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Results of Initial Operations in Non-small Cell Lung Cancer Patients With Single-level N2 Disease

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Running head: Lung cancer with single level N2

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Abstract

Background: There is still debate regarding the use of surgery in the management of non-small cell lung cancer patients with N2 disease. The primary aim of the present study was analysis of the results of initial operations in non-small cell lung cancer patients with single-level N2 disease.

Methods: Ninety-four patients with the disease who underwent initial surgery consisting of complete resection of the primary site plus systematic lymphadenectomy were examined. We also immunohistochemically examined lymphatic vessel density (LVD) and vascular endothelial growth factor (VEGF)-C expression.

Results: The overall 5- and 10-year survival rates for the 94 patients were 27.1% and 12.1%, respectively, with a median survival of 22 months. When stratified by skipping status, the 5-year survival rates for the patients in skip-N2 and non-skip-N2 groups were 33.4% and 19.8%, respectively (p=0.0189). Skip metastasis, T factor, subcarinal lymph node metastasis, and adjuvant chemotherapy were recognized as independent prognostic indicators. In both skip- and non-skip-N2 groups, distant relapse was the dominant pattern of recurrence. While the peritumoral LVD was associated with VEGF-C expression in tumors, lymphangiogenic profile appeared to be different between skip-N2 and non-skip-N2 tumors, suggesting different nodal metastatic process.

Conclusions: Lung cancer patients with single-level N2 disease are a oncologically heterogeneous cohort. Although further studies involving randomized comparisons are required, the

poor outcomes found here indicate that the initial operation has yet to be validated for patients with this disease.

Introduction

Nodal involvement is crucial for the oncological outcome in patients with non-small cell lung cancer. Induction chemo- or chemoradiotherapy followed by salvage surgery are well-accepted forms of treatment for patients with ordinal N2 disease. On the other hand, recent clinical trials have begun to demonstrate absolute survival benefit of postoperative adjuvant chemotherapy in selected cases of non-small cell lung cancer patients, and recent guidelines generally recommend adjuvant chemotherapy for patients with completely resected stage IB-III non-small cell lung cancer. In this situation, in contrast to alternate induction treatment followed by surgery, the use of an initial operation plus postoperative adjuvant chemotherapy in the management of patients with resectable single-level N2 disease has become a matter of debate. Although studies of the molecular events underlying nodal metastasis in lung cancer are not yet conclusive, some investigators have also postulated a subgroup of skip N2 disease with more favorable therapeutic results as compared to those with non-skip N2 disease [1-7]. In the present study, we reviewed our experience with non-small cell lung cancer patients with single-level N2 disease who underwent initial operation and performed a retrospective analysis of the surgical results. As a possible nodal metastatic process, we

further explored the lymphangiogenic profile in this unique subgroup of N2 disease.

Materials and Methods

Patients

Among 1,751 primary non-small cell lung cancer patients who underwent operations at Kanazawa University Hospital between 1981 January and 2004 May, 325 patients were diagnosed as having mediastinal nodal involvement after resection of the primary site with systematic nodal dissection of both the hilar and mediastinal lymph nodes. Of those with N2 disease, 94 patients (52 men and 42 women) with a median age of 67 years (range, 36-82 years) had single-level N2 disease. All of these 94 patients underwent initial operation consisting of complete resection of the primary site plus systemic lymphadenectomy. Until around 2001, clinical assessment of nodal metastasis was performed according to CT findings and ^{201}Tl scan results. From about 2001, FDG-PET has been routinely performed in place of ^{201}Tl scan as a less invasive diagnostic tool. Lymph node biopsy through mediastinoscopy was not performed routinely and was performed selectively in patients with clinical N2 diagnosis based on the CT findings and ^{201}Tl/PET scan results. Thirty-three of 94 patients were diagnosed as having clinically single-level N2 disease by mediastinoscopy. For patients with clinical single-level N2 disease, we elected to perform initial operation. All patients gave written informed consent before surgery. Operative procedures included lobectomy in 77 cases

(chest wall resections were combined in 4 cases, bronchoplastic procedures were used in 3 cases, circumferential aortic resection was combined in one case), bilobectomy in 9 cases, pneumonectomy in 6 cases, and segmentectomy in 2 cases. The pathological types were as follows: 52 cases with adenocarcinoma, 37 cases with squamous cell carcinoma, 3 cases with large cell carcinoma, and 2 cases with adenosquamous carcinoma. The pathological stages according to the TNM classification [8] were, 86 cases in stage IIIA and 8 cases in stage IIIB. The mediastinal lymph nodes were identified and numbered (level 1-9) by the lymph node map [9]. Fifty of these 94 patients were free from metastasis in the hilar and intrapulmonary lymph nodes as determined by histopathological examination (skip-N2) and the others showed combined hilar or intrapulmonary nodal metastasis (non-skip N2). The basic clinical features of the patients are summarized in Table 1.

Immunohistochemistry and assessment of lymphatic vessel density (LVD)

The primary antibodies used in the study were an anti-vascular endothelial growth factor (VEGF)-C polyclonal antibody (Santa Cruz Biothechnology, Inc, Santa Cruz, CA, USA) diluted 200-fold and D2-40 monoclonal antibody (Nichirei Corporation, Tokyo, Japan) diluted 100-fold. After reviewing the hematoxylin and eosin-stained slides of the tumor specimens, we selected blocks of the invasive edge in the tumor area. Paraffin-embedded tumor tissues were sectioned into a 4-μm thickness and were deparaffinized and immunohistochemical staining was performed using the

labeled streptavidin-biotin method, as described previously [10.11].

The lymphatic vessel densities were assessed in both intratumoral and peritumoral (within and 500 μ m outside the tumor border) lesions. The areas of highest lymphatic vascularization were chosen under low power (100x magnification), 3 areas with the highest LVD were identified. The lymphatic vessel count was performed with a computer-aided image processing application (Win ROOF, Mitani Corp., Fukui, Japan). Lymphatic vessel counts from 3 areas were averaged and LVD was defined. VEGF-C staining was considered positive when more than 10% of the tumor area was stained.

Statistics

The association between variables was analyzed with the χ^2 test. The Mann-Whitney U test for differences in mean values was used for comparison of nominal data. To assess the prognostic effects on overall survival, age, tumor size, and LVD were classified as high or low relative to the median value. Survival curves were obtained by the Kaplan-Meier method and compared univariately by the log-rank test. Zero time was the date of surgical treatment. Skip metastasis, age (≥67 vs. <67 years), sex, T factor (T1,2 vs. T3,4), pathological type, location of the primary tumor (right vs. left, upper lobe vs. lower lobe), tumor size (≥3.5 vs. <3.5 cm), clinical N2, adjuvant treatment, location of metastatic mediastinal lymph node (subcarinal lymph node metastasis, upper mediastinum vs. lower

mediastinum), and LVD were included in the univariate assessment of prognostic indicators. Clinicopathological and biological factors found to have a P value of less than 0.20 in the univariate analysis were entered into stepwise multiple logistic regression analysis for the determination of statistically significant, independent prognostic factors. Mean values are shown ± the standard error.

Results

The Upper lobe was more frequently the primary site in the skip-N2 group as compared with the non-skip-N2 group (p=0.0041). There were no significant differences in gender, age, tumor size, location of the primary site (right vs. left), histological type, percentage of patients with postoperative adjuvant therapy (systemic chemotherapy with/without radiation therapy), pathological stage (IIIA vs. IIIB), or the number of patients with clinical N2 disease between the two groups (Table 1).

With respect to the mediastinal spread of metastasis, if the primary tumor was located in an upper lobe, nodal metastasis was mainly detected in the upper mediastinal area (54/57 patients). In patients with the primary tumor in the left lower lobe, nodal involvement was detected predominantly in the lower mediastinal area in non-skip-N2 group (11/11 patients). However, the dominant area affected was the upper mediastinal area (paraaortic or subaortic) in the skip-Ns group (3/5 patients). In patients with the primary site in the right lower lobe, although metastases were detected mainly in

the lower mediastinal area (16/20 patients), the upper mediastinal area was also affected in the non-skip-N2 group (4/12 patients)(Figure 1).

In total, the overall 5-year and 10-year survival rates for the 94 patients with single-level N2 disease who underwent complete resection were 27.1% and 12.1%, respectively, with a median survival of 22 months (Figure 2). The effects of skip metastasis on survival were evaluated, and the median survival periods in skip-N2 and non-skip-N2 groups were 31 months and 20 months, respectively. The 5-year survival rates were 33.4% and 19.8%, respectively. The difference was statistically significant (p=0.0189)(Figure 3). Univariate analysis indicated that male gender (p=0.020), non-skip single-level metastasis (p=0.015), pathological type other than adenocarcinoma (p=0.007), T3,4 factor (p=0.0003), clinical N2 (p=0.033), the presence of subcarinal lymph node metastasis (p=0.043), and no postoperative adjuvant chemotherapy (p=0.048) were significantly associated with a poorer outcome. Stratified by skip and non-skip metastasis, the components were different between skip-N2 and non-skip-N2 group, with the exception of T factor (Table 2). As a result of multivariate analysis, non-skip metastasis, T3,4 factor, subcarinal lymph node metastasis, and no postoperative adjuvant chemotherapy were independent negative prognostic indicators. Stratified by skip and non-skip metastasis, peritumoral LVD showed independent negative prognostic impact on overall survival in non-skip-N2 group (Table 3).

Among the patients who died of lung cancer, the pattern of recurrence could be identified in 46

patients. In the skip-N2 group, among 19 patients whose recurrent pattern could be identified, 13 had developed distant metastases, 3 had relapsed due to local recurrence, and 3 had developed both patterns of recurrence at the time of diagnosis. In the non-skip-N2 group, among 27 patients with identified recurrent pattern, 17 had developed distant metastases, 4 had relapsed due to local recurrence, and 6 had developed both patterns at the time of diagnosis. Clearly, distant relapse was the dominant pattern of recurrence in both groups.

In comparison with the consecutive H&E-stained sections, staining for D2-40 was detected predominantly in lymphatic vessels both within the tumor and in the peritumoral area (Figure 4). No immunostaining was identified in the tumor cells or blood vessls, as described previously. The mean LVD within tumor area in the skip-N2 group tended to be greater than that in the non-skip-N2 group [72.8±4.5 (range 23-194) vs. 63.2±4.5 (range 15-171), p=0.074]. The mean LVD within the peritumor area in skip-N2 and non-skip-N2 groups were 64.4±4.1 (range 12-141) and 53.0±3.0 (range 17-100), respectively. The difference was statistically significant (p=0.037). The impact of LVD as a prognostic indicator was not statistically significant in the total patient group. However, in skip-N2 patients, LVD in the peritumoral area appeared to have a weaker relationship with outcome, although the value only trended toward significance.

VEGF-C antigens were mainly identified in the cytoplasm of tumor cells (Figure 5). The percentage of positive tumors were 56.0% (28/50) in skip-N2 group and only 29.6% (12/44) in

non-skip-N2 group (p=0.0126). The relationship between VEGF-C expression and LVD was summarized in Table 4. Peritumoral LVD and VEGF-C expression were strongly associated (p=0.0035) in skip-N2 group, but not in non-skip-N2 group. No association was found between intratumoral LVD and VEGF-C expression.

Discussion

In the present study, a relatively regular distribution of the spread of mediastinal nodal metastasis was found in both skip and non-skip groups. That is, if the primary tumor was located in the upper lobe, nodal metastasis was detected mainly in the upper mediastinal area, while in patients with the primary tumor in the left lower lobe, nodal involvement was detected frequently in the lower mediastinal area. However, 7 patients (19.4% of the patients in whom the primary site was located in the lower lobe) had isolated nodal spread in the upper mediastinal area from the lower lobe and 3 patients (5% of those in whom the primary site was in the upper lobe) had nodal metastasis in the lower mediastinal area from the upper lobe. The lymphatic channel draining directly into the mediastinal area has been proposed as a possible metastatic route of skip mediastinal spread. Based on an autopsy study, Riquet and his colleagues demonstrated incidences of direct passage into the mediastinal nodes of 22.2% and 25% on the right and left sides, respectively. Interestingly, they also reported that direct passage was observed more frequently in the segments of the upper lobes [12].

The observation in the present study that the upper lobe was the predominant site of primary lung cancer with mediastinal skip nodal metastasis may represent the anatomical distribution of these direct passages as suggested by Riquet [7]. This relationship between the location of the primary site and the frequency of mediastinal skip nodal metastasis was also documented by other investigations[3,7,13,14]. From the viewpoint of sentinel lymph node mapping, the results of recently launched clinical trials support the anatomical concept. While the percentage of skip metastasis in lung cancer patients with N2 disease has been reported to be around 20-40% [1-7], sentinel node mapping in non-small cell lung cancer patients in various stages revealed a similar percentage of mediastinal (N2) sentinel nodes of 16.7-31% with a high sensitivity rate of more than 90% [16-18]. Arguably, the direct drainage system appears the most plausible main channel of skip mediastinal nodal spread of cancer cells.

The concept that N2 nodes with a direct drainage system should be included in N1 is of interest as an answer to the questions regarding the relatively preferable surgical results in the skip-N2 group. However, some peculiar clinical features cannot be explained only by the anatomical and mechanical drainage system. As the results of immnunohistochemical staining of N1 lymph nodes with anti-cytokeratin antibodies, one previous report indicated that microscopic spread of cancer cells could be detected in 17.6% of patients with skip-N2 disease [4]. Using immunohistochemical staining for cytokeratin in 3,081 lymph node samples obtained from 181 non-small cell lung cancer

patients diagnosed as being in stage I by conventional histopathological examination, we previously found nodal micrometastasis in 44 patients [19]. Twenty-six of these patients had micometastases in a single level of the mediastinal area. Of these 26 patients, 19 had a skip pattern and 7 showed a non-skip pattern with involvement of N1 micrometastases (data not shown). Here, if the small amount of cancer cells detected in N1 lymph nodes were the trace or footprint of cancer dissemination, some cancer cells may pass through the sentinel node and grow in other regional nodes. This possibility warrants further study and the possible dual lymphatic pathways with coexisting N1(-)N2 and N1(+)N2 remains to be defined [7].

As most of skip metastases are restricted to a single region, the disease is likely to be localized. However, 5-year survival rates of patients with skip N2 disease have been reported to be 21-51% even after complete resection [1-7]. In our series, the 5-year survival rate of the patients with skip-N2 disease was 33.4% despite complete resection with systematic lymphadenectomy Consistent with previous reports [1-7], survival of patients with non-skip-N2 disease was worse than that of patients with skip-N2 disease. The present study also showed that distant metastasis is the dominant pattern of recurrence. In comparison with patients with nodal micrometastasis, the 5-year survival rate of the 26 patients with single-level mediastinal nodal micrometastasis was 57.7% despite systematic lymph node dissection, although survival was significantly better than the 94 patients with single-level metastasis presented here ($p=0.0033$)(data not shown). Besides T factor, among the

various clinicopathological factors, while histological type and adjuvant therapy showed significance in the skip-N2 group, tumor size and clinical N2 status showed statistical significance in the non-skip-N2 group. These observations suggested that it is necessary to consider non-small cell lung cancer patients with single station N2 disease as an oncologically heterogeneous subgroup with different biological behavior, and a number of patients in this group have a risk of systemic disease even if the nodal metastasis is the skip pattern.

Newly formed tumor vessels have been shown to have structural and functional abnormalities such as loss of vascular hierarchy, arterio-venous shunting. Larger numbers and greater surface area of immature neo-vessels are thought to be a driving force of cancer metastasis via blood or lymphatic routes. It is interesting to note that, among the members of the vascular endothelial growth factor (VEGF) family, VEGF-C/D and VEGF receptor (VEGFR)-2/3 pathways are involved in induction of “lymphangiogenesis” and these specific pathways involved in lymphangiogenesis have been shown to be different from those in hemangiogenesis [20]. Of more direct clinical relevance, recent reports further indicated that lymphangiogenesis is an indicator of lymph node metastasis in various malignant solid tumors such as cutaneous melanoma, head and neck squamous cell carcinoma, malignant pleural mesothelioma, by direct counting of lymphatics [21-23]. The findings in of basic research have suggested that peritumoral functional lymphatics are important for lymphatic metastasis [24]. In lung cancer, although the association of lymphangiogenesis with nodal

metastasis is still an open question, previous research conducted in our laboratory confirmed a significant association between tumor-induced VEGF–C and VEGFR-3 expression in vascular endotheluial cells and lymphatic vessel invasion and lymph node metastasis in non-small cell lung cancer [25]. We have also confirmed a positive association between tumor-induced VEGF-C and nodal micrometastasis, the earliest phase of cancer nodal spread, in non-small cell lung cancer [11]. In the present study, for direct assessment of lymphatic vessels using paraffin-embedded tumor tissue specimens, we used a monoclonal antibody D2-40, which was originally introduced as an antibody against testicular oncofetal and testicular germ cell tumors antigens. It has already been shown to be a specific marker of tumor-associated lymphatic endothelium with no cross-reactivity with blood vessel endothelium [26-29]. To our knowledge, this is the first time this antibody has been applied to non-small cell lung cancer specimens to assess LVD. Consistent with the observations of previous studies, lymphatic vessels were clearly detected both within the tumor and in the peritumoral area in lung cancer tissue. Interestingly, in the assessment of LVD in the peritumor area, we found a significant difference in LVD and VEGF-C expression between the skip-N2 and non-skip-N2 groups. The results demonstrated here suggested that the dependency of nodal metastasis on lymphangiogenesis is different between skip and non-skip nodal metastasis. Although the LVD assessed is unrelated to outcome in the total patient population, survival of patients with high LVD tended to be worse than that of patients with low LVD in the skip-N2 group. The

interrelation between LVD and outcome was similar to that between blood vessel density and outcome. Recent studies have postulated different angiogenic profiles, i.e., angiogenic and non-angiogenic, in non-small cell lung cancer [30-32]. In non-angiogenic tumors, cancer cell nests fill the alveolar space without destruction of parenchyma, co-opting the septal blood vessels[30]. Some investigators suggested that non-angiogenic profile was associated with negative prognostic indicators and angiogenesis-related parameters, such as tumor vascular density, can only be of prognostic value in tumors with angiogenic profile [32]. Similarly, skip-N2 and non-skip-N2 tumors may have different lymphangiogenic profiles, resulting in different LVD and corresponding different outcomes.

In conclusion, non-small cell lung cancer patients with single-level N2 are included in the heterogeneous cohort of patients. Although the survival of patients with skip-N2 was superior to that of patients with non-skip-N2, surgical results after the initial operation were not satisfactory. Therefore, for patients with single-level N2, the same tactic as used for patients with ordinal N2 is warranted. While the anatomical/mechanical lymphatic drainage systems appear to pertain to the formation of isolated skip mediastinal nodal metastases, our results further suggested differences in metastatic process with different lymphangiogenic profiles between skip and non-skip single-level N2 patients. Further studies are required to characterize the

potential causal relationship between lymphangiogenesis and lymphatic metastasis in detail.

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Figure Legends

Fig. 1. Intramediastinal spread of metastasis related to the primary tumor sites. RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe. Open circles, skip metastasis; closed circles, non-skip metastasis.

Fig. 2. Kaplan-Meier survival plots for 94 NSCLC patients with single-level N2 disease. The survival curve is combined with lines represent the 90% confidence interval.

Fig. 3. Kaplan-Meier survival plots for 94 NSCLC patients with single-level N2 disease subdivided according to skip (n=50) or non-skip (n=44) status. The difference in survival between the two groups was significant (p=0.015). The survival curves are combined with lines represent the 90% confidence interval.

Fig. 4. Immunohistochemical staining for D2-40 (A). In comparison with consecutive H&E-stained section (B), D2-40 staining was detected predominantly in lymphatic vessels. No immunostaining was identified in the tumor cells or blood vessels.

Fig. 5. Immunohistochemical staining for VEGF-C. VEGF-C antigens were mainly identified in the cytoplasm of tumor cells.

Table 1. Basic Clinical and Pathological Characteristics of The 94 Lung Cancer Patients With Single-level N2

Variable	Total	Skip	Non-skip
Patients (n)	94	50	44
Sex (n)			
Male	52	29	23
Female	42	21	21
Mean age (y)	65.5±9.5	66.5±8.1	64.4±10.9
Mean tumor size (cm)	4.0±2.0	4.0±1.8	4.0±2.3
Location-1 (n)			
Right	48	28	20
Left	46	22	24
Location-2 (n)			
Upper lobe	57	36	21
Middle lobe	1	1	0
Lower lobe	36	13	23
Histological types (n)			
Adenocarcinoma	52	27	25
Squamous cell carcinoma	37	20	17
Others	5	3	2
T factor (n)			
T1	25	16	9
T2	49	25	24
T3	12	6	6
T4	8	3	5
Pathological stage (n)			
IIIA	86	47	39
IIIB	8	3	5
Clinical N factor (n)			
N0	54	32	22
N1	7	2	5
N2	33	16	17
Adjuvant treatment (n)	65	33	32

Table 2. Univariate Analysis of Prognostic Factors

Factors	Total		Skip		Non-skip	
	P value	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)
Skip metastasis	0.015	0.3537 (1.059-1.733)				
Age	0.331	0.889 (0.701-1.128)	0.355	0.852 (0.602-1.195)	0.363	0.853 (0.608-1.207)
Sex	0.020	1.328 (1.045-1.702)	0.122	1.309 (0.930-1.878)	0.102	1.326 (0.944-1.888)
Histological type (adeno vs others)	0.007	1.382 (1.090-1.756)	0.013	1.535 (1.092-2.187)	0.153	0.784 (0.560-1.096)
T factor (T1,2 vs T3,4)	0.0003	3.019 (1.703-5.161)	0.001	2.964 (1.092-2.187)	0.014	2.683 (1.234-5.521)
Tumor size	0.005	1.409 (1.105-1.811)	0.087	0.741 (0.517-1.043)	0.044	1.432 (1.009-2.048)
Clinical N2	0.033	1.301 (1.020-1.653)	0.345	1.184 (0.827-1.671)	0.035	1.444 (1.026-2.034)
Subcarinal (#7) LN metastasis	0.043	1.928 (1.022-3.410)	0.105	0.446 (0.193-1.209)	0.132	0.516 (0.237-1.242)
Adjuvant therapy	0.048	1.319 (1.001-1.714)	0.039	1.507 (1.019-2.196)	0.681	0.921 (0.638-1.391)
Location-1 (right vs left)	0.730	0.958 (0.752-1.216)	0.723	1.063 (0.756-1.515)	0.632	1.086 (0.773-1.533)
Location-2 (upper vs lower)	0.098	0.814 (0.641-1.040)	0.233	0.792 (0.554-1.174)	0.867	0.971 (0.690-1.358)
Location of LN Metastasis (upper med. vs lower med.)	0.125	0.825 (0.650-1.056)	0.296	0.811 (0.572-1.186)	0.619	0.919 (0.657-1.287)
*`LVD (1) (within tumor)	0.577	0.872 (0.536-1.410)	0.829	0.927 (0.463-1.839)	0.557	0.816 (0.409-1.612)
LVD (2) (peritumor)	0.220	0.756 (0.457-1.239)	0.166	0.593 (0.265-1.234)	0.184	0.604 (0.293-1.280)

***LVD, lymphatic vessel density**

Table 3. Multivariate Analysis of Prognostic Factors

Factors	Total		Skip		Non-skip	
	P value	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)
Skip metastasis	0.032	1.311 (1.022-1.686)				
T factor (T1,2 vs T3,4)	<0.001	3.873 (2.127-6.866)	0.025	2.923 (1.153-6.862)	0.004	3.913 (1.572-9.358)
Subcarinal (#7) LN metastasis	0.001	3.10 (1.578-5.799)	0.025	3.318 (1.176-8.197)	0.004	4.606 (1.555-12.347)
Adjuvant therapy	0.011	1.439 (1.087-1.880)	0.060	1.475 (0.982-2.191)		
*LVD (peritumor)					0.021	2.644 (1.159-6.042)

*LVD, lymphatic vessel density

Table 4. Lympatic Vessel Density (LVD) and VEGF-C Expression in Tumors

	Total (n=94)		Skip (n=50)		Non-skip (n=44)	
	Peritumoral mean LVD	Intratumoral mean LVD	Preitumoral mean LVD	Intratumoral mean LVD	Peritumoral mean LVD	Intratumoral mean LVD
VEGF-C (+)	70.6±4.4	74.8±5.3	74.6±5.6	77.6±6.5	61.2±6.0	68.7±9.5
VEGF-C (-)	49.7±2.6	63.3±3.9	49.8±4.3	66.6±6.1	49.7±3.4	60.9±5.1
P value	0.0004	0.127	0.0035	0.423	0.128	0.517

*LVD, lymphatic vessel density