

Clinicopathologic factors influencing postoperative prognosis in patients with small-sized adenocarcinoma of the lung

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Objective: Recent technologic developments in computed tomography have increased the incidence of surgical intervention for small-sized lung cancer. Although indications of a sublobar resection for early disease have been discussed, we occasionally encounter locally advanced small-sized lung cancer with node metastasis. The present study aimed to clarify the histopathologic factors influencing nodal involvement and prognosis of such patients.

Methods: We studied 97 patients who underwent complete resection for an adenocarcinoma of 2 cm or less in diameter. Lymph node metastasis and necrosis were microscopically evaluated, whereas immunohistochemical studies were also performed with Ki-67 and D2-40 for proliferation activity and lymphatic invasion, respectively. In addition, carcinoembryonic antigen expression in the tumor and its level in serum were investigated. Survival analysis was then conducted by using these clinicopathologic factors.

Results: The 5-year disease-free survival rate was 90%. Nodal involvement was significantly frequent in patients with tumors showing microscopic necrosis, a Ki-67 labeling index of greater than 5%, and an increased serum carcinoembryonic antigen level. Furthermore, 5-year disease-free survival was worse in patients with lesions showing microscopic necrosis (68%), a Ki-67 labeling index of greater than 5% (76%), and lymphatic invasion detected with D2-40 staining (77%). Multivariate analysis identified lymphatic invasion detected with D2-40 to be an independent predictor for postoperative recurrence.

Conclusions: These results indicate that microscopic necrosis, Ki-67 labeling index, and serum carcinoembryonic antigen level are predictors of nodal involvement. Careful postoperative follow-up examinations for recurrence are required for patients with tumors that show microscopic necrosis, high Ki-67 labeling index, and lymphatic invasion, even in those with stage IA disease.

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With the development of high-resolution computed tomography (CT) and its application for cancer screening, small-sized peripheral lung cancer is more frequently encountered. A bronchioloalveolar carcinoma that shows ground-glass opacity (GGO) in CT imaging without vessel or lymphatic spread determined histologically has been recognized as a less-invasive subgroup of small-sized adenocarcinomas.^{1,2} The Noguchi classification, which was first reported in 1995 and is popular in Japan, classifies peripheral adenocarcinomas as those of 2 cm or less in diameter.³ Because of their low-grade malignant feature, several studies have evaluated the results of a lesser pulmonary resection by using preoperative information in regard to GGO area or tumor diameter and found it to be safe on the basis of similar outcomes compared with outcomes in patients who underwent a lobectomy.⁴⁻⁷ However, it is well known that lymph node metastasis is not rare in patients with lung

Abbreviations and Acronyms

CEA	= carcinoembryonic antigen
CT	= computed tomography
GGO	= ground-glass opacity
LI	= labeling index

cancer with tumors smaller than 2 cm because the frequency of nodal involvement was reported to be 15% to 28% for tumors sized 2 cm or less and 15% for solid subcentimeter lesions.⁸⁻¹⁰ Thus it is important to identify patients with locally advanced cancer with nodal metastasis originating from small-sized lung cancer to make proper decisions regarding operative mode and adjuvant treatment.

In the present study we analyzed several potential prognostic factors, including histologic necrosis, Ki-67 labeling index (LI), carcinoembryonic antigen (CEA) expression, serum CEA level, and degree of lymphatic invasion based on the D2-40 antibody for lymphangioendothelium, in patients with a pulmonary adenocarcinoma of 2 cm or less in diameter. Furthermore, we evaluated the capability of those factors to predict advanced disease with a worse outcome before or during the operation. We believe that the clinicopathologic predictors revealed in the present study can contribute to the improvement of outcome in patients with a small-sized adenocarcinoma who undergo a complete resection.

Materials and Methods**Patients**

We retrospectively reviewed the records of 565 patients who underwent thoracic surgery for non-small cell lung cancer between 1992 and 2003 at Osaka University Hospital. Of those, 97 who underwent a complete resection of a peripheral adenocarcinoma of 2 cm or less in diameter were enrolled, whereas patients treated with induction therapy or with multiple primary lesions were excluded. None were indicated for a mediastinoscopy. The patient characteristics are shown in Table 1. Postoperative follow-up was completed in 90 patients, and the mean follow-up period was 55 months. Prethoracotomy histologic diagnosis was obtained in 69 (70%) patients: in 43 by using a CT-guided percutaneous needle biopsy, in 25 by using a transbronchial lung biopsy, and in 1 by using sputum cytology. A lobectomy with systematic hilar and mediastinal lymph node dissection was performed in principle. A limited resection was indicated for poor-risk cases, including patients with low pulmonary function, cardiovascular comorbidity, advanced age, and severe diabetes mellitus, as well as for those with a pure GGO lesion smaller than 1.5 cm in diameter. Postoperative staging was performed according to TNM classification.¹¹

Histologic Evaluation

Informed written consent for research use of the surgical specimens was obtained from all of the patients. Surgically resected specimens were fixed in 10% formalin and routinely processed for paraffin embedding. Histologic sections were cut into 4- μ m

TABLE 1. Characteristics of 97 patients who underwent complete resection of a small-sized adenocarcinoma (from our database: 1993–2003)

Age (y)	38–82; average, 63.1
Sex	
Male	53
Female	44
Tumor diameter	
<1 cm	15
1–1.5 cm	13
1.5–2 cm	69
Mode	
Lobectomy	77
Segmentectomy	16
Partial resection	4
p Stage	
IA	82
IB	4
IIA	2
IIB	2
IIIA	7

slices, stained with hematoxylin and eosin, and reviewed by 2 pathologists (T.T. and T.N.), who evaluated the presence of microscopic necrosis. Immunohistochemical studies of the paraffin sections were also performed by using the Envision+ system (DAKO, Carpinteria, Calif) for Ki-67 and CEA (DAKO, Kyoto, Japan) and a Vectastain ABC kit (Vector Laboratories, Burlingame, Calif) for D2-40. Antigen retrieval was performed by heating the sections after immersion in Target Retrieval Solution, High pH (DAKO) in a pressure cooker for 15 minutes. In addition, sections were incubated with primary antibodies (monoclonal antibody for Ki-67 [MIB-1] at a concentration of 1:50 for Ki-67, II-7 at 1:75 for CEA, and D2-40 at 1:120; DAKO) and then lightly counterstained with hematoxylin. Immunohistochemistry with the anti-Ki-67 (clone MIB-1) monoclonal antibody was also performed to estimate the proliferative activity of each tumor, with neoplastic epithelial cells showing intranuclear staining for Ki-67 judged to be positive. The number of Ki-67–positive cells among 200 tumor cells was counted, and the percentage was used as the Ki-67 LI. The Ki-67–high group was defined as those with a Ki-67 LI of greater than 5%, whereas the Ki-67–low group had a Ki-67 LI at 5% or lower. CEA expression was graded as negative (–), no staining observed or membrane staining observed in less than 10% of tumor cells (weakly positive, +), weak to moderate complete membrane staining observed in more than 10% of tumor cells (strongly positive, ++), and strong complete membrane staining observed in more than 10% of tumor cells. Lymphatic invasion was determined to be positive when cancer cells were present surrounded by lymphangioendothelium with D2-40 expression.

Statistical Analyses

Survival probability was calculated by using the Kaplan–Meier method. The prognostic influence of the variables on survival was analyzed by using a Wilcoxon test and Cox regression. A χ^2 or Fisher exact test was used to compare ratios between 2 groups.

Results

Results of Surgical Intervention

The 5-year overall and disease-free survival rates were 93% and 90%, respectively. Lymph node metastasis was detected in 11 (11%) patients, and the pathologic stage was pIA (T1 N0 M0) in 82, IB (T2 N0 M0) in 4, IIA (T1 N1 M0) in 2, IIB (T2 N1 M0) in 2, and IIIA (T1 N2 M0) in 7 (Table 2). The 5-year disease-free survival rate was 95% for patients without lymph node metastasis, which was better than that (59%) for patients with nodal involvement ($P < .001$). According to tumor diameter, the 5-year disease-free survival rate was 100% for patients with a tumor of 1 cm or less in diameter, 92% for those with a tumor of 1 to 1.5 cm in diameter, and 89% for those with a tumor of 1.5 to 2 cm in diameter.

Microscopic Necrosis

Irrespective of the presence or absence of necrosis recognized at a macroscopic level, tumor cell necrosis was present at the microscopic level in 17 (18%) patients (Figure 1, A). Among those patients with necrotic findings in the tumor, 5 (29%) had lymph node metastasis, whereas only 6 (8%) of the 78 patients without necrotic findings showed nodal involvement ($P = .022$, Table 2). The 5-year disease-free survival for patients with microscopic necrosis in the tumor was 68%, which was significantly worse than that for patients without necrosis ($P < .001$; Figure 2, A). Analysis of the 82 patients with stage IA disease without nodal involvement showed a significant difference in regard to disease-free period between those with positive and negative results for necrosis ($P = .035$; Figure 2, B).

Ki-67 LI

Ki-67 values varied from no staining to more than 30% positive cells in small-sized adenocarcinomas. Thirty (31%) patients had a Ki-67 LI of greater than 5% (Ki-67-high group), in whom 15 tumors showed a value of greater than 10% (Figure 1, B). Lymph node metastasis was detected in 8 (27%) of the 30 patients in the Ki-67-high group, which was more frequent compared with that for the Ki-67-low

group ($P = .003$, Table 2). The 5-year disease-free survival rate for the Ki-67-high group was 76%, which was significantly worse than that (99%) for the Ki-67-low group ($P < .001$; Figure 3, A). Even after analyzing patients with stage IA disease without lymph node metastasis, there was a significant difference in disease-free period between the Ki-67-high and Ki-67-low groups ($P = .037$; Figure 3, B).

CEA Expression by Means of Immunohistochemistry and Serum CEA Level

CEA expression varied from negative to moderately and strongly positive (Figure 1, C). In immunohistochemistry results tumor cells expressed CEA in 72 (74%) of the patients, among whom 22 (23%) had strongly positive (++) results. Lymph node metastasis was detected in 5 (23%) of 22 patients with CEA (++) results and 6 (8%) of 75 patients with CEA (–) and (+) results ($P = .118$). Furthermore, patients with an increased serum CEA level had frequent nodal involvement (Table 2). Prethoracotomy increase of serum CEA levels was observed in 24 (25%) of 97 patients. Among those patients, nodal metastasis was detected in 6 (25%), which was a higher percentage than those with a normal CEA level (Table 2). Of 13 patients with both increased serum CEA levels and strong CEA staining of the tumors, 5 (38.5%) had lymph node metastasis. In addition, analysis of the relationship between CEA expression in the tumor shown by means of immunohistochemistry and preoperative serum CEA level demonstrated a significant correlation (Table 3). Strong CEA expression was found in 13 (54%) of 24 patients with increased serum CEA levels. Conversely, 13 (59%) of 22 patients with CEA (++) expression had an increase of serum CEA levels. As for disease-free survival, we found no significant difference between CEA expression level in the tumor and serum CEA level.

Lymphatic Invasion Evaluated With D2-40 Staining of Lymphangioendothelium

Lymphatic invasion was detected in 18 (19%) of the patients by using immunohistochemistry with D2-40 for visualizing lymphatic vessels (Figure 1, D). The 5-year disease-free survival for those with lymphatic invasion was 77%, which was significantly worse than that (95%) for patients without lymphatic invasion ($P < .001$; Figure 4, A). When analyzing patients with stage IA disease without lymph node involvement, the 5-year disease-free survival for those with lymphatic invasion tended to be poor compared with that for patients without lymphatic invasion ($P = .061$; Figure 4, B). No significant correlation was found between lymph node metastasis and lymphatic invasion in the present group of patients.

Multivariate Analysis for Prognostic Influence

Multivariate analysis with microscopic necrosis, Ki-67 LI, lymphatic invasion detected with D2-40, serum CEA level,

TABLE 2. Relationships between lymph node metastasis and microscopic necrosis, Ki-67 labeling index, and serum carcinoembryonic antigen level

	LN positive	LN negative	P value
Necrosis (+)	5 (29%)	12	
Necrosis (–)	6 (8%)	74	.022
Ki-67 LI <5%	3 (4%)	64	
Ki-67 LI >5%	8 (27%)	22	.003
Increased CEA level	6 (25%)	18	
Normal CEA level	5 (7%)	68	.025

The cutoff level for serum CEA is 5 ng/mL. The Fisher exact test was used for statistical analysis. LN, Lymph node, LI, labeling index; CEA, carcinoembryonic antigen.

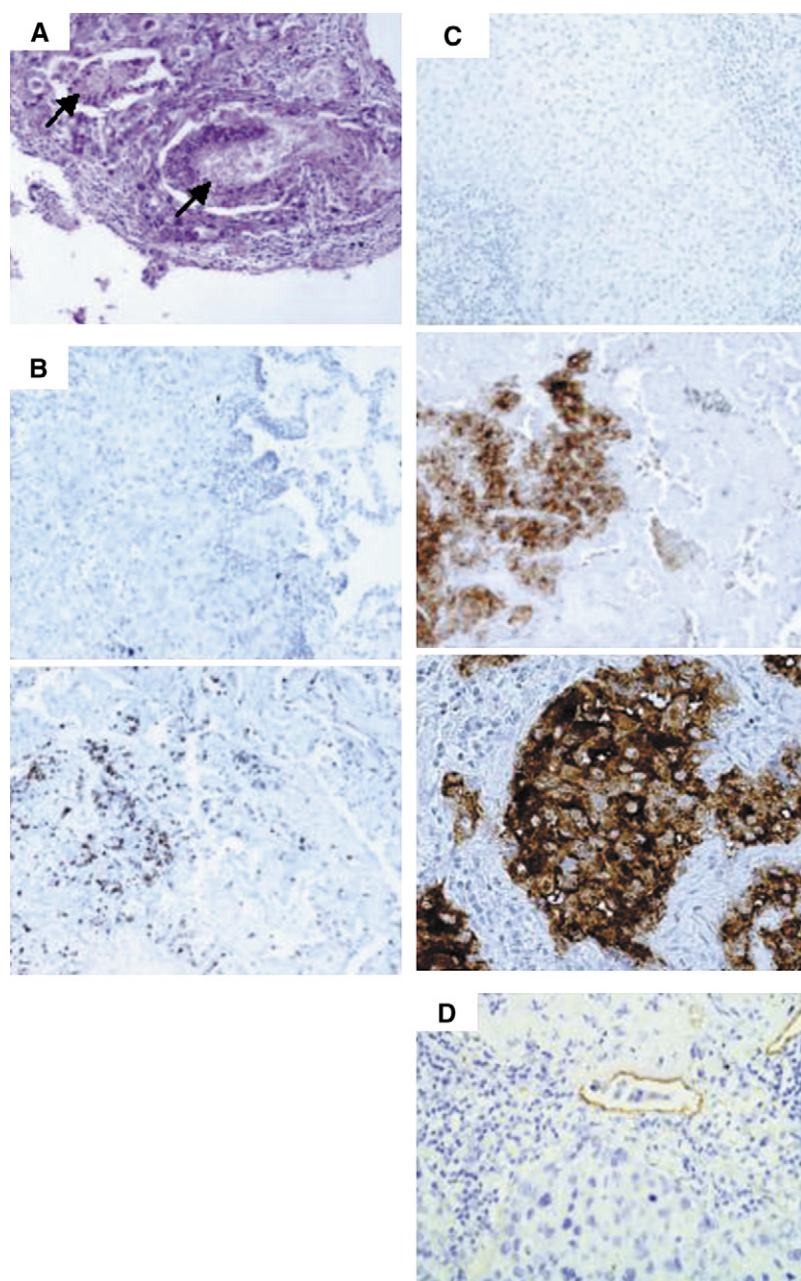


Figure 1. A, Microscopic necrosis (arrow): hematoxylin and eosin staining, original magnification $\times 40$. B, Ki-67 staining: original magnification $\times 40$. Top, Few cancer cells were stained with MIB-1 in this specimen. Immunohistochemistry results classified the tumor as having a Ki-67 labeling index of less than 5% (Ki-67–low group). Bottom, A proportion of cancer cells was positively stained. Ki-67 labeling index was judged as 20% (Ki-67–high group). C, Carcinoembryonic antigen expression: original magnification $\times 40$. Top, Negative staining; middle, moderate expression; bottom, strong expression. D, Immunohistochemistry with D2-40 to evaluate lymphatic invasion: original magnification $\times 40$. Lymphatic invasion by cancer cells was revealed by D2-40 staining of the lymphatic vessels.

and p stage as variables identified only lymphatic invasion as an independent predictor for postoperative recurrence (Table 4).

Discussion

In the present clinicopathologic study, which focused on small-sized pulmonary adenocarcinomas of 2 cm or less in diameter, we identified the presence of microscopic necrosis, high Ki-67 LI, and increased serum CEA level as predictors of lymph node metastasis, whereas microscopic necrosis,

higher Ki-67 LI, and lymphatic invasion detected with D2-40 staining were negative factors for disease-free survival. A lesser pulmonary resection for a peripheral small-sized lung cancer with careful node exploration has become an accepted therapy.^{6,7} However, when serum CEA levels are increased in a preoperative examination or if microscopic necrosis is found in preoperative biopsy results or intraoperative rapid diagnosis by using frozen sections, a standard operation with a lobectomy with node dissection should be considered. In addition, patients with a tumor showing

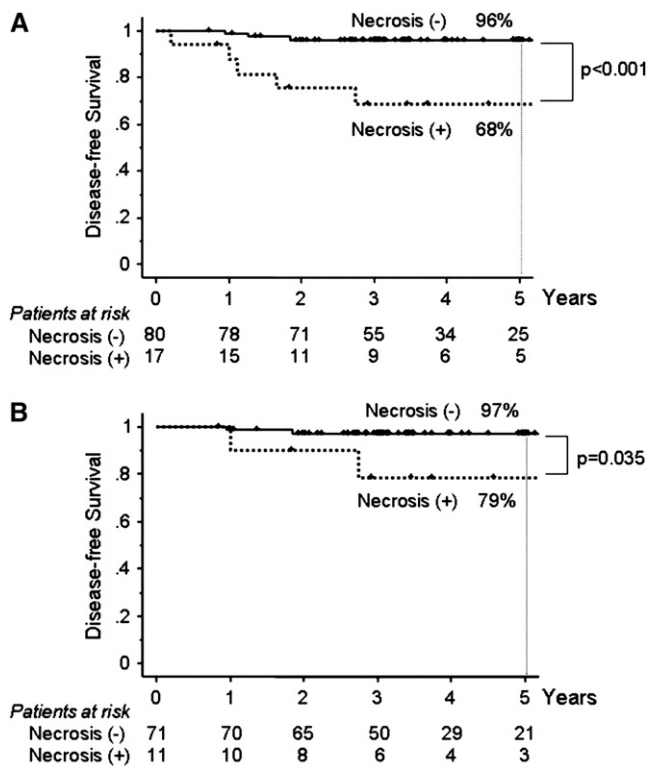


Figure 2. A, Disease-free survival for all patients according to microscopic findings of necrosis. Patients with necrosis in the tumor showed a worse outcome compared with those without necrosis. B, Disease-free survival for patients without nodal metastasis according to microscopic findings of necrosis. The difference in the disease-control ratio was statistically significant.

microscopic necrosis, higher Ki-67 LI, or lymphatic invasion might be a candidate for adjuvant therapy, even if the lesion is small and diagnosed as pI.

Kessler and colleagues¹² reported that a high percentage of necrosis was a negative prognostic factor in addition to blood vessel invasion in patients with resected non-small cell lung carcinomas, although 66% of those had squamous cell carcinomas. In another report a significant correlation of necrosis with T stage, tumor size, or invasiveness was documented.¹³ Necrosis is speculated to be caused by chronic cellular hypoxia as a result of rapid tumor progression, and thus it is reasonable that necrotic findings have a negative prognostic effect. In the present study we found a predictive value of microscopic necrosis for nodal involvement and postoperative disease progression in patients with a small-sized adenocarcinoma, in whom macroscopic necrosis is seldom recognized. Furthermore, in our analysis of pIA disease, microscopic necrosis was shown to be a useful prognostic factor for recurrence. Because such microscopic necrosis can be observed intraoperatively in frozen sections and rapidly diagnosed, clinical application is expected.

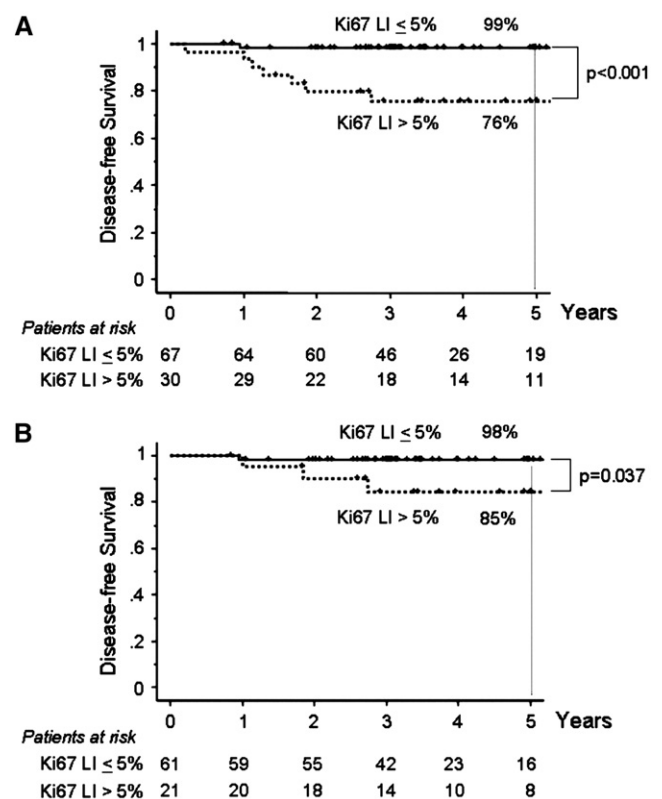


Figure 3. A, Disease-free survival for all patients according to Ki-67 labeling index (LI). The Ki-67-high group showed a significantly worse outcome compared with the Ki-67-low group. B, Disease-free survival for patients without lymph node metastasis according to Ki-67 LI. The difference in the disease-control ratio was statistically significant.

Ki-67 is generally known as a nuclear protein that is expressed on various cells in other than the G0 phase of the cell cycle. A significant correlation between Ki-67 expression and lymph node metastasis was previously reported for other malignancies,^{14,15} and the prognostic significance of Ki-67 expression for disease-free survival was shown in cases of resected non-small cell lung cancer.¹⁶ As for Ki-67 expression in early lung cancer, dysplasia and

TABLE 3. Relationship between carcinoembryonic antigen expression in tumor and preoperative serum carcinoembryonic antigen level

	IHC (++)	IHC (-) to (+)
Serum increased CEA level	13	11
Serum normal CEA level	9	64

The cutoff level is less than 5 ng/mL. $P < .001$, Fisher exact test. IHC, Immunohistochemistry for tumor; CEA, carcinoembryonic antigen.

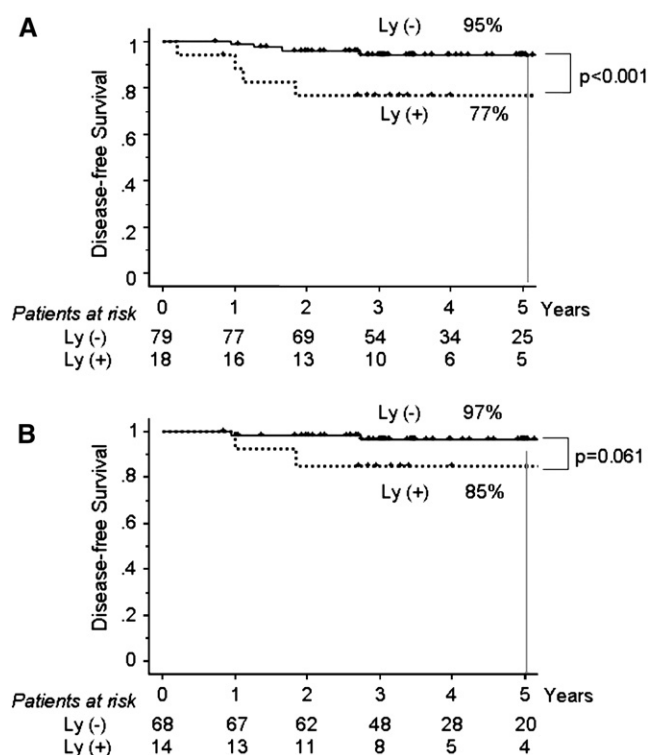


Figure 4. A, Disease-free survival for all patients according to lymphatic invasion (Ly) shown by D2-40 immunohistochemistry results. The Ly (+) group had a significantly worse outcome compared with the Ly (-) group. B, Disease-free survival for patients with pIA disease without lymph node metastasis according to Ly. Disease-free survival for the Ly (-) group tended to be better.

carcinoma in situ, which are precursor lesions for squamous cell carcinoma, and atypical adenomatous hyperplasia have been analyzed,^{17,18} although prognostic significance was not identified in those studies. The present results revealed that a higher Ki-67 LI in a small-sized adenocarcinoma was a clinical predictor for both nodal involvement and postoperative recurrence. We also found that Ki-67 LI was a useful predictor for recurrence in our analysis of patients with pIA disease. Thus an immunohistochemical evaluation of Ki-67 by using preoperative biopsy materials or resected specimens would be useful in clinical practice.

We previously reported that the preoperative serum CEA level can predict nodal involvement in small-sized non-small cell lung cancer lesions smaller than 2 cm.¹⁹ Sawabata and coworkers²⁰ also noted that lymph node metastasis was found in 23% of cases of cI non-small cell lung cancer. Therefore we examined CEA expression in tumors and levels in serum to identify the utility of CEA measurement as a predictor for advanced disease in patients with a small-sized pulmonary lesion. However, local CEA expression levels in the

TABLE 4. Multivariate analysis of clinicopathologic factors for postoperative recurrence in patients with resected adenocarcinoma of 2 cm or less in diameter

Variables	Hazard ratio	95% CL	P value
Microscopic necrosis			
Negative	1.000		
Positive	2.462	0.596–10.167	.2130
Ki-67 labeling index			
<5%	1.000		
>5%	3.985	0.708–22.440	.1169
Lymphatic invasion detected with D2-40 antibody			
Negative	1.000		
Positive	4.767	1.069–21.264	.0406
Preoperative serum CEA level			
WNL	1.000		
>5 ng/mL	1.422	0.329–6.136	.6373
p Stage			.1094
IA–IB	1.000		
IIA–IIB	2.660	0.465–15.233	.2718
IIIA	6.781	1.059–43.425	.0433

CL, Confidence limit; CEA, carcinoembryonic antigen (cutoff value is 5 ng/mL); WNL, within normal limit.

tumor had no relationship with nodal metastasis or disease-free survival in the present cases, although it was reported in another study that all patients with cI disease with an adenocarcinoma who showed a diffuse expression of CEA had a poor prognosis and a lower CEA normalization ratio after surgical intervention.²¹ We consider that measurement of serum CEA levels is a more convenient and useful strategy than immunohistochemical analysis of CEA levels in the tumor for practical management of patients with a small-sized adenocarcinoma.

The D2-40 antibody has been recently reported to be more sensitive for the detection of lymphatic invasion compared with conventional hematoxylin and eosin staining in several types of malignancy, such as gastric, breast, colorectal, and cervical carcinomas.^{22–25} Although some of those previous studies revealed a relationship between lymphatic invasion detected with D2-40 staining and lymph node metastasis,^{22–24} we found no significant relationship in the present study. Nevertheless, lymphatic invasion detected with D2-40 staining had an independent prognostic effect in our cases of small-sized pulmonary adenocarcinoma. We propose that D2-40 staining, which is reliable for evaluation of lymphatic invasion, is useful to predict postoperative relapse in patients who have undergone resection for a small-sized adenocarcinoma.

Although we identified several pathologic predictors for disease relapse in the present study, the preoperative availability of these useful factors has not been established, which is the main limitation of this study. Diagnostic imaging, such

as positron emission tomography–CT, can provide information regarding oncologic behavior before surgical intervention.²⁶ In addition, gene profiling with DNA-chip technology might be another methodology to predict the outcome of patients with resected malignancy. Additional studies of preoperative diagnosis are required to elucidate the means to select small-sized adenocarcinomas with malignant potential.

In summary, histologic findings for microscopic necrosis, Ki-67 LI, CEA expression, and lymphatic invasion, in addition to serum CEA level, should be considered for practical management of patients with a small-sized adenocarcinoma of the lung.

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