Intraoperative pleural lavage cytology after lung resection as an independent prognostic factor for staging lung cancer

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Objective: Although malignant pleural effusion or dissemination is regarded as T4 per TNM classification of lung cancer, the prognostic significance in staging of pleural lavage cytologic examination remains undetermined. The purpose of this study was to clarify the utility of pleural lavage cytologic staging as a prognostic factor in patients with non-small cell lung cancer.

Methods: In 1271 patients with lung cancer who underwent curative resection, intraoperative pleural lavage cytologic examination was performed at thoracotomy (first cytologic examination), immediately after pulmonary resection and mediastinal lymph node dissection (second cytologic examination), and after last washing of pleural cavity (third cytologic examination). Positive first cytologic result represented cytologic positive result before lung resection; positive second and third cytologic results were regarded as cytologic positive results after lung resection.

Results: Eighty-nine patients (7.0%) had positive findings of pleural lavage cytologic examination before or after lung resection. Five-year survivals were 44.1% for patients with positive results before lung resection and 23.4% for patients with positive results after lung resection, both significantly worse than that for patients with negative results. Multivariate analyses revealed that positive lavage result after lung resection was an independent prognostic factor. We found significantly greater pleural recurrence among patients with positive pleural lavage cytologic results after lung resection than among those with negative results.

Conclusions: In addition to TNM classification, results of pleural lavage cytologic examination after lung resection should be considered when staging non-small cell lung cancer. Adjuvant systemic therapy may improve outcome for patients with positive results.

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Intraoperative pleural lavage cytologic examination (PLC) has been suggested as a possible prognostic factor for patients with non-small cell lung cancer (NSCLC).¹⁻⁷ Although there have been differences in the timing of instillation, volume, and type of carrier solution, a consistent finding is the uniformly poor prognosis of patients with positive results.⁸ We performed intraoperative PLC on more than 1200 patients at the same institution since 1985 and analyzed the relationship between positive cytologic findings and several pathologic factors. Malignant pleural effusion or pleural dissemination is regarded as T4 according to the TNM classification⁹; however, the prognostic significance of PLC remains undetermined in terms of staging. The aim

of this study was to clarify the utility of PLC as a prognostic factor for patients with NSCLC.

MATERIALS AND METHODS

From September 1985 to December 2005 in our institution, thoracotomy was performed in 1490 patients with pathologic stage IA to IIIA NSCLC. Because we excluded patients with effusion at the time of the operation, extreme adhesions, extreme chest wall invasion, or lack of curative resection, this study consisted of 1271 patients with lung cancer. All patients underwent intraoperative PLC at three times as previously reported⁴: (1) at thoracotomy immediately on entering the pleural space (1st PLC), (2) at thoracotomy on completion of pulmonary resection and mediastinal lymph nodes (2nd PLC), and (3) at thoracotomy after the last washing of the pleural cavity with 5000 mL physiological saline solution before closing (3rd PLC). At each lavage, the pleural cavity was filled with 500 mL physiologic saline solution, which was aspirated into a glass bottle with heparin. About 400 mL of the supernatant of the lavage fluid was discarded after settlement, and the remainder was centrifuged at 1500 rpm for 5 minutes. Cytologic examination was performed with the Papanicolaou method. Papanicolaou classes IV and V were regarded as positive cytologic findings.

Pathologic staging was assessed according to TNM staging (International Union Against Cancer),⁹ and the histologic type of the tumor was assessed according to the World Health Organization classification.¹⁰ Pathologic TNM classification, pleural status, differences in histologic type, and lymphatic and vascular invasions were examined. The Japanese Lung Cancer Society¹¹ guidelines were used to classify pleural status into four groups as follows: p0, visceral pleura is not invaded by the tumor; p1, tumor has reached but not invaded through the visceral pleura; p2, tumor has invaded the visceral pleura but has not involved the parietal pleura; and p3, tumor has invaded the parietal pleura and chest wall.

Survival was defined as the interval in months between the day of operation and the date of death from any cause or of the last follow-up. Cancer recurrence was divided into three categories according to the sites of initial

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Abbreviations and Acronyms

CI	= confidence interval
NSCLC	= non-small cell lung cancer
PLC	= pleural lavage cytologic examination

recurrence: locoregional, pleural, and distant. Pleural recurrence was defined as any recurrent disease within the hemithoracic pleura.

An institutional review board approved this retrospective study, and written informed consent for the surgical intervention was obtained from each patient. The χ^2 test was used to assess the statistical significance of the differences according to PLC status and clinicopathologic factors. Survival curves were calculated according to the method of Kaplan and Meier,¹² and the log-rank test was used to compare the survival curves. Variables related to survival were analyzed with the Cox proportional hazards regression model¹³ (StatView version 5.0; SAS Institute, Inc, Cary, NC).

RESULTS

Patient clinicopathologic characteristics are shown in Table 1. The median follow-up was 5.7 years. Eighty-nine patients, 7.0% of the study group (n = 1271), had positive findings on PLC before or after lung resection. As shown in Table 2, 67 patients (5.3%) had positive PLC results at thoracotomy (1st PLC). Thirty-two patients (2.5%) had positive results at thoracotomy on completion of pulmonary resection and mediastinal lymph nodes (2nd PLC), at thoracotomy after the last washing of the pleural cavity before closing (3rd PLC), or both. Positive 1st PLC result was regarded as a positive PLC result before lung resection, whereas positive 2nd and 3rd PLC results were regarded as positive PLC results after lung resection. Ten patients had positive PLC results both before and after lung resection.

There were significant differences between patients with negative and positive PLC results before lung resection in pathologic stage, pleural invasion, T status, and vascular invasion (Table 3). Furthermore, significant differences were observed between patients with negative and positive PLC results after lung resection in pathologic stage, T status, and vascular invasion (Table 4). The 5-year survivals were 44.1% (95% confidence interval [CI] 31.2%–57.0%) for patients with positive PLC results before lung resection and 21.9% (95% CI 7.6%–34.2%) for patients with positive PLC results after lung resection, both of which were significantly worse than the 58.3% (95% CI 55.2%–61.4%) survival for patients with negative PLC results (Figures 1 and 2). To evaluate PLC result as a prognostic factor, multivariate analyses were performed with variables regarded as clinical prognostic factors for patients with NSCLC. These analyses revealed that a positive PLC result after lung resection was an independent prognostic factor (Table 5). Age, pathologic stage, lymphatic invasion, and vascular invasion were also independent prognostic factors.

We found recurrences in 384 of 1182 patients (32.5%) with negative PLC results, in 32 of 67 patients (47.8%)

TABLE 1. Patient characteristics (n = 1271)

Characteristic	No.
Sex	
Male	870
Female	401
Pathologic stage	
IA	393
IB	335
IIA	44
IIB	232
IIIA	267
Histologic type	
Adenocarcinoma	699
Squamous cell carcinoma	398
Others	174

with positive PLC results before lung resection, and in 20 of 32 patients (62.5%) with positive PLC results after lung resection. The 5-year disease-free survivals were 36.9% (95% CI 22.2%–51.6%) for patients with positive PLC results before lung resection, 34.1% (95% CI 14.3%–54.9%) for patients with positive PLC results after lung resection, and 38.1% (95% CI 34.0%–42.2%) for patients with negative PLC results. There was no significant difference among these survivals. Recurrence patterns among patients with positive PLC results are shown in Table 6. We found a significantly higher rate of pleural recurrence in patients with positive PLC results after lung resection than among those with negative PLC results.

DISCUSSION

The survival in the group with positive PLC results was significantly lower than that in the group with negative PLC results, indicating that intraoperative PLC is a useful technique for estimating the prognosis of patients with lung cancer, as has been previously suggested.^{1-7,14} In agreement with the findings of Enatsu and colleagues,¹⁵ positive PLC results after lung resection had a greater impact on

TABLE 2. Results of pleural lavage cytologic examination

Cytologic examination				Results	
1st PLC	2nd PLC	3rd PLC	Total patients	Before resection	After resection
Negative	Negative	Negative	1182	Negative	Negative
Positive	Negative	Negative	57	Positive	Negative
Positive	Negative	Positive	2	Positive	Positive
Positive	Positive	Negative	4	Positive	Positive
Positive	Positive	Positive	4	Positive	Positive
Negative	Negative	Positive	4	Negative	Positive
Negative	Positive	Negative	13	Negative	Positive
Negative	Positive	Positive	5	Negative	Positive

PLC, Pleural lavage cytologic result; 1st PLC, at thoracotomy immediately on entering pleural space; 2nd PLC, after completion of pulmonary resection and mediastinal lymph node dissection; 3rd PLC, after last washing of pleural cavity before closing.

TABLE 3. Clinicopathologic characteristics of patients with positive results of pleural lavage cytologic examination before lung resection

Factor	Positive (n = 67)	Negative (n = 1204)	Total (n = 1271)	P value
Sex				.2969
Male	42 (4.8%)	828	870	
Female	25 (6.2%)	376	401	
Pathologic stage				.0397
I	29 (4.0%)	699	728	
II	17 (6.2%)	259	276	
IIIA	21 (7.9%)	246	267	
Histologic type				.1408
Adenocarcinoma	44 (6.3%)	655	699	
Squamous cell carcinoma	14 (3.5%)	384	398	
Other	9 (5.2%)	165	174	
Pathologic pleural involvement				<.0001
p0	26 (3.4%)	731	757	
p1	10 (4.5%)	212	222	
p2	18 (14.7%)	105	123	
p3	13 (7.7%)	156	169	
Pathologic T status				.0002
T1	10 (2.1%)	469	479	
T2	43 (6.8%)	592	635	
T3	14 (8.9%)	143	157	
Pathologic N status				.0937
N0	36 (4.5%)	772	808	
N1	13 (5.4%)	229	242	
N2	18 (8.1%)	203	221	
Lymphatic invasion (n = 1192)				.1751
Positive	42 (6.0%)	655	697	
Negative	21 (4.2%)	474	495	
Vascular invasion (n = 1193)				.0048
Positive	47 (6.9%)	639	686	
Negative	16 (3.2%)	491	507	

Values represent numbers of patients.

TABLE 4. Clinicopathologic characteristics for patients with positive results of pleural lavage cytologic examination after lung resection

Factor	Positive (n = 32)	Negative (n = 1239)	Total (n = 1271)	P value
Sex				.6728
Male	23 (2.6%)	847	870	
Female	9 (2.2%)	392	401	
Pathologic stage				.6991
I	16 (2.2%)	712	728	
II	8 (2.9%)	268	276	
IIIA	8 (3.0%)	259	267	
Histologic type				.9785
Adenocarcinoma	18 (2.6%)	681	699	
Squamous cell carcinoma	10 (2.5%)	388	398	
Other	4 (2.3%)	170	174	
Pathologic pleural involvement				.1674
p0	14 (1.8%)	743	757	
p1	6 (2.7%)	216	222	
p2	4 (3.3%)	119	123	
p3	8 (4.7%)	161	169	
Pathologic T status				.0016
T1	3 (0.6%)	476	479	
T2	21 (3.3%)	614	635	
T3	8 (5.1%)	149	157	
Pathologic N status				.6299
N0	18 (2.2%)	790	808	
N1	8 (3.3%)	234	242	
N2	6 (2.7%)	215	221	
Lymphatic invasion (n = 1192)				.2886
Positive	21 (3.0%)	676	697	
Negative	10 (2.0%)	485	495	
Vascular invasion (n = 1193)				.0468
Positive	24 (3.5%)	662	686	
Negative	7 (1.4%)	500	507	

survival than did positive PLC results before lung resection. Multivariate analyses also revealed that a positive PLC result after lung resection as an independent prognostic factor was as significant as established prognostic factors, including T status and N status. The 5-year survival after surgery was 23.4% for patients with positive PLC results after lung resection, whereas the 5-year survivals for patients with stages IIIA and IIIB were 31.4% and 21.7%, respectively. Kotoulas and associates¹⁶ reported that a positive PLC result after lung resection is a T4 element, upgrading the stage to IIIB. Thus PLC after lung resection as an addition to the TNM classification is recommended to help determine the prognosis of patients with NSCLC.

Buhr and colleagues³ reported that pleural lavage was done before lung resection to determine whether tumor cells were disseminated into the pleural cavity.³ Exfoliation of tu-

mor cells through subpleural lymphatics and vessels to the pleural space has been reported to be a cause of positive PLC results before lung resection.^{5,7} There was a significant relationship between positive PLC results before lung resection and pleural invasion, making PLC at thoracotomy useful for estimation of pleural invasion. Similarly to the findings of Nakagawa and associates,¹⁴ p2 was more frequently observed than p3 in patients with positive PLC results before lung resection, suggesting that tumor that has reached but not invaded through the visceral pleura is important for cancer cells to desquamate into the pleural space. In our study, 32 of 67 patients (47.8%) with positive PLC results before lung resection had recurrence, and 3 of these (9%) had carcinomatous pleuritis, a higher rate of recurrence in the pleural cavity than was seen among patients with negative PLC results (2%).

	Patients at risk					
Negative	1182	832	546	354	227	179
Positive	67	41	23	18	13	9

FIGURE 1. Survival curves for patients with positive versus negative results of pleural lavage cytologic examination before lung resection.

Buhr and colleagues³ also reported that PLC after lung resection indicated whether surgical manipulation had led to exfoliation of tumor cells into the pleural cavity. Kotoulas and associates¹⁶ reported that the main causes of positive PLC results after lung resection are the exfoliation of cancer cells into the pleural cavity from infiltrated mediastinal lymph nodes and the spreading of the cancer cells of infiltrated mediastinal lymph nodes as a result of surgical manipulations during the mediastinal dissections. We also found significant differences in pathologic stage, T status, and vascular invasion between patients with positive and negative PLC results after lung resection, indicating that surgical manipulation of a large tumor with vessel invasion might lead to exfoliation and release of cancer cells into the pleural cavity.

	Patients at risk					
Negative	1182	832	546	354	227	179
Positive	32	16	10	5	3	3

FIGURE 2. Survival curves for patients with positive versus negative results of pleural lavage cytologic examination after lung resection.

TABLE 5. Multivariate analysis of potential prognostic factors

Variable	Hazard ratio	95% Confidence interval	P value
Age			
≥65 y vs <65 y	1.895	1.592–2.256	<.0001
Pathologic stage			
IIIA vs I	2.438	1.969–3.019	<.0001
II vs I	1.626	1.305–2.027	<.0001
Histologic type			
Other vs adenocarcinoma or squamous	1.113	0.936–1.323	.2245
Lymphatic invasion			
Positive vs negative	1.270	1.041–1.551	.0188
Vascular invasion			
Positive vs negative	1.562	1.269–1.921	<.0001
PLC before resection			
Positive vs negative	1.072	0.753–1.525	.7003
PLC after resection			
Positive vs negative	2.313	1.516–3.531	.0001

PLC, Pleural lavage cytologic result.

ity. Thirty-two patients showed positive PLC results after resection of lung and mediastinal lymph nodes; 17 of these had negative results after the last washing of the pleural cavity, whereas the remaining 15 patients showed positive PLC results even after the last washing (Table 2). The 5-year survivals were 29.4% for patients with positive PLC results before the last washing and 15.2% for patients with positive PLC results after the last washing. The frequency of recurrence in the pleura was greater among patients with positive PLC results after lung resection than in other groups. These data suggest that a positive PLC result after lung resection is indicative of cancer cells in the pleural cavity, which in turn may result in local recurrence at pleura.

The Japanese Clinical Oncology Group¹⁷ conducted a randomized trial to determine the efficacy of intraoperative intrapleural hypotonic cisplatin treatment for patients with positive PLC results. Survivals were not significantly different between the treated and control groups. Satoh and colleagues¹⁸ analyzed recurrence patterns in patients with positive PLC results and reported that 72% had distant metastasis.¹⁸ We found distant metastasis in 36 of patients with

TABLE 6. Recurrence of disease in patients with positive and negative results of pleural lavage cytologic examination

Recurrence	Positive		Negative (n = 1182)
	Before resection (n = 67)	After resection (n = 32)	
Total	32	20	384
Pleural	3 (9%)	5 (25%)	7 (2%)
Distant	24 (75%)	11 (55%)	324 (84%)
Locoregional	5 (16%)	4 (20%)	53 (14%)

By χ^2 test, $P < .05$ for positive results before resection versus negative results and $P < .0001$ for positive results after resection versus negative results.

positive PLC results (69.2%) who had recurrence, indicating that a positive PLC result is a sign of systemic disease. Therefore curative resection followed by adjuvant systemic therapy may be necessary to improve outcome for patients with positive PLC results. Furthermore, we should verify intraoperative intrapleural treatment for patients with NSCLC who have positive PLC results after lung resection to reduce the rate of pleural recurrence.

In conclusion, we have shown that a positive PLC result after lung resection can be used as an independent prognostic factor for staging NSCLC, together with such established prognostic factors as pathologic T status and N status. In addition adjuvant systemic therapy may be necessary to improve outcome for patients with positive PLC results.

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