nodal involvement	Negative/positive	2.98	2.20	4.03	<0.001	1.27	0.79	2.00	0.32
Pre-PLC	Negative/positive	2.31	1.36	3.93	0.001	1.09	0.56	2.12	0.79
Post-PLC	Negative/positive	3.27	2.08	5.14	<0.001	2.12	1.19	3.77	0.01
Pathologic staging	I/II &III	3.28	2.44	4,42	<0.001	2.26	1.43	3.59	<0.001

(Ad = adenocarcinoma, CI = confidence interval, HR=Hazard ratio).

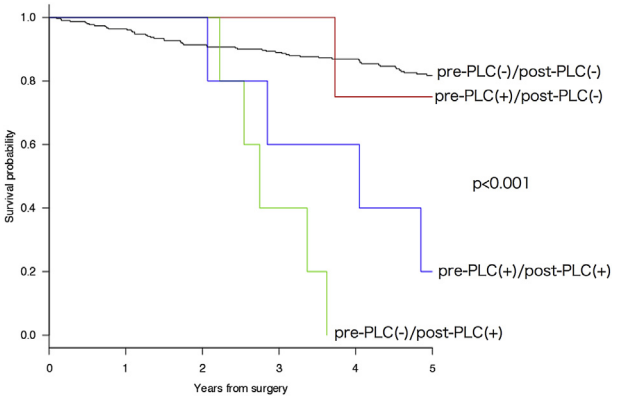

Figure 5 The overall survival was significantly worse in the patients with a positive post-PLC regardless of the pre-PLC status in stage I disease ($p < 0.001$).

facilitate generalizing the routine testing of the post-PLC by simplifying the procedure.

Conflict of interest

Nothing to declare.

References

1. Li YN, Shi HZ, Liang QL, Yang HB, Huang GM. Prognostic significance of pleural lavage cytology in patients with lung cancer: a meta-analysis. *Lung Cancer*. 2008;60:183—192.
2. Lim E, Clough R, Goldstraw P, et al. Impact of positive pleural lavage cytology on survival in patients having lung resection for non-small-cell lung cancer: an international individual patient data meta-analysis. *J Thorac Cardiovasc Surg*. 2010;139:1441—1446.
3. Wang CM, Ling ZG, Wu YB, et al. Prognostic value of pleural lavage cytology in patients with lung cancer resection: an updated meta-analysis. *PLoS One*. 2016;11:e0157518.
4. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol*. 2007;2:706—714.
5. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48:452—458.
6. Kondo H, Asamura H, Suemasu K, et al. Prognostic significance of pleural lavage cytology immediately after thoracotomy in patients with lung cancer. *J Thorac Cardiovasc Surg*. 1993;106:1092—1097.
7. Higashiyama M, Doi O, Kodama K, et al. Pleural lavage cytology immediately after thoracotomy and before closure of the thoracic cavity for lung cancer without pleural effusion and dissemination: clinicopathologic and prognostic analysis. *Ann Surg Oncol*. 1997;4:409—415.
8. Dresler CM, Fratelli C, Babb J. Prognostic value of positive pleural lavage in patients with lung cancer resection. *Ann Thorac Surg*. 1999;67:1435—1439.
9. Okada M, Tsubota N, Yoshimura M, Miyamoto Y, Maniwa Y. Role of pleural lavage cytology before resection for primary lung carcinoma. *Ann Surg*. 1999;229:579—584.
10. Satoh Y, Hoshi R, Ishikawa Y, Horai T, Okumura S, Nakagawa K. Recurrence patterns in patients with early stage non-small cell lung cancers undergoing positive pleural lavage cytology. *Ann Thorac Surg*. 2007;83:197—202.
11. Shoji F, Yamazaki K, Kouso H, Mori R, Takeo S. The impact of pleural lavage cytology both before and after lung resection on recurrence of non-small cell lung cancer. *Ann Thorac Surg*. 2016;101:2141—2146.
12. Enatsu S, Yoshida J, Yokose T, et al. Pleural lavage cytology before and after lung resection in non-small cell lung cancer patients. *Ann Thorac Surg*. 2006;81:298—304.
13. Taniguchi Y, Nakamura H, Miwa K, et al. Prognostic significance of pleural lavage cytology after thoracotomy and before closure of the chest in lung cancer. *Interact Cardiovasc Thorac Surg*. 2009;9:102—106.
14. Kotoulas C, Lazopoulos G, Karaiskos T, et al. Prognostic significance of pleural lavage cytology after resection for non-small cell lung cancer. *Eur J Cardio Thorac Surg*. 2001;20:330—334.
15. Mazza F, Ferrari E, Maineri P, Dozin B, Ratto GB. Pleural lavage cytology predicts recurrence and survival, even in early non-small cell lung cancer. *Surg Today*. 2015;45:322—328.
16. Kameyama K, Okumura N, Miyaoka E, et al. Prognostic value of intraoperative pleural lavage cytology for non-small cell lung cancer: the influence of positive pleural lavage cytology results on T classification. *J Thorac Cardiovasc Surg*. 2014;148:2659—2664.

17. Hofman V, Bonnetaud C, Ilie MI, 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—835.
18. Krebs MG, Sloane R, Priest L, et al. Evaluation and prognostic significance of circulating tumor cells in patients with non-small-cell lung cancer. *J Clin Oncol*. 2011;29: 1556—1563.
19. Hashimoto M, Tanaka F, Yoneda K, et al. Significant increase in circulating tumour cells in pulmonary venous blood during surgical manipulation in patients with primary lung cancer. *Interact Cardiovasc Thorac Surg*. 2014;18:775—783.
20. Sawabata N, Funaki S, Hyakutake T, Shintani Y, Fujiwara A, Okumura M. 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? *Surg Today*. 2016;46: 1402—1409.
21. Ichinose Y, Tsuchiya R, Koike T, et al. A prematurely terminated phase III trial of intraoperative intrapleural hypotonic cisplatin treatment in patients with resected non-small cell lung cancer with positive pleural lavage cytology: the incidence of carcinomatous pleuritis after surgical intervention. *J Thorac Cardiovasc Surg*. 2002;123:695—699.
22. Nakamura T, Suzuki K, Mochizuki T, et al. Prognostic significance and possibility in guiding adjuvant therapy of the pleural lavage cytology in patients with non-small cell lung cancer. *Interact Cardiovasc Thorac Surg*. 2009;8:321—324.
23. Rami-Porta R, Wittekind C, Goldstraw P. International Association for the Study of Lung Cancer Staging C. Complete resection in lung cancer surgery: proposed definition. *Lung Cancer*. 2005;49:25—33.
24. Aokage K, Yoshida J, Ishii G, et al. The impact on survival of positive intraoperative pleural lavage cytology in patients with non-small-cell lung cancer. *J Thorac Cardiovasc Surg*. 2010; 139:1246—1252, 1252 e1241.