Preoperative lymphocyte count is an independent prognostic factor in node-negative non-small cell lung cancer

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Abstract

A number of prognostic factors have been reported in non-small cell lung cancer (NSCLC). Although lymph node metastasis is the most poorly predictive value in completely resected NSCLC, a significant number of patients have a fatal recurrence even in node-negative curative NSCLC. Recently inflammatory response has been shown as a predictive value in NSCLC. Neutrophils and lymphocytes play an important role in cancer immune response. In this study, we retrospectively examined the impact of preoperative peripheral neutrophil and lymphocyte counts on survival, and investigated the relationships of these factors to clinicopathological factors in node-negative NSCLC. A total 237 patients were evaluated. When the cut-off value of neutrophil count was 4500 mm^{-3} with a maximum log-rank statistical value, overall 5-year survival rates were 79.7% for the low-neutrophil-count group and 69.5% for the high-neutrophil-count group (P = 0.04). When the cut-off value of lymphocyte count was 1900 mm^{-3} with a maximum log-rank statistical value, overall survival rates were 67.9% for the low-lymphocyte group and 87.7% for the high-lymphocyte

group ($P < 0.001$). High-neutrophil-counts were associated with tumor size ($P = 0.002$) and pleural invasion ($P < 0.001$). Low-lymphocyte-counts were correlated with vascular invasion ($P = 0.018$) and recurrence of NSCLC ($P = 0.01$). Multivariate analysis showed that the lymphocyte count was an independent prognostic factor (hazard ratio: 3.842; 95% confidence interval: 1.827 – 8.078; $P < 0.001$), but the neutrophil count was not ($P = 0.185$). We conclude that a peripheral lymphocyte count, which is associated with vascular invasion, is an independent prognostic factor in node-negative NCSLC.

Keywords: non-small cell lung cancer, node-negative, prognostic factor, lymphocyte count, neutrophil count, vascular invasion

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common causes of cancer-related death. A number of prognostic factors have been proposed for patients with NSCLC, such as age, sex, performance status, tumor size, pleural invasion, lymphatic invasion, vascular invasion, lymph node metastasis, and distant metastasis [1-4]. Although lymph node metastasis is recognized as the worst prognostic factor in completely resected NSCLC [2, 3], a significant number of patients have systemic recurrence even in node-negative curative NSCLC. Recently, the systemic inflammatory response has also been shown to have predictive value [5-7]. The neutrophil-lymphocyte ratio has been found to be an important indicator of adverse prognosis in colorectal cancer [8, 9], gastric cancer [10, 11], and NSCLC [12, 13]. In addition, neutrophils have been implicated in the promotion of aerogenous metastasis in patients with bronchioalveolar carcinoma [14], and lymphocytes recognized as playing a fundamental role in cell-mediated immunologic destruction of host cancer cells [15]. The purpose of the present study, therefore, was to examine the impact of

preoperative peripheral neutrophil and lymphocyte counts on survival and to investigate the relationships of the neutrophil and lymphocyte counts to clinicopathological factors in node-negative NSCLC.

Materials and methods

Patients

We conducted a retrospective analysis of patients diagnosed with NSCLC who underwent surgery at the Tsukuba University Hospital between January 2000 and December 2009. Data from 455 patients were obtained from the hospital's database. Two hundred eighteen patients were excluded owing to positive lymph node metastasis (n = 94), unmeasured differential leukocyte count (n = 59), incomplete radical surgery (n = 42), preoperative treatment (n = 18), suspicion of granulocyte-colony stimulating factor-producing tumor with leukocytosis (n = 3), and autoimmune disease (n = 2). Thus, 237 patients were included in this study. No patients had infection, such as pneumonia. A peripheral venous blood sample was collected from each patient within a month before surgery. A Blood test was performed using

fully automated blood cell counting system and confirmed by laboratory technicians. Histological classification was carried out according to the WHO guidelines [16]. The tumors were staged according to the TNM classification of malignant tumors [17]. Vascular invasion was detected by using elastica van Gieson staining and lymphatic invasion was determined using D2-40 staining.

Statistical analysis

Univariate analysis was performed using the Kaplan-Meier method, and statistical significances between survival curves were assessed by the log-rank test. Overall survival was determined from the date of surgery to the date of death or last follow-up. In selecting the optimal cutoff value for determining the effect of the neutrophil and lymphocyte count on overall survival, a running log-rank test was performed at intervals of 100 mm^{-3} between the 5th percentile and the 95th percentile of neutrophil and lymphocyte counts. The cutoff value was defined when the log-rank statistical value was maximum [18]. To examine the association with clinicopathological factors, the chi-square test (or Fisher's exact test when

appropriate) was used for categorical variables, and the *t* test for continuous variables. To assess the independent predictive value of survival of different variables, multivariate analysis was performed using the Cox proportional hazards model. The result was considered to be significant when the *P* value was less than 0.05. Statistical analyses were performed using PASW Statistics 18 (SPSS, Chicago, IL, USA).

Results

Patients' characteristics

The clinicopathological characteristics of the patients are shown in Table 1. There were 147 men and 89 women. The patients' age at the time of operation ranged from 29 to 89 years (mean age, 66.9 years). The mean follow-up was 43 months (range, 1-118 months). Thirteen patients were performed postoperative adjuvant chemotherapy and 29 patients had recurrences of lung cancer. The overall 5-year survival rate of all 237 patients was 77.7%.

Optimal cutoff values for neutrophil and lymphocyte counts

As for the neutrophil count, the maximum log-rank statistical value was 4.210 (P = 0.04) when the cutoff value was 4500 mm^{-3}. Kaplan-Meier survival curves according to the optimal cutoff value are shown in Figure 1A. The overall 5-year survival rates were 79.7% for the low-neutrophil-count group (neutrophil count ≤ 4500 mm^{-3}; n = 191) and 69.5% for the high-neutrophil-count group (neutrophil count > 4500 mm^{-3}; n = 46). As for the lymphocyte count, the maximum log-rank statistical value was 13.19 (P < 0.001) when the cutoff value was 1900 mm^{-3}. Kaplan-Meier survival curves according to the optimal cutoff value are shown in Figure 1B. The overall 5-year survival rates were 67.9% for the low-lymphocyte-count group (lymphocyte count ≤ 1900 mm^{-3}; n = 110) and 87.7% for the high-lymphocyte-count group (lymphocyte count > 1900 mm^{-3}; n = 127).

Relationships between preoperative neutrophil and lymphocyte counts and clinicopathological factors

The relationships between the neutrophil and lymphocyte counts and clinicopathological factors are shown in Table 2 and 3. The high neutrophil

count was significantly associated with tumor size (P = 0.002) and pleural invasion (P < 0.001), which influenced tumor status and staging. The high neutrophil count was also significantly correlated with C-reactive protein (P < 0.001). On the other hand, the low lymphocyte count was significantly correlated with vascular invasion (P = 0.018) and recurrence of NSCLC (P = 0.01). As for age, sex, performance status, smoking index, comorbidity and past history, surgical procedure, histological type, albumin, and cause of death, no significant differences were detected between the groups.

Multivariate analysis

The results are shown in Table 4. The lymphocyte count was an independent prognostic factor for overall survival (hazard ratio: 3.842; 95% confidence interval: 1.827 – 8.078; P < 0.001). Other independent prognostic factor was tumor size (hazard ratio: 3.568; 95% confidence interval: 1.748 – 7.282; P < 0.001). The neutrophil count, albumin, C-reactive protein, pleural invasion, vascular invasion, and lymphatic invasion were predictive values only in univariate analysis, but not in multivariate analysis.

Discussion

The results of this study suggest that a high peripheral lymphocyte count is an independent positive prognostic factor in node-negative NSCLC. Previously, the lymphocyte count, an inexpensive, reproducible, and widely available blood test, was found to have an independent prognostic significance in pancreatic [19, 20] and breast cancers [21]. In NSCLC, Hespanhol *et al* reported that the lymphocyte count might be a valuable contribution to prognosis estimation in stage IIIB or IV patients [22], and Muers *et al* proposed a lymphocyte count as one of the predictive models in patients who had not received curative treatment [23]. To the best of our knowledge, this is the first report of the prognostic significance of lymphocyte counts in early-stage NSCLC with curative treatment, and with additional investigation of the relationships with clinicopathological factors. Lymphocytes play a fundamental role in cell-mediated immunologic destruction of various cancers [15, 24, 25]. An increased number of tumor-infiltrating lymphocytes (TILs) is correlated with a favorable prognosis in cancer [26]. Cluster-of-differentiation (CD) 8^+ T cells have a

pivotal role in tumor growth control by cytotoxic T-cell killing and apoptosis [27, 28], and $CD4^+$ T cells play a central role in orchestrating the immune response to cancers [29]. In NSCLC, the role of TILs is still controversial. Some reports showed that TILs correlated with improved prognosis [28-33], while others reached the opposite conclusion [34]. In our study, the peripheral low lymphocyte count was significantly associated with vascular invasion. Al-Shibli *et al* showed that a low level of stromal $CD8^+$ lymphocyte infiltration was associated with an increased incidence of angiolymphatic invasion [31], and Ruffini *et al* showed that TILs were more frequent in tumors with microscopic vascular invasion [32]. The pathogenesis is unclear, but there might be a relationship between lymphocytes and vascular invasion, which is one of the important prognostic factors in NSCLC [1, 35]. In our study, the fact that the low lymphocyte count had a significant correlation with vascular invasion would support this relationship.

In the present study, only the univariate analysis showed that a high neutrophil count was a poor prognostic factor in node-negative NSCLC. An

increasing neutrophil count has been identified as an independent prognostic factor in patients with advanced NSCLC [4, 13]. The difference between those previous reports and the present study is tumor stage. Sarraf *et al* also did not find any association between the absolute value of the neutrophil count on survival after complete resection of early-stage NSCLC [12]. In this study, the high neutrophil count was significantly associated with tumor size and pleural invasion. Therefore, a high neutrophil count may indicate only the severity or nature of inflammation occurring within or around the tumor. Neutrophil-Lymphocyte ratio was previously reported as a prognostic factor in NSCLC [12, 13]. In our study, Neutrophil-Lymphocyte ratio was a significant prognostic factors in univariate analysis, but not in multivariate analysis in preliminary study (data not shown), probably because Neutrophil-Lymphocyte ratio was a direct confounding factor of lymphocyte count.

C-reactive protein and albumin have also been shown to be independent prognostic factors in NSCLC [5-7, 22, 23, 36]. In the present study, the C-reactive protein and albumin were significant prognostic factors in

univariate analysis, but not in multivariate analysis. The C-reactive protein was associated with the neutrophil count. Therefore it is considered that the C-reactive protein may reflect an inflammatory response to the tumor as well as the neutrophil count. Hypoalbuminemia is reported to associate with inflammation and malnutrition especially in advanced cancer [5, 37]. However, in the present study, albumin correlated with neither neutrophil count nor lymphocyte count, and few patients were malnourished.

We investigated patients with node-negative NSCLC. We considered that the analysis could be difficult in node-positive NSCLC because patients with node-positive NSCLC were performed various postoperative treatment. In fact, the neutrophil or lymphocyte count had no impact on survival in node-positive NSCLC (data was not shown). In the present study, 20 out of 43 patients (47%) died from non-NSCLC. Therefore we also analyzed the impact of prognosis using disease specific survival and disease free survival as endpoint. The results showed that lymphocyte count was still a significant prognostic factor and neutrophil count was not significant by univariate analysis (data was not shown).

In conclusion, the results of this study indicate that a preoperative peripheral lymphocyte count is an independent prognostic factor in node-negative NSCLC. The subject for future investigation is to determine the mechanism of association between lymphocytes and prognosis by clarifying the relationships among peripheral lymphocyte counts, TILs, and vascular invasions.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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A short descriptive title of Figure 1A

Figure 1A. Overall survival curves at the optimal cutoff value of the neutrophil counts (4500 mm^{-3})

Legends of Figure 1A.

The overall 5-year survival rates were 79.7% for the low-neutrophil-count group (neutrophil count≤ 4500 mm^{-3}; n = 191), which was significantly higher than 69.5% for the high-neutrophil-count group (neutrophil count > 4500 mm^{-3}; n = 46).

A short descriptive title of Figure 1B.

Figure 1B. Overall survival curves at the optimal cutoff value of the lymphocyte counts (1900 mm^{-3})

Legends of Figure 1B

The overall 5-year survival rates were 67.9% for the low-lymphocyte-count group (lymphocyte count$\leq$ 1900 mm^{-3}; n = 110), which was significantly lower than 87.7% for the high-lymphocyte-count group (lymphocyte count > 1900 mm^{-3}; n = 127).

A short descriptive title of Table 1.

Table 1. Clinicopathological characteristics of patients

Legends of Table 1

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; Lob, lobectomy or bilobectomy; Seg, segmentectomy; Wedge, wedge resection; Ad, adenocarcinoma; Sq, squamous cell carcinoma; Large, large cell carcinoma.

A short descriptive title of Table 2

Table 2. Relationship between preoperative peripheral neutrophil counts and clinicopathological factors

Legends of Table 2

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; COPD, chronic obstructive pulmonary disease; Lob, lobectomy or bilobectomy; Seg, segmentectomy; Wedge, wedge resection; Ad, adenocarcinoma; Sq, squamous cell carcinoma; Large, large cell carcinoma.

A short descriptive title of Table 3.

Table 3. Relationship between preoperative peripheral lymphocyte counts and clinicopathological factors

Legends of Table 3

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; COPD, chronic obstructive pulmonary disease; Lob, lobectomy or bilobectomy; Seg, segmentectomy; Wedge, wedge resection; Ad, adenocarcinoma; Sq, squamous cell carcinoma; Large, large cell carcinoma.

A short descriptive title of Table 4.

Table 4. Univariate and multivariate analyses of prognostic factors in node-negative NSCLC.

Legends of Table 4

CI, Confidence interval.

Figure 1A

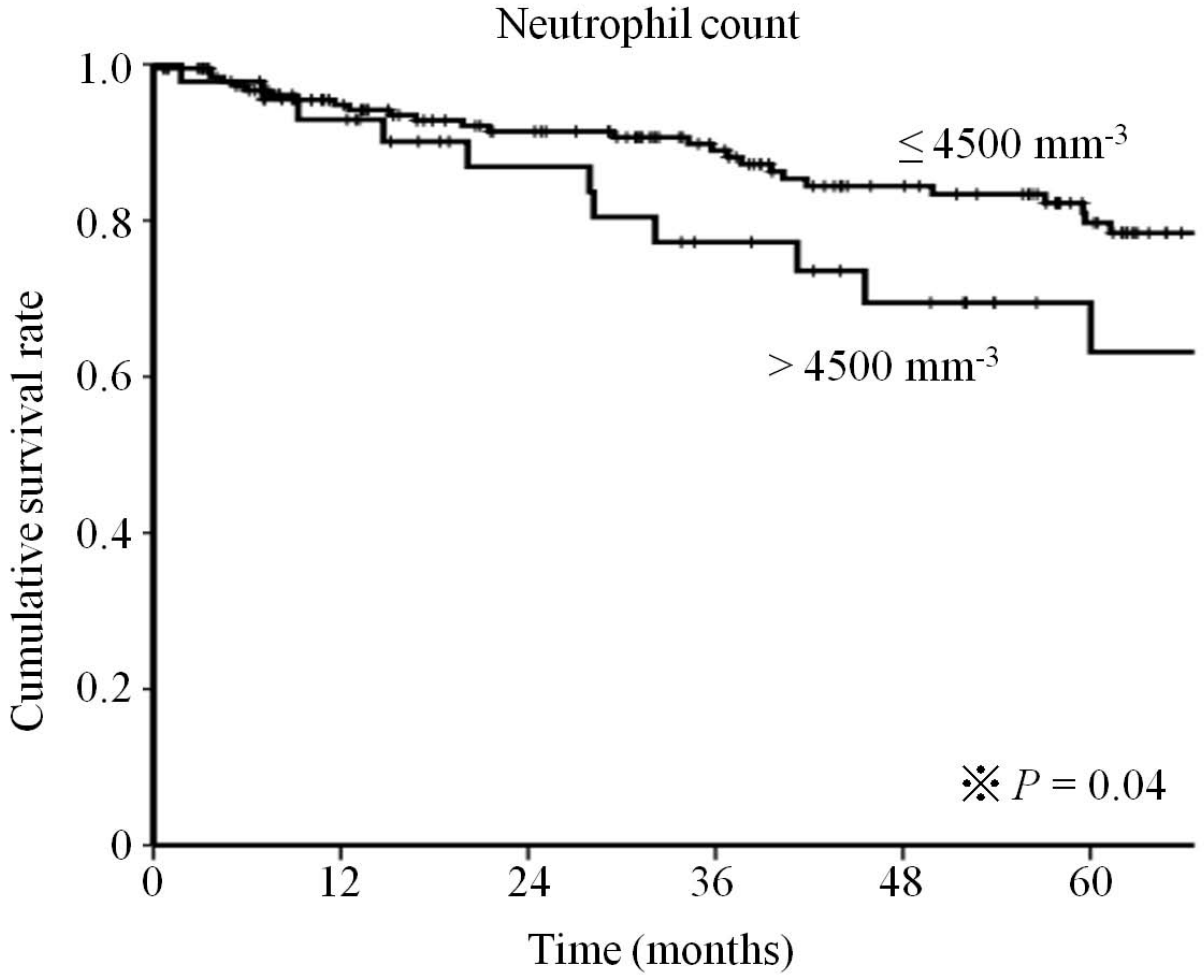
Neutrophil count
1.0
0.8
0.6
0.4
0.2
0
Cumulative survival rate
≤ 4500 mm-3
> 4500 mm-3
※ P = 0.04
0
12
24
36
48
60
Time (months)

Figure 1B

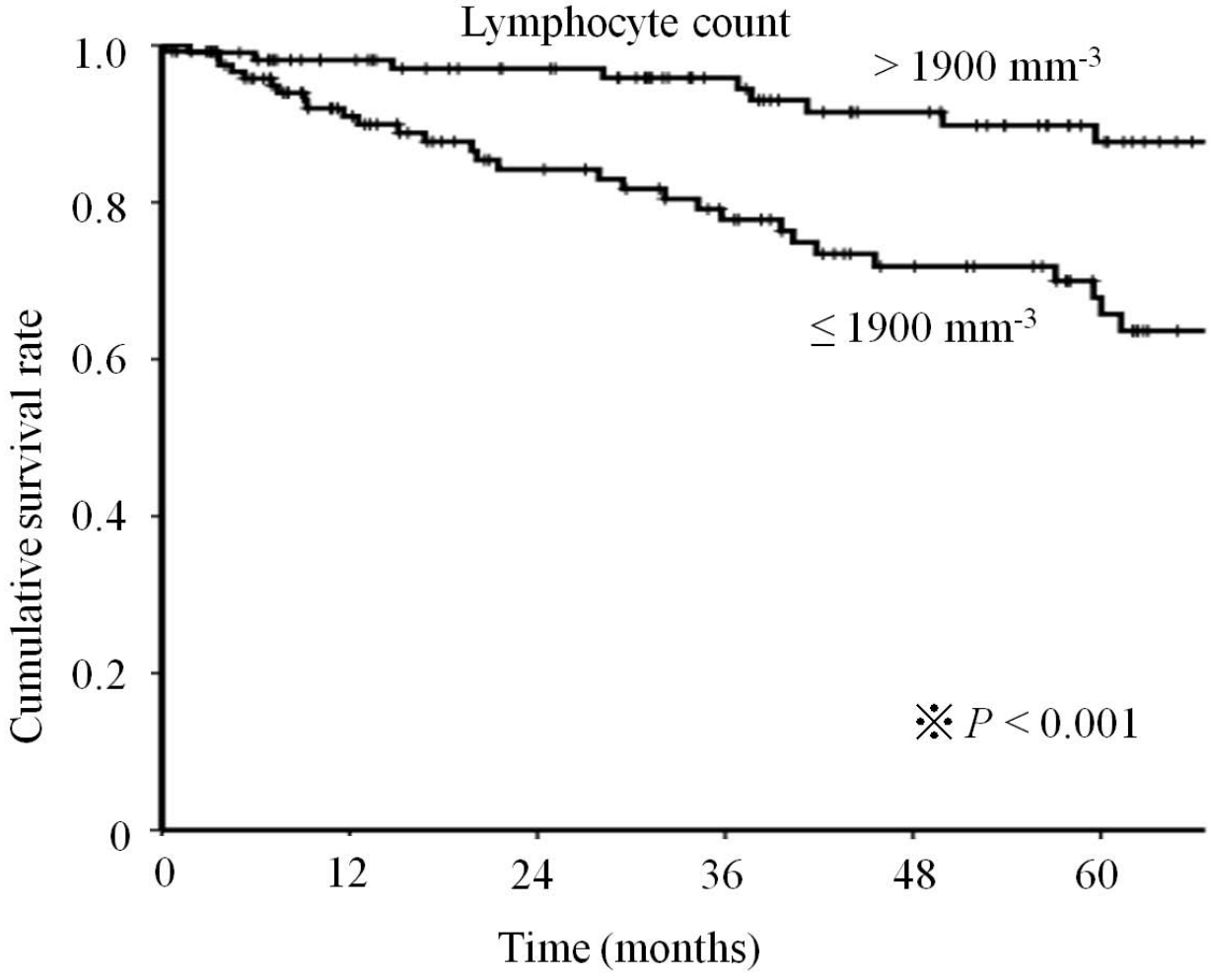
Lymphocyte count
> 1900 mm-3
≤ 1900 mm-3
※ P < 0.001
Cumulative survival rate
1.0
0.8
0.6
0.4
0.2
0
0
12
24
36
48
60
Time (months)

Table 1. Clinicopathological characteristics of patients

	Total n = 237
Age (mean ± SD), years	66.9 ± 10.1
Sex (male / female)	147 / 89
ECOG performance status (0 / 1 / 2)	173 / 15 / 1
Smoking index (mean ± SD)	733 ± 776
Resected side (right / left)	137 / 100
Surgical procedure (Pneumonectomy / Lob / Seg / Wedge)	1 / 189 / 24 / 23
Histological type (Ad / Sq / Large / others)	159 / 53 / 12 / 13
Pathological staging (I / II / III)	204 / 32 / 1
Tumor status (T1 / T2 / T3 / T4)	136 / 80 / 20 / 1
Tumor size (mean ± SD), mm	26.8 ± 17.2
Pleural invasion (positive / negative)	59 / 178
Vascular invasion (positive / negative)	78 / 157
Lymphatic invasion (positive / negative)	58 / 177
Leukocyte counts (mean ± SD), mm^{-3}	6300 ± 1800
Neutrophil counts (mean ± SD), mm^{-3}	3700 ± 1400
Lymphocyte counts (mean ± SD), mm^{-3}	1900 ± 710
Albumin (mean ± SD), g/dL	4.0 ± 0.4
C-reactive protein (mean ± SD), mg/dL	0.45 ± 1.63
Postoperative adjuvant Chemotherapy	13
Recurrences of NSCLC	29
Cause of death (lung cancer / others / unknown)	21 / 20 / 2

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; Lob, lobectomy or bilobectomy; Seg, segmentectomy; Wedge, wedge resection; Ad, adenocarcinoma; Sq, squamous cell carcinoma; Large,

Table 2. Relationship between preoperative peripheral neutrophil counts and clinocopathological factors

	Neutrophil Counts		
	≤ 4500 mm^{-3} n = 191	> 4500 mm^{-3} n = 46	*P* value
Age (mean ± SD), years	66.5 ± 9.8	67.1 ± 10.3	0.722
Sex (male / female)	114 / 76	33 / 13	0.140
ECOG performance status (0 / 1 / 2)	144 / 12 / 1	30 / 3 / 0	0.867
Smoking index (mean ± SD)	692 ± 788	908 ± 702	0.089
Comorbidity and past history			
COPD	8	5	0.083
Interstitial pneumonia	5	3	0.187
Old pulmonary tuberculosis	10	2	0.578
Asthma	10	1	0.334
Hypertension	72	15	0.52
Diabetes mellitus	21	4	0.443
Hyperlipidemia	26	5	0.62
Ischemic heart disease	13	2	0.414
Arrhythmia	15	3	0.524
Surgical procedure (Pneumonectomy / Lob / Seg / Wedge)	0 / 151 / 20 / 20	1 / 38 / 4 / 3	0.178
Histological type (Ad / Sq / Large / others)	132 / 39 / 8 / 12	27 / 14 / 4 / 1	0.180
Pathological staging (I / II / III)	169 / 21 / 1	35 / 11 / 0	0.064
Tumor status (T1 / T2 / T3 / T4)	119 / 60 / 11 /	17 / 20 / 9 / 0	0.002
Tumor size (mean ± SD), mm	25.1 ± 15.2	33.9 ± 22.8	0.002
Pleural invasion (positive / negative)	38 / 153	21 / 25	<0.001
Vascular invasion (positive / negative)	58 / 132	20 / 25	0.075
Lymphatic invasion (positive / negative)	45 / 145	13 / 32	0.467
Leukocyte counts (mean ± SD), mm^{-3}	5600 ± 1200	8900 ± 1600	<0.001
Lymphocyte counts (mean ± SD), mm^{-3}	1800 ± 700	2100 ± 800	0.026
Albumin (mean ± SD), g/dL	4.0 ± 0.4	4.0 ± 0.4	0.697
C-reactive protein (mean ± SD), mg/dL	0.22 ± 0.42	1.44 ± 3.48	<0.001
Postoperative adjuvant Chemotherapy	11	2	0.521
Recurrences of NSCLC	23	6	0.852
Cause of death (lung cancer / others / unknown)	15 / 14 / 1	6 / 6 / 1	0.819

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; COPD, chronic obstructive pulmonary disease; Lob, lobectomy or bilobectomy; Seg, segmentectomy; Wedge, wedge resection; Ad, adenocarcinoma; Sq, squamous cell carcinoma; Large, large cell carcinoma.

Table 3. Relationship between preoperative peripheral lymphocyte counts and clinocopathological factors

	Lymphocyte Counts		
	≤ 1900 mm^{-3} n = 127	> 1900 mm^{-3} n = 110	*P* value
Age (mean ± SD), years	68.1 ± 10.1	66.5 ± 10.1	0.063
Sex (male / female)	75 / 51	72 / 38	0.348
ECOG performance status (0 / 1 / 2)	92 / 10 / 1	82 / 5 / 0	0.385
Smoking index (mean ± SD)	659 ± 761	820 ± 787	0.110
Comorbidity and past history			
COPD	4	9	0.090
Interstitial pneumonia	6	2	0.192
Old pulmonary tuberculosis	6	6	0.798
Asthma	8	3	0.192
Hypertension	48	39	0.709
Diabetes mellitus	9	16	0.062
Hyperlipidemia	12	19	0.075
Ischemic heart disease	9	6	0.607
Arrhythmia	10	8	0.862
Surgical procedure (Pneumonectomy / Lob / Seg / Wedge)	0 / 101 / 12 / 14	1 / 88 / 12 / 9	0.621
Histological type (Ad / Sq / Large / others)	77 / 33 / 7 / 10	82 / 20 / 5 / 3	0.100
Pathological staging (I / II / III)	105 / 22 / 0	99/ 10 / 1	0.106
Tumor status (T1 / T2 / T3 / T4)	65 / 51 / 11 / 0	71 / 29 / 9 /1	0.097
Tumor size (mean ± SD), mm	28.6 ± 16.8	24.9 ± 17.6	0.098
Pleural invasion (positive / negative)	36 / 91	23 / 87	0.402
Vascular invasion (positive / negative)	50 / 75	28 / 82	0.018
Lymphatic invasion (positive / negative)	35 / 90	23 / 87	0.208
Leukocyte counts (mean ± SD), mm^{-3}	5600 ± 1500	7100 ± 1800	<0.001
Neutrophil counts (mean ± SD), mm^{-3}	3500 ± 1300	3800 ± 1600	0.080
Albumin (mean ± SD), g/dL	4.0 ± 0.4	4.0 ± 0.3	0.307
C-reactive protein (mean ± SD), mg/dL	0.46 ± 1.92	0.44 ± 1.22	0.905
Postoperative adjuvant Chemotherapy	7	6	0.985
Recurrences of NSCLC	22	7	0.010
Cause of death (lung cancer / others / unknown)	17 / 14 / 1	4 / 6 / 1	0.521

SD, standard deviation; ECOG, Eastern Cooperative Oncology Group; COPD, chronic obstructive pulmonary disease; Lob, lobectomy or bilobectomy; Seg, segmentectomy; Wedge, wedge resection; Ad, adenocarcinoma; Sq, squamous

Table 4. Univariate and multivariate analyses of prognositic factors in node-negative NSCLC.

Variable		n	Univariate analysis 5-year survival rate	*P* -value	Multivariate analysis Adjusted hazard ratio (95% CI)	*P* -value
Neutrophil counts	> 4500 mm^{-3}	46	69.5	0.040	1.64 (0.789 - 3.405)	0.185
	≤ 4500 mm^{-3}	191	79.7		1	
Lymphocyte counts	≤ 1900 mm^{-3}	127	67.9	< 0.001	3.84 (1.827 - 8.078)	< 0.001
	> 1900 mm^{-3}	110	87.7		1	
Albumin	≤ 3.5 g/dL	27	59.7	0.020	1.04 (0.455 - 2.398)	0.918
	> 3.5 g/dL	210	80.3		1	
C-reactive protein	> 0.1 mg/dL	101	68.5	0.009	1.84 (0.951 - 3.573)	0.070
	≤ 0.1 mg/dL	130	84.9		1	
Tumor size	> 3.0 cm	71	57.4	< 0.001	3.57 (1.748 - 7.282)	< 0.001
	≤ 3.0 cm	166	87.4		1	
Pleural invasion	positive	59	67.3	0.014	1.010 (0.504 - 2.021)	0.979
	negative	178	81.1		1	
Vascular invasion	positive	78	65.2	< 0.001	1.559 (0.788 - 3.085)	0.202
	negative	157	83.4		1	
Lymphatic invasion	positive	58	63.2	0.004	1.763 (0.933 - 3.333)	0.081
	negative	177	82.6		1	

CI, Confidence interval