OPEN

Impact of Extratumoral Lymphatic Permeation on Postoperative Survival of Non–Small-Cell Lung Cancer Patients

Yuki Matsumura, MD, Tomoyuki Hishida, MD,* Yoshihisa Shimada, MD,* Genichiro Ishii, MD,† Keiju Aokage, MD,* Junji Yoshida, MD,* and Kanji Nagai, MD**

Introduction: Lymphatic permeation has been reported as a prognostic factor for patients with resected non–small-cell lung cancer (NSCLC). Lymphatic canals are located in both intratumoral and extratumoral areas. Since 2001, we have prospectively evaluated lymphatic permeation based on its location. The purpose of this study was to determine the survival impact of extratumoral lymphatic permeation in patients with resected NSCLC by analyzing the long-term follow-up data.

Methods: We reviewed 1069 consecutive patients with NSCLC who underwent complete resection between 2001 and 2006. Lymphatic permeation was classified as follows: ly0, absence of lymphatic permeation; ly1, intratumoral; and ly2, extratumoral.

Results: There were 845 patients (79%) with ly0, 134 (12%) with ly1, and 90 (9%) with ly2. Ly2 was more frequently observed in patients with advanced disease and intrapulmonary metastases than ly0–1. The 5-year overall survival (OS) rates of the ly0, ly1, and ly2 groups were 75%, 63%, and 34%, respectively. The OS rate was significantly worse in the ly2 group compared with OS rate in the ly0 ($p < 0.01$) and ly1 groups ($p < 0.01$). In multivariate analyses, ly2 proved to be an independent poor prognostic factor (hazard ratio, 1.73; $p < 0.01$). OS and recurrence-free survival of patients with T1 and T2 tumors with ly2 were not statistically different from that of the patients with T3 tumor (OS, $p = 0.43$ and $p = 0.77$; recurrence-free survival, $p = 0.94$ and $p = 0.94$, respectively).

Conclusions: The adverse prognostic impact of lymphatic permeation was remarkably different whether it is detected in intratumoral or extratumoral lymphatic canals. We recommend that lymphatic permeation in resected NSCLC should be evaluated by considering its location.

Key Words: Non–small-cell lung cancer, Surgery, Lymphatic permeation, Prognostic factor.

(*J Thorac Oncol.* 2014;9: 337–344)

*Department of Thoracic Surgery, National Cancer Center Hospital East, Chiba, Japan; and †Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Chiba, Japan.

Disclosure: The authors declare no conflict of interest.

Presented as a moderated poster at the 20th European Conference on General Thoracic Surgery in Essen, Germany, June 10–13, 2012.

Address for correspondence: Tomoyuki Hishida, MD, Department of Thoracic Surgery, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa, Chiba 277–8577, Japan. E-mail: thishida@east.ncc.go.jp

ISSN: 1556-0864/14/0903-0337

Lung cancer is one of the most prevalent and lethal cancers worldwide. Surgery is the most effective treatment modality for patients with localized non–small-cell lung cancer (NSCLC); however, many patients develop recurrence even after complete resection. Many studies have reported that clinicopathological factors are associated with the survival of patients who undergo surgical treatment for NSCLC.[1–5] Among these factors, vessel involvement, that is, vascular invasion and lymphatic permeation, has been well investigated.[3,6,7] Some investigators have collectively studied vascular invasion and lymphatic permeation as microscopic vessel invasion.[8] However, others have reported that lymphatic permeation had a survival impact which is different from that of vascular invasion.[9,10] The current Tumor–Node–Metastasis (TNM) classification of lung cancer describes vascular invasion and lymphatic permeation as *optimal descriptors* which deserve future consideration of incorporation into the staging system.[11] Lymphatic canals are distributed in intratumoral and extratumoral areas in resected lung cancer specimens. Lymphatic permeation may have a different impact on the outcome according to its location. Since 2001, we have been classifying lymphatic permeation in patients with resected NSCLC into the following three categories: absence of lymphatic permeation (ly0), intratumoral lymphatic permeation (ly1), and extratumoral lymphatic permeation (ly2). We had previously reported that patients with ly2 NSCLC significantly developed more recurrence than patients with ly1 tumor.[12] However, the follow-up duration was relatively short, and the 5-year survival data were not available at that time. We continued to classify lymphatic permeation, and thus the longer-term follow-up data are now available. In this study, we report the 5-year overall survival (OS) data for patients with surgically resected ly2 NSCLC and the clinical significance of these findings.

PATIENTS AND METHODS

Patient Selection

Between August 2001 and December 2006, a total of 1069 consecutive patients underwent surgical resection for NSCLC by segmentectomy or greater lung resection with lymph node dissection in our institution and were retrospectively enrolled in this study. This study was approved by the institutional review board in June 2012, and the need to obtain written informed consent was waived. Patients

who underwent preoperative chemotherapy, radiotherapy, or incomplete resection were excluded. All patients underwent preoperative evaluation, including physical examination, chest radiography, and chest and upper abdomen computed tomography (CT) scan. Magnetic resonance imaging of the brain, bone scintigraphy, and positron emission tomography were performed for patients who were suspected to have stage IB or more advanced disease on chest CT scans.

Histopathological Examination

Surgical specimens were immediately fixed in 10% formalin, and then cut horizontally at approximately 5-mm intervals. As our routine process, we have created paraffin-embedded sections of all cut surfaces containing the main tumor, irrespective of tumor location and its size. The serial 4-μm sections were stained with hematoxylin and eosin (HE) for routine histopathological workup. Histological typing of the primary tumor was performed in accordance with the World Health Organization classification.[13] The pathological stage was determined on the basis of the 7th TNM classification of the Union for International Cancer Control. Victoria blue van Gieson staining to visualize elastic fibers was also routinely performed for all sections containing tumor cells to evaluate vascular invasion, lymphatic permeation, and pleural invasion. Lymphatic permeation was suspected when floating tumor cells were identified in vessels with no supporting smooth muscles or when elastic fibers were identified.[12,14,15] If lymphatic permeation was suspected in the HE sections, we also performed immunohistochemical staining with anti-D2-40 antibody to confirm the visualization of the lymphatic vessels (Fig. 1). We classified lymphatic permeation into the following three categories: ly0, absence of lymphatic permeation; ly1, presence of intratumoral lymphatic permeation (Fig. 1*A* and *B*); and ly2, presence of extratumoral lymphatic permeation (Fig. 1*C* and *D*). Ly2 were frequently identified around the subpleural spaces near the main tumor or bronchovascular bundles connecting the main tumor to hilum. Tumors with both ly1 and ly2 were classified as ly2. The lymphatic permeation status was prospectively evaluated by more than two pathologists and reviewed for the current study by one of the authors (G.I.).

Follow-Up and Evaluation of Recurrence and Survival

After the surgery, patients were followed-up at our outpatient clinic. Patients were evaluated every 3 to 6 months during the first 2 years after surgery and every 6 to 12 months thereafter. The follow-up evaluation included physical examination, chest radiography, and blood examination, including tumor markers. Whenever any symptoms or signs of recurrence were detected, further evaluations were performed, including CT scans of the chest and abdomen, brain magnetic resonance imaging, and bone scintigraphy. Integrated positron emission tomography and CT scans were also performed for selected patients. Treatment after recurrence was determined by a board comprising thoracic surgeons, oncologists, and radiologists. The date of recurrence was defined as the date of histological proof or, in cases diagnosed on the basis of clinicoradiological findings, as the date of identification by a physician. Recurrences were categorized into locoregional recurrence and distant metastasis. Locoregional recurrence included recurrence in the bronchial stump, chest wall, residual lung field, chest cavity, and lymph nodes in the hilar, mediastinal, and cervical areas. Distant metastasis was defined as metastasis to extrathoracic organs, including the brain, bone, liver, adrenal gland, and others. Patients with both locoregional recurrence and distant metastases were classified as having distant metastases.

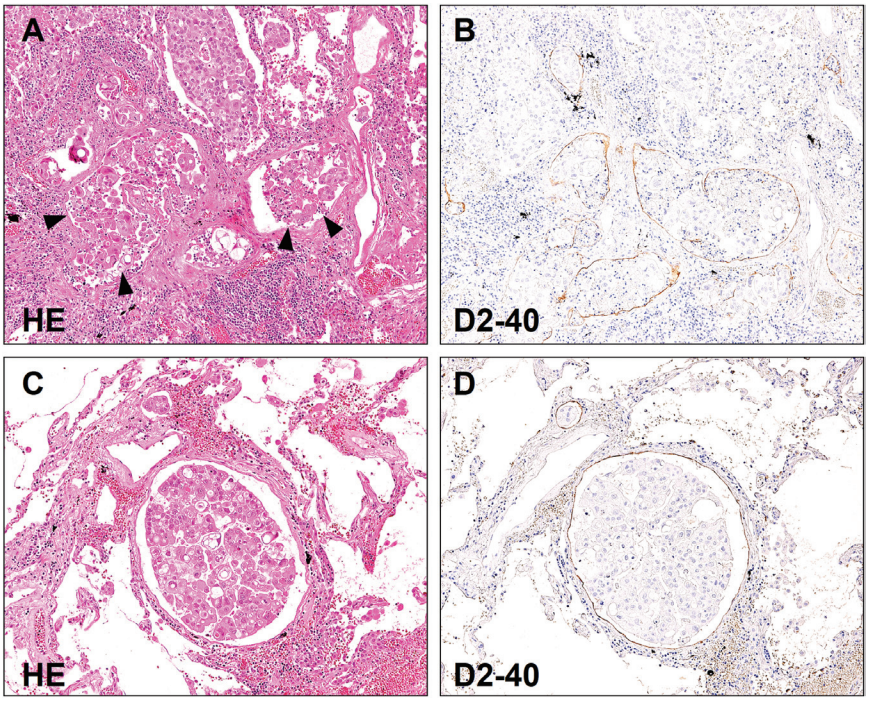

FIGURE 1. Microscopic findings of intratumoral (*A* and *B*) and extratumoral (*C* and *D*) lymphatic permeation. *A*, Intratumoral lymphatic permeation is indicated by arrows (HE stain, original magnification ×100). *B*, Intratumoral lymphatic permeation detected by anti-D2-40 immunostaining of lymphatic vessels (×100). Extratumoral lymphatic permeation by HE stain (*C*) (×100) and anti-D2-40 immunostaining (*D*) (×100). HE, hematoxylin and eosin.

OS was defined as the interval in months between the date of surgical intervention and that of death due to any cause or the last follow-up. Observations were censored at the last follow-up when the patient was alive or was lost to follow-up. The length of the recurrence-free period was calculated in months from the date of resection to that of the first recurrence or last follow-up.

Statistical Analysis

In this study, we compared the clinicopathological factors according to the lymphatic permeation status (ly0, ly1, and ly2). The relationship between ly status and the recurrence pattern was also evaluated. The prognostic impact of ly2 status was also evaluated in relation to other clinical and pathological factors by univariate analyses. The significant factors in univariate analysis were then enrolled in multivariate analysis. Relationship between pathological T factor and ly2 status was also evaluated as optional analysis. Fisher's exact test or Pearson's χ^2 test were used to determine significant differences in patient characteristics between the two groups. For univariate analyses, the Kaplan–Meier method was used to estimate all cumulative survival rates, and the log-rank test was used to calculate differences in variables. Cox proportional hazards regression models were used to perform multivariate analysis. Statistical analysis was performed using SPSS statistical software (Dr. SPSS II for Windows, standard version 11.0; SPSS Inc., Chicago, IL).

RESULTS

Clinicopathological Characteristics of All Patients

Table 1 shows the clinicopathological characteristics of all 1069 patients. The median age was 65 years (range, 20–90), and 672 patients (63%) were men. The median tumor size was 2.8 cm (range, 0.4–47). Pathological stage was I in 714 patients (67%), II in 192 patients (18%), and III in 163 patients (15%), respectively. Adenocarcinoma was the most common histological type (741; 69%), followed by squamous cell carcinoma (243; 23%). Lymphatic permeation was detected in 224 patients (21%), whereas pleural invasion and vascular invasion were observed in 333 patients (31%) and 487 patients (46%), respectively. The number of patients who received adjuvant chemotherapy was 18 (3%) in stage I, 14 (7%) in stage II, and 17 (10%) in stage III, respectively.

TABLE 1. Clinicopathological Characteristics of All Patients

Characteristics	No. of Patients (%) n = 1069
Median age (yr)	
Range	65 (20–90)
Sex	
Men	672 (63)
Women	397 (37)
Smoking (pack-years)	
≥40	417 (39)
<40	652 (61)
CEA (ng/ml)	
>5	386 (36)
≤5	683 (64)
Median tumor size (cm)	
Range	2.8 (0.4–47)
pStage	
I	714 (67)
II	192 (18)
III	163 (15)
Histological type	
Adenocarcinoma	741 (69)
Squamous cell carcinoma	243 (23)
Large cell carcinoma	53 (5)
Others	32 (3)
Pleural invasion	
Positive	333 (31)
Negative	736 (69)
Vascular invasion	
Positive	487 (46)
Negative	582 (54)
Lymphatic permeation	
Positive (ly1 and ly2)	224 (21)
Negative (ly0)	845 (79)
Intrapulmonary metastasis	
Positive	36 (3)
Negative	1033 (97)

CEA, serum carcinoembryonic antigen level (preoperative).

Clinicopathological Characteristics According to Lymphatic Permeation Status

Table 2 shows the relationships between clinicopathological variables and the lymphatic permeation status. The number of patients classified as ly0, ly1, and ly2 were 845 (79%), 134 (12%), and 90 (9%), respectively. The clinical factors, such as sex, age, and smoking history, were not significantly different among each ly group. The surgical procedures were 16 segmentectomies (ly0: 15, ly1: 0, and ly2: 1), 1005 lobectomies (ly0: 805, ly1: 125, and ly2: 75), and 48 pneumonectomies (ly0: 25, ly1: 9, and ly2: 14), respectively. In ly2 patients, there were significantly more patients having advanced nodal disease (pN1–2; 77% versus 46%; $p < 0.01$), advanced pathological stage (stage II to III; 81% versus 57%; $p < 0.01$), and tumors with intrapulmonary metastases (15% versus 5%; $p = 0.02$) when compared with ly1 patients. Ly0, ly1, and ly2 were observed in 588 (79%), 87 (12%), and 66 (9%) patients with adenocarcinoma, respectively, whereas ly0, ly1, and ly2 were observed in 195 (80%), 31 (13%), and 17 (7%) patients with squamous cell carcinoma, respectively. There was no significant difference in the distribution of the lymphatic permeation status between patients with adenocarcinoma and those with squamous cell carcinoma.

Survivals

The median follow-up duration was 85 months (range, 1–124 months). During the study period, a total of 401 patients

TABLE 2. Clinicopathological Characteristics According to Lymphatic Permeation

	ly0 (%) n = 845	ly1 (%) n = 134	ly2 (%) n = 90	*p* ly0 vs. 1–2	*p* ly0–1 vs. 2
Sex					
Men	522 (62)	87 (65)	63 (69)	0.22	0.57
Age (yr)					
Median (range)	67 (20–90)	66 (22–85)	66 (41–83)	0.56	0.92
Smoking					
≥40 p-y	333 (40)	49 (37)	35 (38)	0.54	1.00
FEV1 (liter)					
Median (range)	2.2 (0.6–4.6)	2.2 (0.8–3.7)	2.2 (1.2–3.6)	0.78	0.85
CEA (ng/ml)					
>5	288 (34)	58 (43)	40 (44)	<0.01	0.89
Procedure					
Segment	15 (2)	0	1 (1)		
Lobe	805 (95)	125 (93)	75 (83)	<0.01	<0.01
Pneumo	25 (3)	9 (7)	14 (16)		
Size[a] (cm)					
Median (range)	2.7 (0.4–12)	3.5 (1.2–47)	3.4 (1.0–9.0)	<0.01	0.34
pT status					
pT1	488 (58)	52 (39)	30 (33)	<0.01[b]	0.31[b]
pT2	285 (34)	60 (45)	37 (42)		
pT3/4	72 (8)	22 (16)	23 (25)		
pN status					
pN0	714 (84)	72 (54)	21 (23)	<0.01[c]	<0.01[c]
pN1	84 (10)	36 (27)	29 (32)		
pN2	47 (6)	26 (19)	40 (45)		
pStage					
I	639 (76)	58 (43)	17 (18)	<0.01[d]	<0.01[d]
II	126 (15)	40 (30)	26 (29)		
III	80 (9)	36 (27)	47 (52)		
Histology					
Ad	588 (69)	87 (65)	66 (71)	0.63	0.39
Sq	195 (23)	31 (23)	17 (18)	0.59	0.41
pl					
Present	221 (26)	67 (50)	45 (50)	<0.01	1.00
pm					
Present	15 (2)	7 (5)	14 (15)	<0.01	0.02
V					
Present	320 (38)	95 (71)	72 (80)	<0.01	0.16

[a]Size; median tumor size (range).
[b]T1 vs. T2–4.
[c]N0 vs. N1–2.
[d]I vs. II to III.
p-y, pack-years; FEV, forced expiratory volume; CEA, serum carcinoembryonic antigen level (preoperative); Ad, adenocarcinoma; Sq, squamous cell carcinoma; pl, pleural invasion; pm, intrapulmonary metastasis; v, vascular invasion.

died, that is, 269 patients (32%) with ly0, 67 (50%) with ly1, and 65 (71%) with ly2. Cancer recurrences were detected in 349 patients, that is, 220 (26%) with ly0, 64 (48%) with ly1, and 65 (71%) with ly2. Table 3 shows recurrence patterns according to the lymphatic permeation status. There were 160 locoregional recurrences and 189 distant metastases. Pleural dissemination was more frequent in the ly2 group compared with pleural dissemination in the ly0–1 group (ly0–1, 9%; ly2, 20%; $p = 0.05$).

Figure 2 shows the OS curves according to the lymphatic permeation status. The 5-year OS rates for ly0, ly1, and ly2 groups were 75%, 63%, and 34%, respectively. The survival curve of the ly2 group was significantly inferior to not only that of the ly0 group ($p < 0.01$) but also that of ly1 group ($p < 0.01$). Recurrence-free survival (RFS) and cancer-specific survival in ly2 group were also poorer than those in ly0 or ly1 group (data shown in Supplemental Figures

TABLE 3. Recurrence Pattern According to the Lymphatic Permeation Status

No. of Recurrent pts. (%)	Total n = 349	ly0 n = 220	ly1 n = 64	ly2 n = 65	*p* ly0–1 vs. 2
Locoregional	160 (46)	100 (45)	31 (48)	29 (45)	0.58
Local	8 (2)	6 (3)	1 (2)	1 (2)	1.0
PM[a]	103 (30)	72 (33)	17 (27)	14 (22)	0.13
Dissemination	39 (11)	17 (8)	10 (16)	12 (18)	0.05
Lymph nodes[b]	61 (17)	33 (15)	15 (23)	13 (20)	0.59
Distant metastases[c]	189 (54)	120 (55)	33 (52)	36 (55)	0.89
Brain	90 (26)	51 (23)	17 (27)	22 (34)	0.12
Bone	67 (19)	45 (20)	12 (19)	10 (15)	0.48
Liver	39 (11)	24 (11)	7 (11)	8 (12)	0.83
Adrenal gland	14 (4)	6 (3)	3 (5)	5 (8)	0.15
Others	32 (9)	23 (10)	5 (8)	4 (6)	0.48

[a]PM means intrapulmonary metastasis.
[b]Lymph node metastasis as locoregional recurrence includes metastasis in the hilar, mediastinal, and cervical lymph nodes.
[c]Recurrences in both locoregional and distant sites are included in the distant metastases.

1 and 2, Supplemental Digital Content 1 [http://links.lww.com/JTO/A505] and Supplemental Digital Content 2 [http://links.lww.com/JTO/A506]). When the OS was analyzed by pStages (pStage I and II to III), similar results were obtained to the entire cohort. In pStage I, the 5-year OS rates of ly0, ly1, and ly2 groups were 83%, 79%, and 47%, respectively (Fig. 3). The OS of the ly2 group was significantly inferior compared with that of the ly0 ($p < 0.01$) and ly1 groups ($p = 0.05$). In pStage II to III, the 5-year OS rates of ly0, ly1, and ly2 groups were 47%, 50%, and 25%, respectively. Ly2 was still a significant prognostic factor among the patients with pStage II to III (Fig. 4). Ly2 status also showed significant poor OS than ly0 or ly1 irrespective of pathological nodal (pN) status (data shown in Supplemental Figures 4–6, Supplemental Digital Content 3 [http://links.lww.com/JTO/A507], Supplemental Digital Content 4 [http://links.lww.com/JTO/A508], and Supplemental Digital Content 5 [http://links.lww.com/JTO/A509]).

Table 4 shows the results of the univariate analyses of lymphatic permeation and other conventional clinicopathological variables for prognostic factors. All variables were significantly correlated with OS. Multivariate analyses revealed that age (≥70 years), pT status (pT2–4), pN status (pN1–2), pleural invasion (positive), intrapulmonary metastasis (positive),

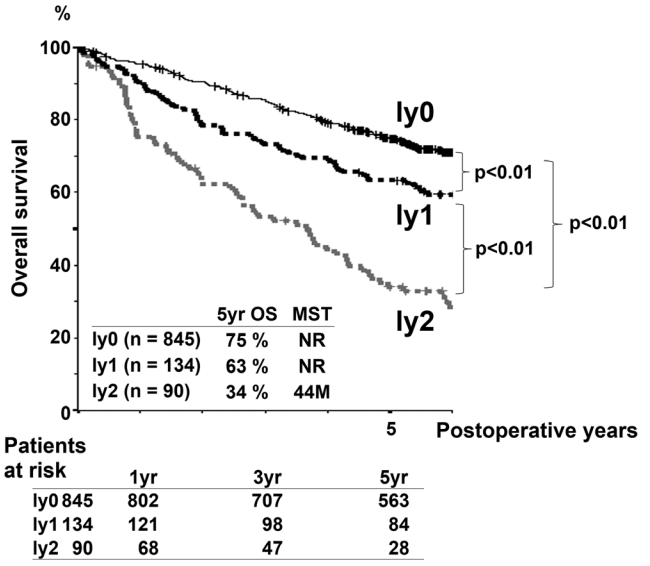

FIGURE 2. OS curves of patients with completely resected non–small-cell lung cancer according to the lymphatic permeation status (ly0, ly1, and ly2). The OS curve of the ly2 group is significantly inferior compared with that of the ly0 and ly1 groups. MST, median survival time; NR, not reached; OS, overall survival.

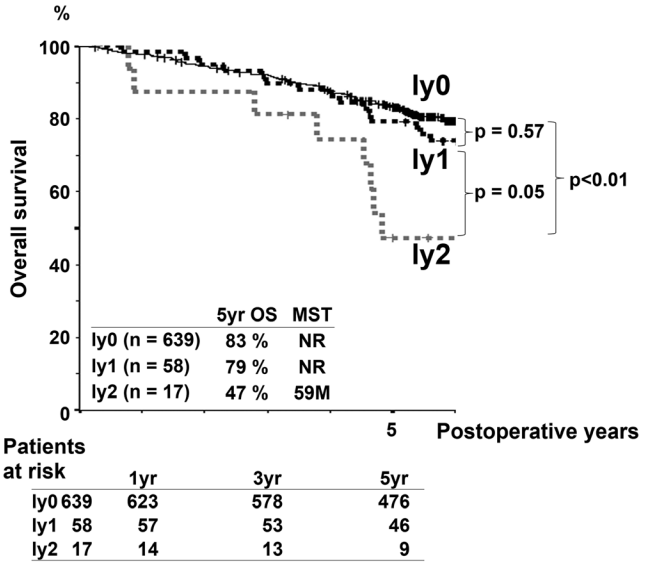

FIGURE 3. OS curves of the patients with pStage I non–small-cell lung cancer according to the lymphatic permeation status (ly0, ly1, and ly2). The OS curve of the ly2 group is significantly inferior compared with that of the ly0 and ly1 groups. MST, median survival time; NR, not reached; OS, overall survival.

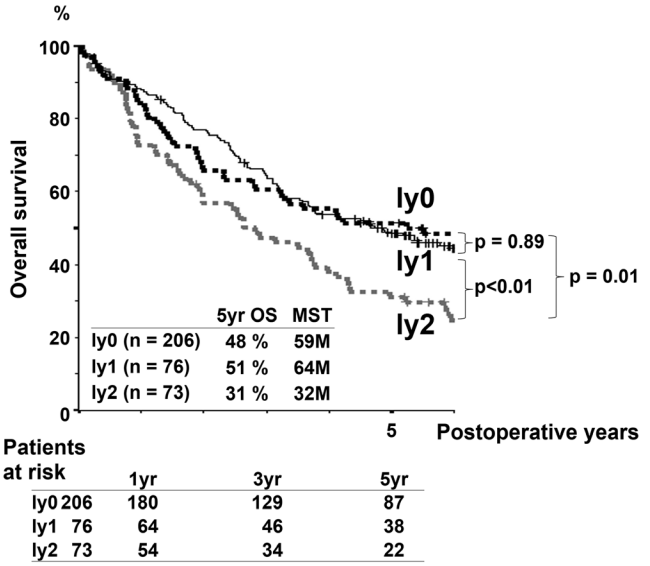

FIGURE 4. OS curves of the patients with pStage II and III non–small-cell lung cancer according to the lymphatic permeation status (ly0, ly1, and ly2). The OS curve of the ly2 group is significantly inferior compared with that of the ly0 and ly1 groups. MST, median survival time; OS, overall survival.

vascular invasion (positive), and ly2 (positive) were independent poor prognostic factors (Table 5). When the presence of lymphatic permeation (ly0 versus ly1–2) was enrolled in multivariate analyses instead of the presence of *extratumoral* lymphatic permeation (ly0–1 versus ly2), it did not reach statistical significance ($p = 0.07$; hazard ratio, 1.27; 95% confidence intervals, 0.98–1.64).

Relationship between pT Factor and Extratumoral Lymphatic Permeation

Ly status is the information obtained from tumor (T) itself. Therefore, we also analyzed the relationship between pT factor and ly2. T1 and T2 tumors were classified into four groups according to the presence or absence of ly2, that is, T1 without ly2 (T1 − ly2), T1 with ly2 (T1 + ly2), T2 without ly2 (T2 − ly2), and T2 with ly2 (T2 + ly2). The RFS curve of each group was compared with each other and that of T3 tumor. The 5-year RFS rates and median survival time of T1 − ly2, T1 + ly2, T2 − ly2, T2 + ly2, and T3 populations were 77% (not reached), 22% (22 months), 52% (66 months), 28% (17 months), and 30% (19 months), respectively (Fig. 5). Significant differences were observed between the T1 − ly2 and T1 + ly2 groups ($p < 0.01$) and between the T2 − ly2 and T2 + ly2 groups ($p < 0.01$). Patients with T1 and T2 with ly2 had poor outcomes, which were not statistically different from those with T3 tumors ($p = 0.94$ for both comparison). When only node-negative patients were analyzed, there were 488 T1 − ly2, eight T1 + ly2, 249 T2−ly2, 11 T2 + ly2, and 51 T3 patients. The number of ly2-positive patients was small (n = 19), but four of eight T1 + ly2 patients and five of 11 T2 + ly2 patients developed relapse. As for the OS curves, the 5-year OS rates and median survival time of T1 − ly2, T1 + ly2, T2 − ly2, T2 + ly2, and T3 were 85% (not reached), 40% (55 months), 63% (93 months), 42% (53 months), and 37% (36 months), respectively (Supplemental Figure 3, Supplemental Digital Content 6, http://links.lww.com/JTO/A510). The same survival differences were observed between each group as shown in RFS analysis.

TABLE 4. Univariate Analysis of Clinicopathological Factors Associated with Overall Survival

Variables	Five-year OS* Rate (%)	*p*
Age (yr)		
<70	75	<0.01
≥70	63	
Sex		
Women	81	<0.01
Men	63	
CEA (ng/ml)		
≤5	76	<0.01
>5	59	
Smoking (pack-years)		
<40	76	<0.01
≥40	60	
pT status		
pT1	83	<0.01
pT2–4	55	
pN status		
pN0	78	<0.01
pN1–2	45	
Pleural invasion		
Absent	79	<0.01
Present	50	
Intrapulmonary metastasis		
Absent	71	<0.01
Present	28	
Vascular invasion		
Absent	84	<0.01
Present	53	
Lymphatic permeation		
Absent (ly0)	75	<0.01
Present (ly1–2)	51	
Extratumoral lymphatic permeation		
Absent (ly0–1)	73	<0.01
Present (ly2)	34	

OS, overall survival; CEA, serum carcinoembryonic antigen level (preoperative).

DISCUSSION

The present study retrospectively evaluated the prognostic impact of extratumoral lymphatic permeation (ly2) in patients with completely resected NSCLC on the basis of our long-term follow-up data. We previously reported that ly2 was a useful prognostic marker,[12] but this study could describe only RFS because of short follow-up period and small number of patients. The present study with enough long follow-up

TABLE 5. Multivariate Analysis of Clinicopathological Factors Associated with Overall Survival

Variables	Hazard Ratio (95% CI)	*p*
Age, yr		
<70	1.00	<0.01
≥70	1.52 (1.24–1.86)	
Sex		
Women	1.00	0.26
Men	1.16 (0.89–1.52)	
CEA (ng/ml)		
≤5	1.00	0.17
>5	1.15 (0.94–1.41)	
Smoking (pack-years)		
<40	1.00	0.25
≥40	1.15 (0.91–1.46)	
pT status		
pT1	1.00	0.01
pT2–4	1.37 (1.07–1.75)	
pN status		
pN0	1.00	<0.01
pN1–2	1.63 (1.30–2.04)	
Pleural invasion		
Absent	1.00	<0.01
Present	1.62 (1.29–2.02)	
Intrapulmonary metastasis		
Absent	1.00	<0.01
Present	2.61 (1.69–4.02)	
Vascular invasion		
Absent	1.00	<0.01
Present	1.70 (1.32–2.20)	
Extratumoral lymphatic permeation		
Absent (ly0–1)	1.00	<0.01
Present (ly2)	1.73 (1.31–2.30)	

CEA, serum carcinoembryonic antigen level (preoperative); CI, confidence interval.

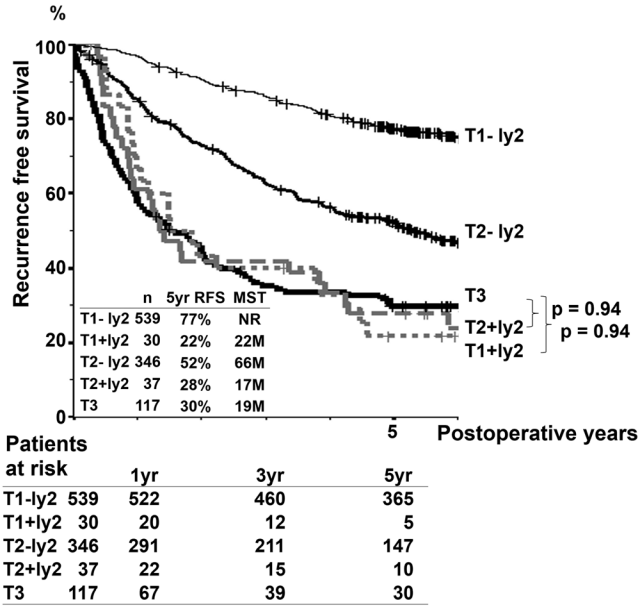

FIGURE 5. RFS curves of the patients with non–small-cell lung cancer according to the T classification and extratumoral lymphatic permeation (ly2) status. The RFS curves of the T1 + ly2, T2 + ly2, and T3 populations are considerably similar to one another. MST, median survival time; NR, not reached; RFS, recurrence-free survival.

period and large cohorts could evaluate OS. This mature survival data confirmed that patients with ly2 had significantly worse OS rates than not only those with ly0 but also those with ly1. Multivariate analysis also demonstrated that ly2 was an independent unfavorable prognostic factor associated with OS, which was as significant as advanced pT and pN status, pleural invasion, and intrapulmonary metastases. However, the presence of lymphatic permeation regardless of its extent (ly1–2) was not a statistically significant prognostic factor ($p = 0.07$). These results indicate that the presence of *extratumoral* lymphatic permeation (i.e., ly2) has a stronger prognostic impact than that of *intratumoral* lymphatic permeation in general (i.e., ly1–2). Tumors classified as ly2 have additional cancer cells in the lymphatic vessels outside the main tumor. Therefore, ly2 tumors should have more chance to metastasize to lymph nodes and distant organs via lymphatic flow compared with ly0 or ly1 tumors. In our cohort, ly2 group had more tumors with node involvement (77%) and recurrences (71%) than ly0 group (node involvement, 16%; recurrence, 26%) or ly1 group (46% and 48%, respectively). Our results suggested that ly2 status had remarkable negative prognostic impact and should be included in the staging system.

Ly status is the information obtained from tumor (T) itself but not nodal (N) or metastatic (M) status. Therefore, the survival impact of ly2 status was analyzed by incorporating with the T classification of the TNM staging system. There were no statistically significant differences in OS and RFS between the T1/T2 tumors with ly2 and T3. Even in the small number of pN0 population, almost half the patients with ly2 tumor (4 of 8 T1 + ly2 patients and 5 of 11 T2 + ly2 patients) developed relapse. This analysis was exploratory because there might be other confounding clinicopathological variables, such as pN status for analyzing the association between ly2 and pT factor. Actually, ly2 status was strongly related with pN status. But ly2 was found to be a significant poor prognostic indicator irrespective of pN status, as shown in Supplemental figures. Although our proposal is not complete, these results implied that patients with ly2-positive T1–2 diseases had extremely poor prognoses comparable with T3 tumors. Currently, T1-2N0 disease is not globally indicated for adjuvant chemotherapy using cytotoxic agents. T1–2 N0 + ly2 disease may be upstaged to T3 and also be candidate for adjuvant chemotherapy.

Intrapulmonary metastases significantly occurred more frequently in ly2 tumors compared with that in ly0 or ly1 tumors (ly2, 15%; ly1, 5%; ly0, 2%). Aokage et al.[16] analyzed resected NSCLC with pulmonary metastases and revealed that some pulmonary metastases may be the result of lymphatic tumor spread. They hypothesized that tumor cells in the lymphatic vessels in bronchovascular bundles transmigrate to

the lung parenchyma and develop intrapulmonary metastases. The high frequency of intrapulmonary metastases in the ly2 population in our study may be explained by this hypothesis.

Our results showed that lymphatic permeation was found in 20% (224 of 1095) of patients. But reported frequency of lymphatic permeation in NSCLC was ranged from 15% to 40%.[6,17–20] This discrepancy may be explained by the difficulty in diagnosing lymphatic permeation. Lymphatic vessels lack elastic fibers in their walls, so it is basically hard to identify them by commonly used HE and elastic stains. In addition, when we found tumor cell clusters in elastic fiber-absent canals, it was also difficult to distinguish lymphatic permeation from retraction artifacts caused by tissue processing. These difficulties might cause a scattering frequency of lymphatic permeation. Recently, anti-D2-40 immunohistochemical staining was introduced to help identify lymphatic vessels.[12,14,15,21] Lymphatic vessels are better visualized with this staining, as shown in Figure 1, which enables researchers to more precisely evaluate the presence of lymphatic permeation than without it. But it is not realistic to perform anti-D2-40 staining for all resected specimens because of its cost. Fortunately, *extratumoral* lymphatic permeation (i.e., ly2) seems to be more easily identified on routine HE staining compared with *intratumoral* lymphatic permeation (i.e., ly1) because retraction artifacts are much less frequent in the extratumoral areas. D2-40 immunohistochemical staining may be required only for indistinct cases, particularly in the intratumoral areas.

A limitation of the present study was the type of the analysis. As described, we prospectively observed and classified the lymphatic permeation status by differentiating ly2 and ly1 since 2001, but the analysis itself was retrospective in nature. In addition, this study was based on a single-institution experience including mostly monoethnic patients. Further prospective, large-sized studies are required to confirm our findings. Standard diagnostic methodology should also be established to overcome difficulties in identifying lymphatic permeation.

In conclusion, *extratumoral* lymphatic permeation (i.e., ly2) was found to be an independent prognostic factor for patients with completely resected NSCLC. In this study, ly2 had a stronger unfavorable impact on survival than *intratumoral* lymphatic permeation (i.e., ly1). We recommend a separate evaluation and classification of lymphatic permeation inside and outside the tumors. Further studies are warranted to discuss how to use prognostic impact of ly2 status in the TNM staging system.

REFERENCES

1. Maeda R, Yoshida J, Ishii G, et al. Long-term survival and risk factors for recurrence in stage I non-small cell lung cancer patients with tumors up to 3 cm in maximum dimension. *Chest* 2010;138:357–362.
2. Maeda R, Yoshida J, Ishii G, Hishida T, Nishimura M, Nagai K. Poor prognostic factors in patients with stage IB non-small cell lung cancer according to the seventh edition TNM classification. *Chest* 2011;139:855–861.
3. Naito Y, Goto K, Nagai K, et al. Vascular invasion is a strong prognostic factor after complete resection of node-negative non-small cell lung cancer. *Chest* 2010;138:1411–1417.
4. Li Z, Yu Y, Lu J, et al. Analysis of the T descriptors and other prognosis factors in pathologic stage I non-small cell lung cancer in China. *J Thorac Oncol* 2009;4:702–709.
5. Poncelet AJ, Cornet J, Coulon C, Collard P, Noirhomme P, Weynand B; groupe d'oncologie thoracique des Cliniques Saint-Luc. Intra-tumoral vascular or perineural invasion as prognostic factors for long-term survival in early stage non-small cell lung carcinoma. *Eur J Cardiothorac Surg* 2008;33:799–804.
6. Harada M, Hato T, Horio H. Intratumoral lymphatic vessel involvement is an invasive indicator of completely resected pathologic stage I non-small cell lung cancer. *J Thorac Oncol* 2011;6:48–54.
7. Miyoshi K, Moriyama S, Kunitomo T, Nawa S. Prognostic impact of intratumoral vessel invasion in completely resected pathologic stage I non-small cell lung cancer. *J Thorac Cardiovasc Surg* 2009;137:429–434.
8. Ruffini E, Asioli S, Filosso PL, et al. Significance of the presence of microscopic vascular invasion after complete resection of Stage I-II pT1-T2N0 non-small cell lung cancer and its relation with T-Size categories: did the 2009 7th edition of the TNM staging system miss something? *J Thorac Oncol* 2011;6:319–326.
9. Shoji F, Haro A, Yoshida T, et al. Prognostic significance of intratumoral blood vessel invasion in pathologic stage IA non-small cell lung cancer. *Ann Thorac Surg* 2010;89:864–869.
10. Hishida T, Yoshida J, Maeda R, et al. Prognostic impact of intratumoural microvascular invasion and microlymphatic permeation on node-negative non-small-cell lung cancer: which indicator is the stronger prognostic factor? *Eur J Cardiothorac Surg* 2013;43:772–777.
11. International Association for the Study of Lung Cancer. Staging Handbook in Thoracic Oncology. Orange Park, FL: Editorial Rx Press, 2009.
12. Saijo T, Ishii G, Ochiai A, et al. Evaluation of extratumoral lymphatic permeation in non-small cell lung cancer as a means of predicting outcome. *Lung Cancer* 2007;55:61–66.
13. International Union Against Cancer (UICC). TNM Classification of Malignant Tumours, 7th Ed. International Union Against Cancer: Wiley, John & Sons, 2009.
14. Iwakiri S, Nagai S, Katakura H, et al. D2-40-positive lymphatic vessel density is a poor prognostic factor in squamous cell carcinoma of the lung. *Ann Surg Oncol* 2009;16:1678–1685.
15. Takanami I. Lymphatic microvessel density using D2-40 is associated with nodal metastasis in non-small cell lung cancer. *Oncol Rep* 2006;15:437–442.
16. Aokage K, Ishii G, Ohtaki Y, et al. Dynamic molecular changes associated with epithelial-mesenchymal transition and subsequent mesenchymal-epithelial transition in the early phase of metastatic tumor formation. *Int J Cancer* 2011;128:1585–1595.
17. Funai K, Sugimura H, Morita T, Shundo Y, Shimizu K, Shiiya N. Lymphatic vessel invasion is a significant prognostic indicator in stage IA lung adenocarcinoma. *Ann Surg Oncol* 2011;18:2968–2972.
18. Kozu Y, Maniwa T, Takahashi S, Isaka M, Ohde Y, Nakajima T. Prognostic significance of postoperative serum carcinoembryonic antigen levels in patients with completely resected pathological-stage I non-small cell lung cancer. *J Cardiothorac Surg* 2013;8:106.
19. Renyi-Vamos F, Tovari J, Fillinger J, et al. Lymphangiogenesis correlates with lymph node metastasis, prognosis, and angiogenic phenotype in human non-small cell lung cancer. *Clin Cancer Res* 2005;11:7344–7353.
20. Shimizu K, Yamada K, Saito H, et al. Surgically curable peripheral lung carcinoma: correlation of thin-section CT findings with histologic prognostic factors and survival. *Chest* 2005;127:871–878.
21. Sakuma Y, Takeuchi T, Nakamura Y, et al. Lung adenocarcinoma cells floating in lymphatic vessels resist anoikis by expressing phosphorylated Src. *J Pathol* 2010;220:574–585.