

**Accuracy and prognostic impact of a vessel invasion grading system
for stage IA non-small cell lung cancer**

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Summary

Background: Despite the recognition that lymphatic and blood vessel invasion is an important prognostic factor in lung cancer, there is no common definition for pathological evaluation of vessel invasion. The aim of the present study was to determine whether D2-40 immunostaining can increase the accuracy of detection of lymphatic vessel invasion and whether our new grading system of vessel invasion by “degree” could be used instead of conventional evaluation by “presence” for pathological stage IA non-small cell lung cancer (NSCLC). **Methods:** The vessel invasion classification was re-evaluated in 221 recent paraffin-embedded sections of p-stage IA NSCLC stained by Hematoxylin-Eosin (HE), Elastica-Van-Gieson (EVG), and D2-40. **Results:** After re-assessment using D2-40 immunostaining, 41.2% (31 of 75) of ly1 cases by HE/EVG changed to ly0, and 14.9% (17 of 114) of ly0 cases by HE/EVG changed to ly1. Overall, 4 of 28 ly2 cases on conventional staining were changed to ly1, and 2 were changed to ly0 using D2-40 immunostaining. When the patients were divided into two groups by the presence of vessel invasion (v/ly0 vs. 1, 2, 3), there was no significant difference in cancer-specific survival ($p=0.1107$, 0.0875 , respectively), while when they were divided according to degree of vessel invasion (v/ly0, 1 vs. 2, 3), there was a statistically significant difference ($p=0.0038$, $p=0.0002$, respectively). On multivariate analysis, lymphatic vessel invasion had a significant impact on cancer-specific survival ($p=0.0061$). **Conclusion:** Our results suggest that D2-40 immunostaining provides a precise diagnosis of lymphatic vessel invasion, and our new grading system of vessel invasion by “degree” is accurate and has prognostic value in early lung cancer.

Key words: Non-small cell lung cancer (NSCLC), D2-40, vessel invasion, grading system

1. Introduction

Currently, several oncology groups worldwide have emphasized the use of adjuvant therapy for resected non-small cell lung cancer (NSCLC) [1-4]. Therefore, precise staging and histological typing after resection of lung cancer have been required to choose treatment. The TNM staging system, which is the most powerful prognostic factor for lung cancer thus far, has been re-evaluated using multivariate analysis in some reports [5]. Although the current TNM staging system subdivides stage I by tumor diameter using 30 mm as the cut-off point, recent studies have demonstrated that tumor diameter is a significant prognostic factor after resection of NSCLC [6,7].

In addition to the effect of tumor size on stage I NSCLC, blood and lymphatic vessel invasion (BVI, LVI) has been considered an additional prognostic factor in resected NSCLC [8-12]. Despite the recognition that vessel invasion is an important prognostic factor in lung cancer, many reports have evaluated blood or lymphatic invasion based on the “presence” of tumor in the vessel lumen. In contrast, we have been routinely evaluating vessel invasion in sections of resected lung cancers stained by Hematoxylin-Eosin (HE) and Elastica-Van-Gieson (EVG) using a modified grading system of the Japanese Classification of Gastric Carcinoma [13]. On the other hand, in cases when there has been doubt about the diagnosis based on the histology, EVG, factor VIII-related antigen or platelet-endothelial cell adhesion antigen (CD31) have been used to permit visualization of blood vessel lamina [10,14]. Compared to the blood vessels, the lymphatic vessel system has been relatively poorly studied until recently. There has been difficulty in recognizing LVI due to the lack of specific antibodies for lymphatic endothelium. In recent years, D2-40 was reported to be a new specific monoclonal antibody (a 40,000-kDa O-linked sialoglycoprotein) for lymphatic endothelium and a suitable marker for the identification of tumor emboli in lymph vessels in paraffin sections of many primary tumors [15-22]. However, only a few papers have

reported using D2-40 immunostaining for lung cancer specimens to evaluate LVI [20,23].

The aim of the present study was to determine whether D2-40 immunostaining can increase the accuracy of detecting LVI and whether our new grading system of vessel invasion by “degree” could be used instead of conventional evaluation by “presence” for pathological stage IA non-small cell lung cancer (NSCLC).

2. Patients and methods

2.1. Patients

During the period from 1994 to 2003, 305 patients with pathological stage IA NSCLC underwent complete resection for lung cancer in our institution. Of these 305 patients, 221 patients in whom lymphatic and blood vessel invasion in specimens had been evaluated and who had sufficient information available to determine prognosis were retrospectively reviewed. In each case, formalin-fixed, paraffin-embedded tissue blocks that included the sections that had been originally used for evaluation of vessel invasion were chosen for evaluation. Serial sections were then made, and HE and EVG staining (conventional staining), as well as D2-40 immunostaining using D2-40 monoclonal antibody (Dako Cytomation, Kyoto, Japan) were done. Then, lymphatic vessel invasion was re-evaluated in these specimens with the examiners blinded to clinical information and outcome. Two investigators double-checked each specimen using common criteria that are routinely used in our institution.

The medical records of each patient were reviewed for age, gender, surgical procedure performed (extent of operation), tumor size (maximum tumor dimension), histological type, pleural involvement, LVI, and blood vessel invasion. Preoperative serum carcinoembryonic antigen (CEA) levels were not included in the present study due to incomplete information.

2.2. Immunohistochemical Techniques

Immunohistochemical staining was carried out on formalin-fixed, paraffin-embedded sections. The 5- μ m-thick paraffin sections were deparaffinized in dimethylbenzene and dehydrated through a graded alcohol series. Antigen retrieval was done in citrate buffer (pH 6.0) with microwave oven heating at 600 W for 15 min, followed by washing in

phosphate-buffered saline (PBS). After being immersed in 3% H₂O₂ solution for 15 min to block the endogenous peroxidase, the sections were incubated with mouse monoclonal antihuman D2-40 (diluted 1:50; Dako) for 30 min at room temperature. After washing in PBS buffer, the sections were incubated with peroxidase-labeled polymer conjugated to goat antimouse immunoglobulin (Envision+ kit; Dako) for 30 minutes at room temperature. The staining was visualized with 3,3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. Sections were dehydrated with alcohol and dimethylbenzene, and mounted in a conventional fashion. Negative controls were prepared in all cases by substituting the primary antibody. Other serial sections were stained using HE and EVG for comparison and re-evaluation of lymphatic and blood vessel invasion.

2.3 Classification Criteria

When tumor cells were seen in the vessel lumen, the specimen was considered positive for vessel invasion. The specimens were classified into four grades according to our criteria (Table 1): ly0, no lymphatic invasion; ly1, minimal lymphatic invasion (can barely detect less than 2LVIs); ly2, moderate lymphatic invasion (can relatively easily detect more than 3LVIs); and ly3, marked lymphatic invasion (invasion detected beyond the tumor area). With respect to BVI, the following classification was used: v0, no venous invasion; v1, minimal venous invasion (can barely detect destruction of small vessels); v2, moderate venous invasion (can relatively easily detect destruction of several small vessels or invasion into large vascular endosporium); v3, marked venous invasion (obvious invasion or embolism in large blood vessels). Our classification criteria were modified based on the Japanese Classification of Gastric Carcinoma [13].

2.4. Statistical analysis

Survival time was defined as the interval from the date of surgery for the primary tumor to the last follow-up or until death. All other deaths resulting from non-cancer and unknown causes were excluded in the survival analysis to identify the effect of the tumor itself on the survival rate. Patients who received adjuvant therapy either preoperatively or postoperatively were excluded. The Kaplan-Meier method was used to estimate cancer-specific survival curves, and the differences in survival rates were assessed using the log-rank test. Multivariate analyses were performed using the Cox proportional-hazards model to identify independent prognostic factors. A p value less than 0.05 was considered to indicate statistical significance. All of the statistical analyses were performed using Stat View 5.0 (SAS Institute Inc., Cary, NC, USA)

3. Results

3.1. Patients' clinicopathological characteristics

Overall follow-up ranged from 1 to 133 months (median, 51.3 months). The 3-, 5- and 10-year cancer-specific survival rates were 95.4%, 93.0% and 81.2%, respectively. The characteristics of the 221 patients are summarized in Table 2.

3.2. The difference in the evaluation of lymphatic vessel invasion between conventional staining (HE & EVG staining) and D2-40 immunostaining

The details of LVI were well demonstrated using D2-40 immunostaining, which resulted in several changes from the evaluation of LVI using conventional staining. In some cases, the evaluations of LVI were downgraded on D2-40 immunostaining, including: 1) when the true alveolar structures resembled LVI in well differentiated adenocarcinoma (Fig. 1A, 1B); and 2) when there were tumor nests, which represent tissue detachment from the peripheral stroma due to retraction artifact (Fig. 1C, 1D). On the other hand, in some cases, the evaluations of LVI were upgraded on D2-40 immunostaining, including: 1) when the true alveolar structure surrounding the lymphatic vessel masked identification of true lymphatic invasion (Fig. 2A); 2) when LVI was present in the stroma with dense lymphocytic infiltration (Fig. 2B); 3) when the lymphatic invasion was surrounded by poorly differentiated tumor cell proliferation (Fig. 2C); 4) when the tumor showed lymphatic invasion into the narrow stroma, sandwiched by the well-formed adenocarcinoma lumen (Fig. 2D); and 5) densely packed lymphatic invasion was simulating tumor cell nests. In some squamous cell carcinomas, D2-40 immunostaining highlights the cytoplasm of tumors (Fig. 2E).

The detection of LVI using conventional staining and D2-40 immunostaining in serial sections is compared in Figure 3. All 4 cases of ly3 on conventional staining remained the

same on D2-40 immunostaining. However, 4 of 28 cases of ly2 using conventional staining changed to ly1 on D2-40 immunostaining, and 2 changed to ly0. On the other hand, 31 of 75 cases (41.3%) of ly1 on conventional staining actually had no LVI (ly0) on D2-40 immunostaining, and LVI was newly detected in 18 of 114 cases (15.8%) that had been diagnosed as free of LVI on conventional staining.

Table 3 shows the comparison of frequency of LVI detected by conventional HE staining and D2-40 immunostaining according to the presence (i.e., ly0 vs. ly1, 2, 3) and degree (i.e., ly0, 1 vs. ly2, 3). The degree of LVI on conventional HE staining has superior sensitivity (92.9%), specificity(96.9%) and accuracy (96.4%) to the presence of LVI.

3.3. Correlation between tumor size and vessel invasion

The correlation between tumor size and vessel invasion is summarized in Table 4. Of the 221 patients, 74 (33.5%) had BVI (v1, 2, 3), and 92 (41.6%) had LVI (ly1, 2, 3). Even in the subgroup with tumors smaller than 20 mm in diameter, 35 patients (26.7%) had BVI, and 47 patients (35.9%) had LVI; 4 (3.1%) had high BVI (v2, 3), and 11 (8.4%) had high LVI (ly2, 3). On the other hand, in the subgroup with tumors larger than 21 mm in diameter, 29 patients (32.2%) had BVI and 45 (50.0%) had LVI; 12 (13.3%) had high BVI (v2, 3), and 17 (18.9%) had high LVI (ly2, 3).

3.4. Factors affecting survival

In the present study, tumor size was not significantly related to cancer-specific survival in both groups divided by two cut-off points (15 or 20 mm, $p=0.1600$ or $p=0.5793$, respectively). With respect to vessel invasion, when cancer-specific survival was compared in patients with v0 and with v1, the difference between the groups was not significant ($p=0.7046$). Similarly, there was no significant difference in survival between those with

ly0 and those with ly1 ($p=0.7432$). The patients with v2, 3 or ly2, 3 had a poor prognosis, with 3- and 5-year survivals of 80.8% and 80.6%, respectively.

When the patients were divided into two groups by “absence” (v/ly0) or “presence” (v/ly1, 2, 3), no significant difference in cancer-specific survival was found ($p=0.1107$, 0.0875 , respectively; Fig. 4A, B), while when the patients were divided according to “degree” of vessel invasion (i.e., v/ly0, 1 vs. v/ly2, 3), there were statistically significant differences in cancer-specific survival ($p=0.0038$, $p=0.0002$, respectively; Fig. 4C, D). When the investigation was limited to patients with tumors measuring equal to or less than 20 mm, the prognosis of the group having ly2, 3 was poorer than that of the other group ($p=0.0018$; Fig. 5). The other clinicopathological variables (age, gender, surgical procedure, histological type, and pleural involvement) were not significantly related to cancer-specific survival.

Multivariate analysis of 221 patients with pathological IA NSCLC showed that the degree of LVI was the only significant independent factor related to cancer-specific survival; the degree of BVI, which was a significant predictor of prognosis on univariate analysis, was not found to be a significant factor ($p=0.0061$, $p=0.9832$, respectively; Table 5). The relative risk of cancer-specific death in patients with ly2, 3 was 6.757 (95% CI: 1.724-26.316) compared to patients with ly0, 1.

4. Discussion

Recently, vessel invasion has been considered to be an important prognostic factor in stage I NSCLC or NSCLC without lymph node metastasis [8,10,14,24]. In these reports, LVI was evaluated as either present or absent on conventional HE/GVH staining. However, since specific lymphatic endothelial markers have not been available, and there have been no criteria of definite LVI in lung cancer specimens, the accuracy of LVI has remained controversial.

D2-40 is a new, selective, monoclonal immunohistochemical marker of lymphatic endothelium that can be used to detect lymphatic invasion in conventionally processed, formalin-fixed, and paraffin-embedded tissue specimens [15]. Until now, studies on the utility of D2-40 for the detection of LVI have been reported in cancers of the breast, stomach, colon, prostate, cervix, endometrium, and skin (melanoma, squamous cell carcinoma) [17,18,25,26]. Kahn et al. compared D2-40 immunostaining using HE staining in 50 specimens of patients with breast cancer. For the detection of LVI, they reported that HE staining showed false-negative results in 9 specimens (18%) and false-positive results in 2 specimens (4%) [25]. Arigami et al. compared D2-40 immunostaining and HE staining in 80 specimens of patients with gastric cancer [31]. For the detection of LVI, they reported that HE staining showed false-negative results in 11 specimens (13.8%) and a false-positive result in 1 specimen (11.1%). Both reports suggested that evaluation of LVI was difficult using conventional HE staining. Although D2-40 antibody has been reported as a specific marker for lymphatic endothelial cells, it was recently found that immunoreactivity of D2-40 was detected in the basal cell layer of the squamous epithelium, stromal myofibroblasts, mesothelial cells, and the basal-cell like cells in the marginal area of the squamous cell carcinomas [19, 26-30].

When we were evaluating vessel invasion using HE staining and EVG staining for

lymphatic and blood vessel invasion, it was very difficult to judge whether there was LVI in the following cases: 1) when the structure was in the narrow stroma located between the well-formed adenocarcinoma lumen; 2) when the lymphatic invasion was obscured by dense inflammatory infiltration; 3) when a dense proliferation of tumor cells surrounded the lymphatic invasion; 4) when densely packed lymphatic invasion simulated tumor cell nests; and 5) when retraction artifact of tumor cell nests simulated lymphatic invasion.

In the present study, previously evaluated specimens were re-evaluated using D2-40 immunostaining to confirm the reliability of evaluation by four grades of vessel invasion before the prognostic factors of p-stage IA NSCLC were evaluated. Using our new four-grade classification criteria, we can detect high LVI (ly2, 3) with high reproducibility even on only conventional staining. On the other hand, with respect to low LVI (ly0, 1), many cases in which LVI was overestimated or underestimated on conventional staining were found. In addition, it is obviously more difficult to detect LVI in lung specimens because of the complicated alveolus structure and the narrow interstitial tissue compared with other organ tissues. Therefore, even if there are structures in which LVI is suspected morphologically, the circumference of the structure must be carefully observed.

We used the LVI score obtained using D2-40 immunostaining in the analysis for prognostic factors, because the results of this analysis appeared to have a higher reliability than the analysis using the earlier evaluation based on conventional staining. The present results showed that it was very difficult to strictly differentiate ly0 and ly1,2,3 using only conventional staining, and there was no significant difference in cancer-specific survival between patients with ly0 and those with ly1. This means that it is not important to distinguish between these two groups when looking for a prognostic factor. In other words, the validity of combining ly0 and ly1 cases into a “low grade invasion group” was proven.

Furthermore, there was no significant difference in cancer-specific survival between the

two groups stratified by the presence of LVI, while there was a significant difference between the two groups stratified according to the degree of LVI. On multivariate analysis, the degree of LVI appeared to be a very good prognostic factor. From the above, we concluded that LVI should be evaluated by “degree” and not by “presence” in lung cancer specimens. It is more useful to evaluate the degree, not the presence, of LVI with respect to the objectivity and the reproducibility of the evaluation, as well as with respect to identifying prognostic factors of p-stage IA NSCLC. In the present study, although BVI failed to be a prognostic factor on multivariate analyses, BVI had prognostic significance on univariate analysis. Several studies have suggested that BVI was a prognostic factor in lung cancer [8,10,14,24]. This supports the possibility that evaluating BVI by degree, as with LVI, may be useful.

Due to the remarkable improvement of imaging instruments, such as high-resolution CT, the spread of cancer screening using CT, and the improved accuracy of cancer screening, microscopic lung cancer has recently become more frequently detected. As a result, stage IA lung cancers with a tumor diameter that is equal to or less than 30 mm will account for a greater proportion of cases. The survival rate of stage IA was better in a recent Japanese report than in previous reports [32]. However, in the present study, 3.1% of tumors that were equal to or less than 20 mm had high lymphatic invasion, while 8.4% of such tumors had high BVI. Patients who died from lung cancer did not present with local recurrence but with distant metastases to the pleura, liver, lungs, and other sites. Even in patients with small tumors, their postoperative course must be carefully monitored, and such patients may require postoperative adjuvant chemotherapy. With respect to adjuvant therapy, the usefulness of postoperative chemotherapy for stage IB NSCLC has already been demonstrated [33]. In addition, adjuvant chemotherapy also appears to be useful for stage IA tumors that are larger than 20 mm, though the results were not statistically significant [2].

Therefore, vessel invasion may be an important surrogate marker for choosing adjuvant therapy for patients with early lung cancer.

In conclusion, the use of D2-40 antibody, a new, lymphatic vessel endothelium-specific marker, allows one to make a more precise diagnosis of LVI. Furthermore, from the perspective of the reproducibility and the objectivity of the evaluation of patients with pathological stage IA NSCLC, vessel invasion should be evaluated based not on its “presence” but on its “degree”. Further study and early establishment of the criteria for evaluating vessel invasion in lung cancer are necessary.

Conflict of interest

None.

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Figure legends

Figure 1. False-positive cases on conventional staining.

A lesion mimicking lymphatic vessel invasion on matching HE-stained and D2-40-immunostained sections.

A) The true alveolar structures resemble lymphatic invasion, containing a papillary tumor nest (arrow).

B) D2-40 immunostaining highlights the lymphatic endothelial cells. The above mentioned lesion (arrow) does not have lymphatic endothelial cells.

C) The tumor nests show tissue detachment from the peripheral stroma due to retraction artifact .

D) The cleft between the tumor cells and the stroma does not show D2-40-positive endothelial cells, indicating a false-positive.

Figure 2. False-negative cases on HE staining.

Obscured lymphatic vessel invasion disclosed by D2-40 immunostaining.

A) The true alveolar structure surrounding the lymphatic vessel masks the identification of the true lymphatic invasion highlighted by D2-40 immunostaining.

B) The lymphatic vessel invasion is within the dense lymphocytic infiltration. D2-40 immunostaining highlights the tumor cells in the lymphatic vessel.

C) The lymphatic invasion is surrounded by poorly differentiated tumor cell proliferation. D2-40 immunostaining highlights the lymphatic invasion.

D) The lymphatic invasion is in the narrow stroma, sandwiched by the well-formed adenocarcinoma lumen.

E) Densely packed, expansive lymphatic invasion, simulating a tumor cell nest. D2-40

immunostaining highlights the cytoplasm of the squamous cell carcinomas.

Figure 3. Correction of evaluation of LVI using D2-40.

Four of 28 cases of ly2 using conventional staining changed to ly1 on D2-40 immunostaining, and 2 changed to ly0. Thirty-one of 75 cases of ly1 on conventional staining actually had no LVI on D2-40 immunostaining, and LVI was newly detected in 18 of 114 cases.

Figure 4. Kaplan-Meier cancer-specific survival curves according to the presence of BVI (A) and LVI (B), and according to the degree of BVI (C) and LVI(D).

There was no significant difference in cancer-specific survival between the two groups stratified by the presence of vessel invasion, (A and B).

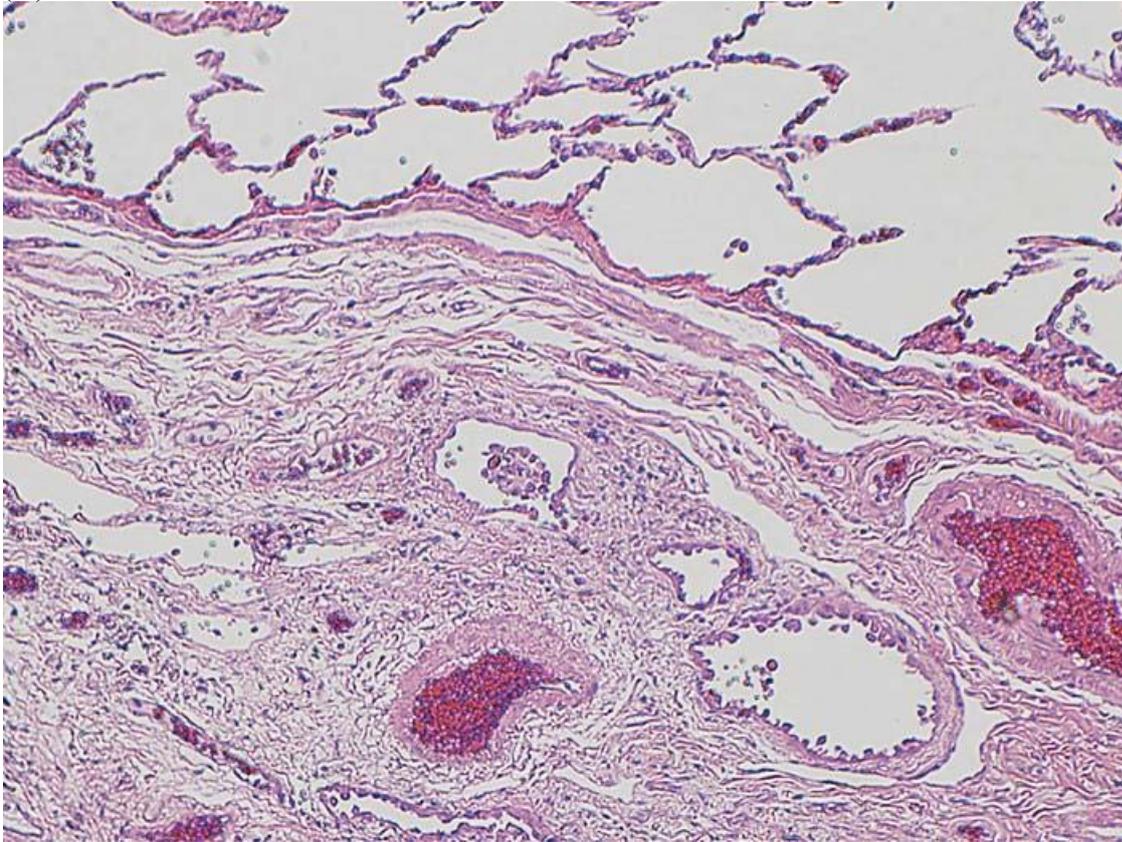
There was a significant difference in cancer-specific survival between the two groups stratified by the degree of vessel invasion, (C and D).

Figure 5. Kaplan-Meier cancer-specific survival curves according to the presence of LVI. (In cases limited to patients with tumors equal to or less than 20 mm).

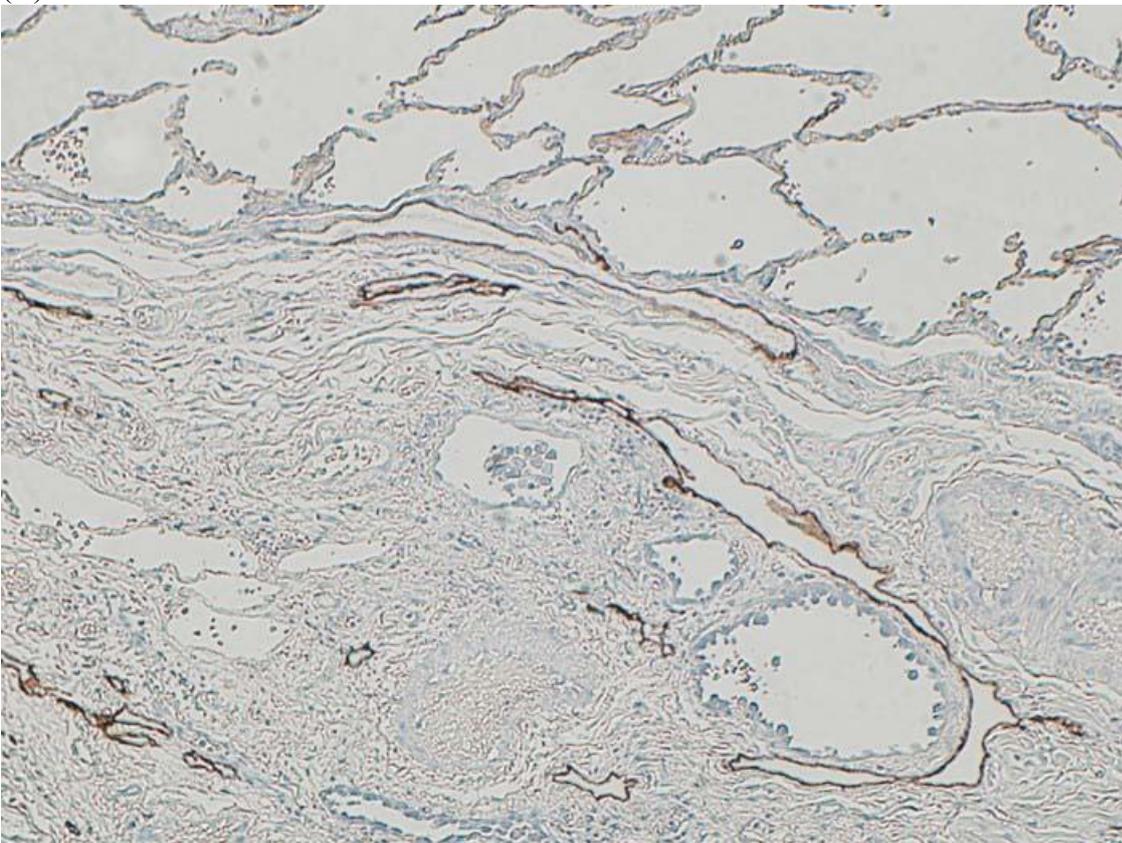
When the investigation was limited to patients with tumors measuring equal to or less than 20 mm, the prognosis of the group having ly2, 3 was poorer than that of the other group (p=0.0018).

Figure 1

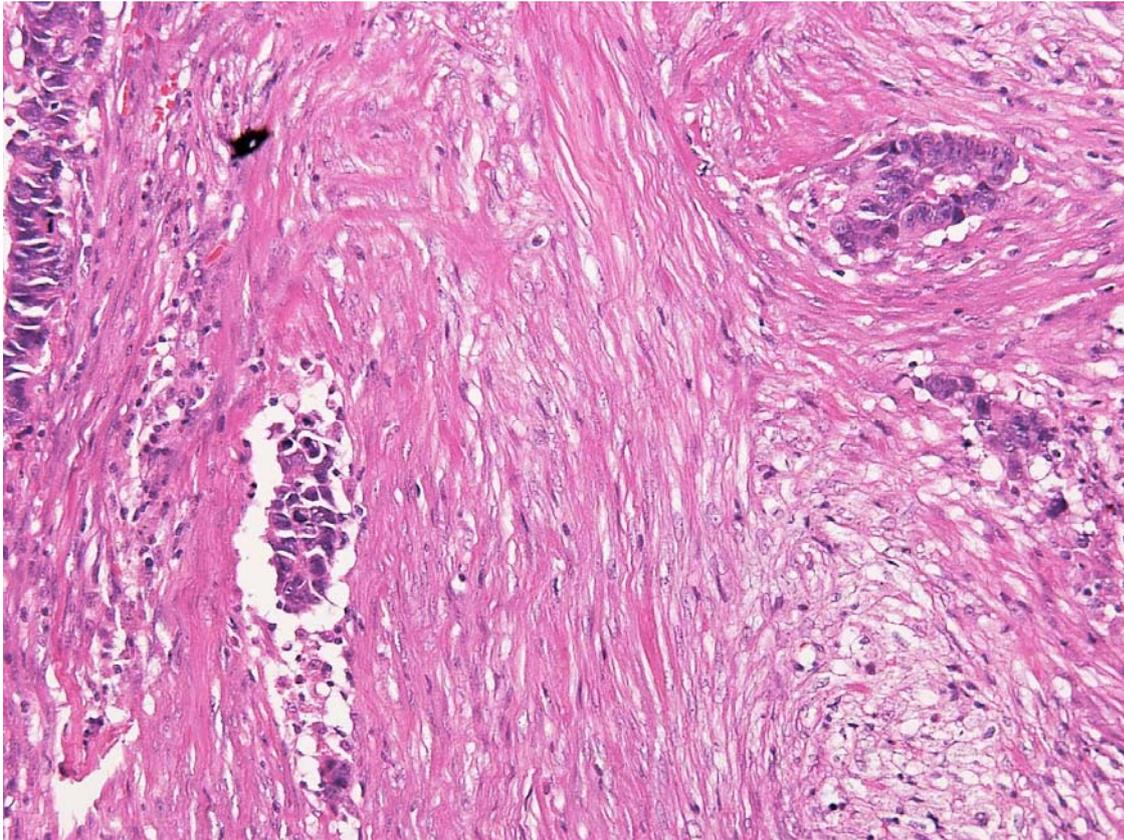
(A)



(B)



(C)



(D)

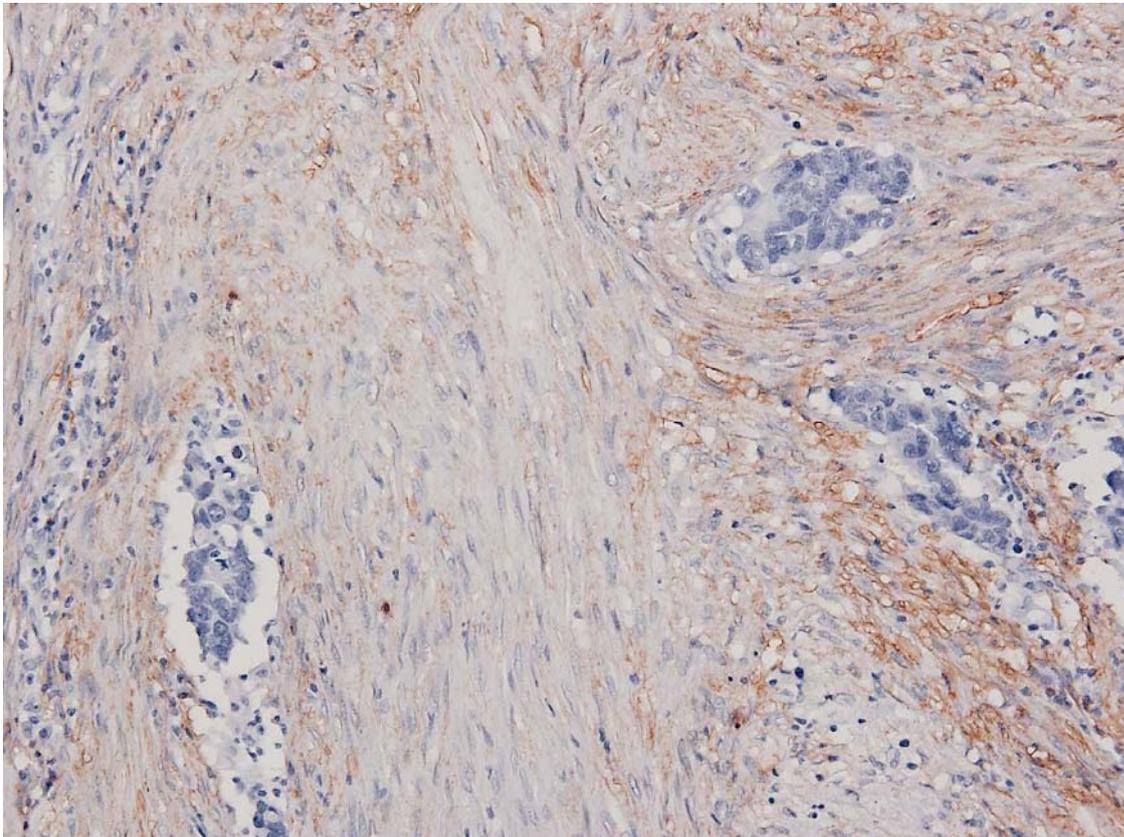
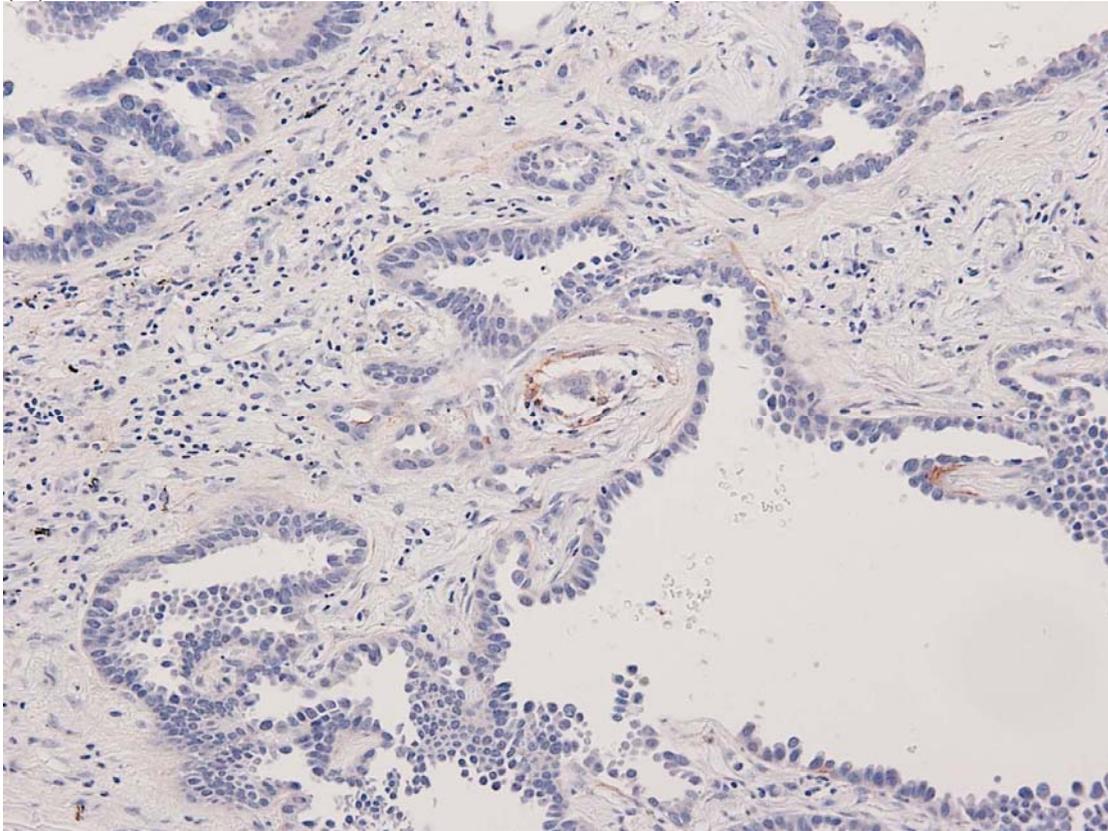
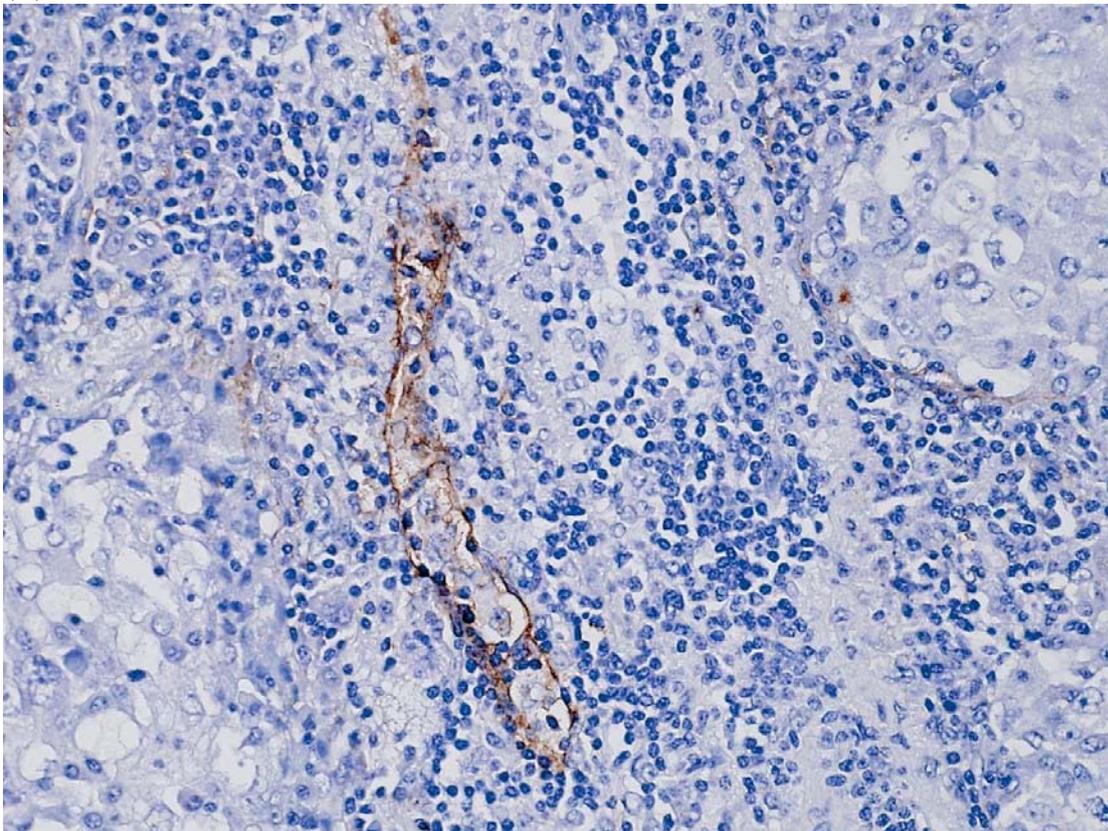


Figure 2

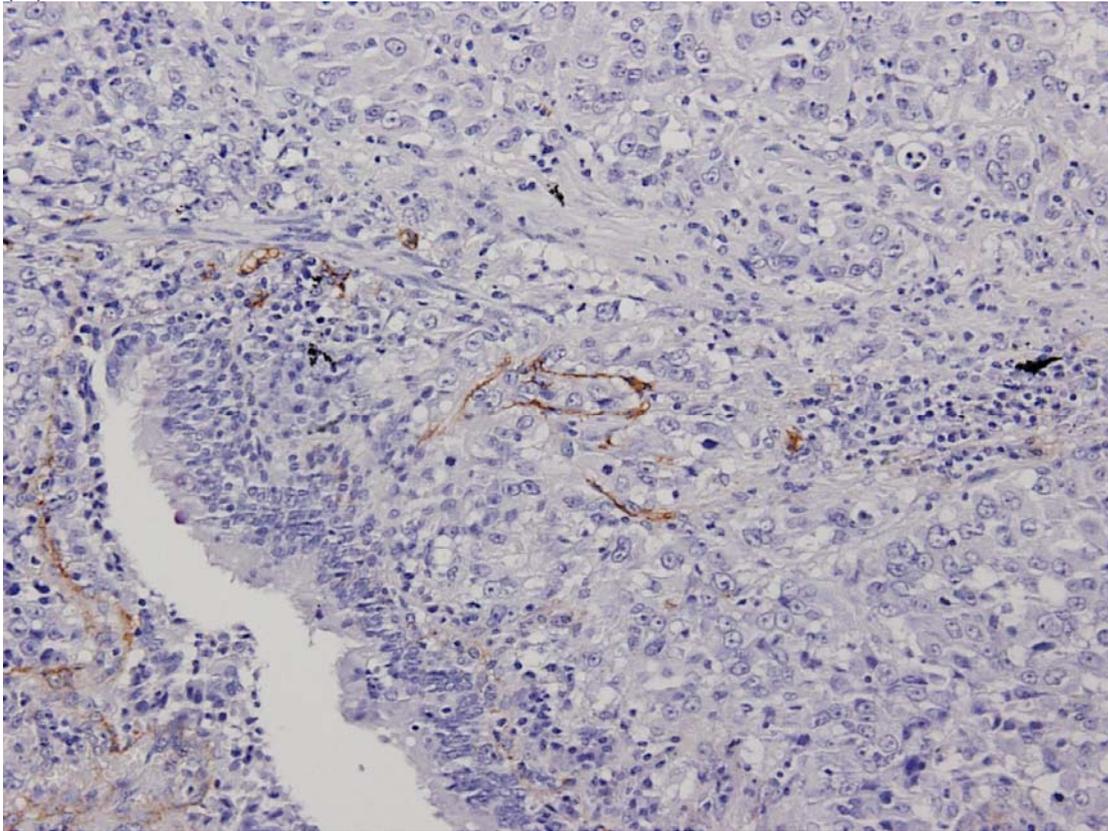
(A)



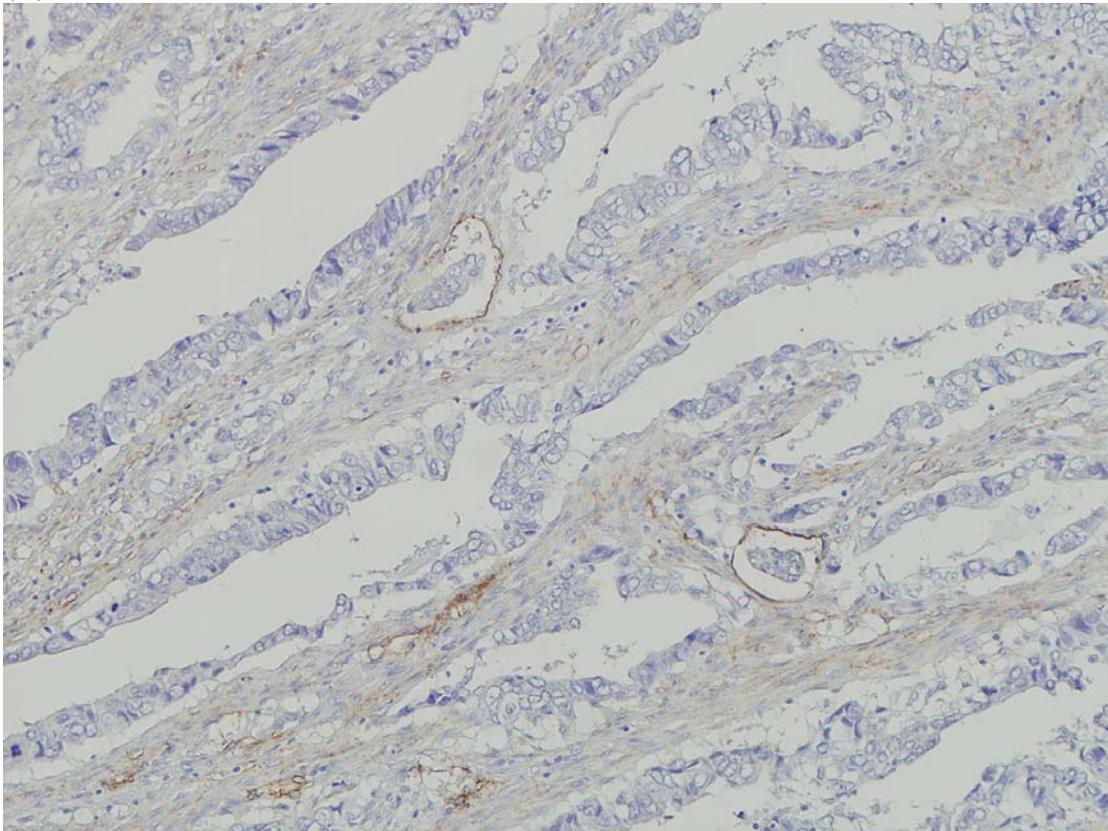
(B)



(C)



(D)



(E)

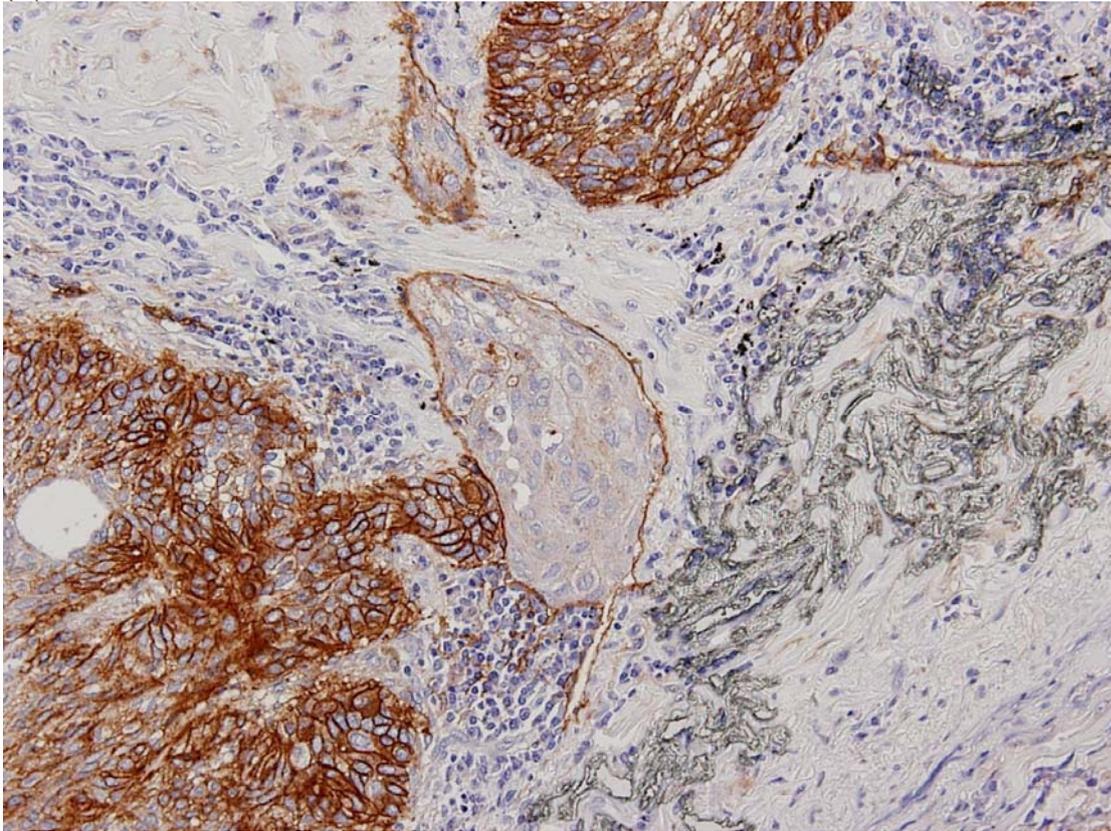


Figure 3

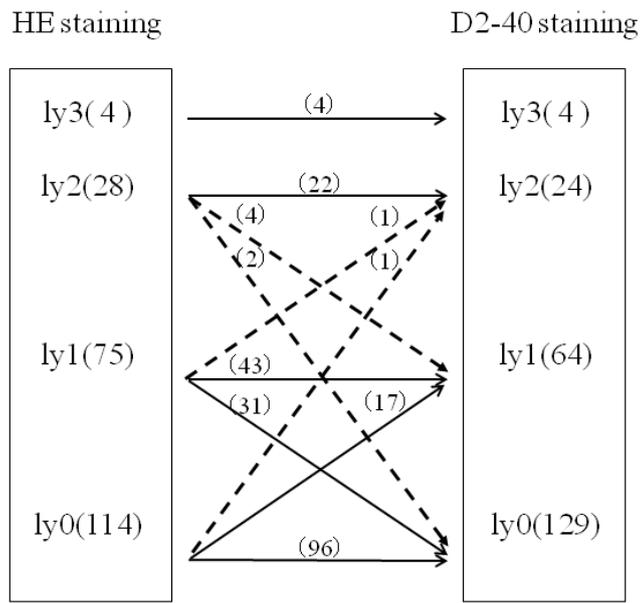


Figure 4

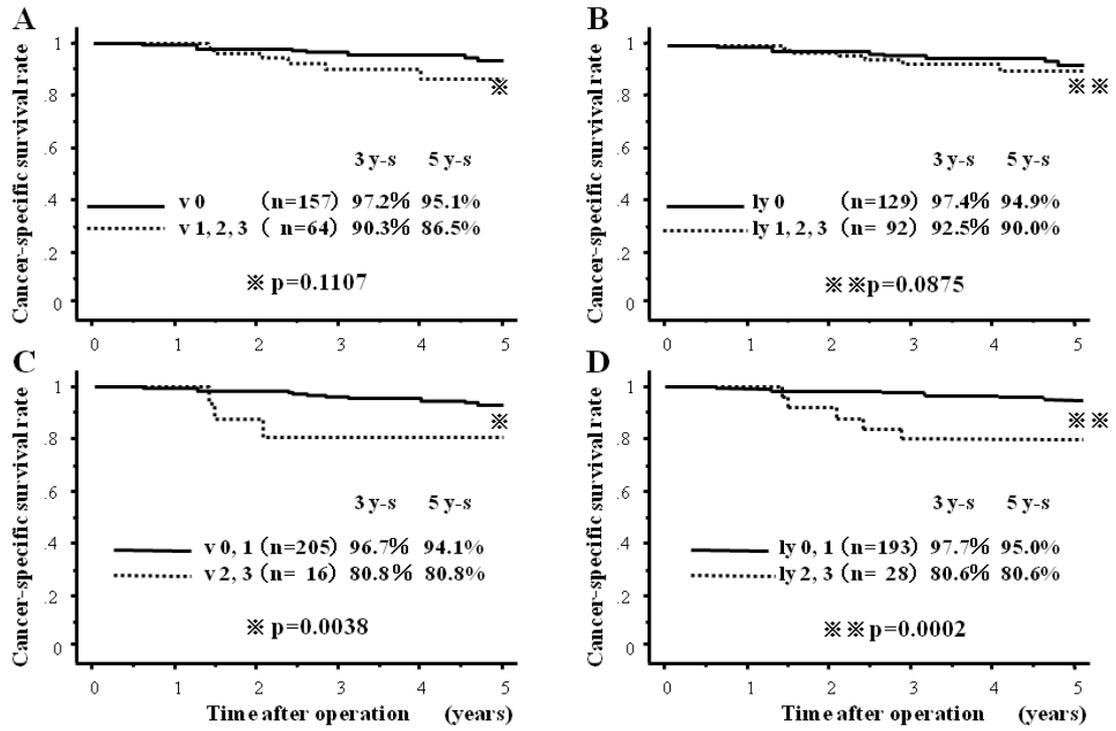


Figure 5

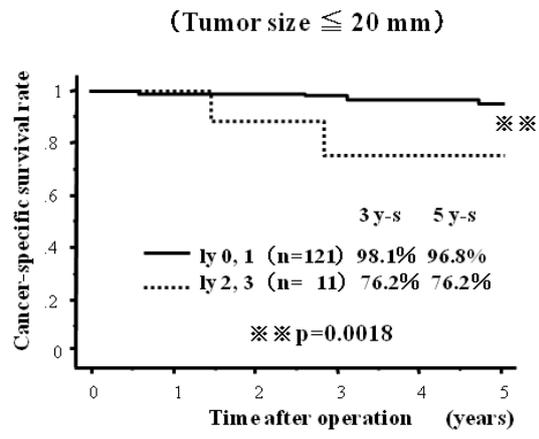


Table 1. Classification Criteria of Vessel Invasion

Lymphatic vessel invasion (LVI)	Blood vessel invasion (BVI)
ly0 : no lymphatic invasion	v0 : no venous invasion
ly1 : minimal lymphatic invasion	v1 : minimal venous invasion
ly2 : moderate lymphatic invasion	v2 : moderate venous invasion
ly3 : marked lymphatic invasion	v3 : marked venous invasion

ly1 : we can barely detect. (less than 2LVIs)

ly2 : we can detect several relatively easily. (more than 3LVIs)

ly3 : we can detect in the region beyond the tumoral area.

v1 : we can barely detect destruction of small vessels.

v2 : we can detect several relatively easily or invasion to big vascular endosporium

v3 : obvious invasion or embolism in big blood vessel.

Table 2. Clinicopathological Characteristics of patients (n=221)

Characteristic	Number	(%)
Age mean 66.8 (median 69, 42 - 88)		
67 >	84	(38.0)
67 ≤	137	(62.0)
Gender		
Male	129	(58.4)
Female	92	(41.6)
Surgical procedure		
Wedge resection	45	(20.4)
Segmentectomy	41	(18.6)
Lobectomy	135	(61.0)
Tumor size (mm)		
Up to 20	131	(59.3)
21 - 30	90	(40.7)
Histological type		
Adenocarcinoma	169	(76.5)
Squamous cell	35	(15.8)
Others	17	(7.7)
Pleural involvement		
p0	196	(88.7)
p1	25	(11.3)

Table 3. Comparison of frequency of LVI detected by HE and D2-40 staining according to the presence and degree

		D2-40 staining							
		present	absent		D2-40 staining				
<i>absent : ly 0</i>					<i>low : ly 0,1</i>				
<i>present : ly 1,2,3</i>					<i>high : ly 2,3</i>				
HE staining	present	76	31	107	HE staining	high	26	6	32
	absent	17	97	114		low	2	187	189
		93	128	221			28	193	221

sensitivity = 76 / 93 (81.7 %)
specificity = 97 / 128 (75.8 %)
accuracy = 173 / 221 (78.3 %)

sensitivity = 30 / 34 (92.9 %)
specificity = 159 / 165 (96.9 %)
accuracy = 189 / 199 (96.4 %)

Table 4. The correlation between tumor size and LVI, BVI

		Tumor size (mm)	
		<20	21-30
LVI	ly0	84 (64.1%)	45 (50.0%)
	ly1	36 (27.5%)	28 (31.1%)
	ly2,3	11 (8.4%)	17 (18.9%)
BVI	v0	96(73.3%)	61 (67.8%)
	v1	31 (23.6%)	17 (18.9%)
	v2,3	4 (3.1%)	12 (13.3%)

Table 5. Multivariate analysis of various prognostic factors

Variables (risk / reference)	CS		
	HR	95% CI	P value
Age 67 ≤ / < 67	1.239	0.390-3.937	0.7164
Gender Male / Female	1.074	0.309-3.728	0.9104
Surgical procedure limited / standard	2.381	0.735-7.719	0.1482
Pleural involvement p1 / p0	2.092	0.546-8.000	0.2817
Histological type Ad / others	0.928	0.168-5.120	0.9318
Sq / others	2.495	0.370-16.841	0.3481
Tumor size (mm) 20 < / ≤ 20	1.045	0.327-3.333	0.9408
LVI ly2, 3 / ly0, 1	6.757	1.724-26.316	0.0061
BVI v2, 3 / v0, 1	0.984	0.215-4.505	0.9832

CS: Cancer-specific survival, HR: Hazard ratio, CI: confidence interval