学位論文

Prognostic impact of tumor-infiltrating lymphocytes and neutrophils in resected non-small cell lung carcinoma

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Original contribution

Prognostic impact of tumor-infiltrating lymphocytes and neutrophils in resected non-small cell lung carcinoma☆,☆☆

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Summary The prognostic impact of tumor-infiltrating lymphocytes (TILs) has been determined in non-small cell lung carcinoma; however, there is no standardized method for counting TILs. In this report, we applied the method proposed by the International Immuno-Oncology Biomarkers Working Group for the assessment of TILs to count the number of tumor-infiltrating neutrophils (TINs). We then analyzed the association between TIL counts, TIN counts, and clinicopathological factors in lung cancer. We retrospectively analyzed a series of 1191 Japanese patients with resected lung adenocarcinoma and squamous cell carcinoma, which were restaged according to the eighth edition of the TNM staging system. Tumors were classified according to the 2015 WHO classification of lung carcinoma. Recurrence-free probability (RFP) and overall survival (OS) were analyzed using the log-rank test and Cox proportional hazard model. Using multivariate analysis for patient outcome in patients with adenocarcinoma, high TIN counts were an independent prognostic factor for worse RFP (hazard ratio [HR]: 1.94, $p < 0.001$) and worse OS (hazard ratio [HR]: 1.75, $p = 0.006$). On the other hand, TIL counts were not related to patient outcome. We have demonstrated that high TINs are unfavorable prognostic markers for resected lung adenocarcinoma. In resected lung squamous cell carcinoma, TIL and TIN counts were not related to patient prognosis. It has been suggested that the immune response to cancer cells may differ depending on the

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histological type. An understanding of how neutrophils are programmed to perform protumor activities is necessary for the future design of targeted immunotherapies.

1. Introduction

Lung cancer is one of the main causes of cancer-related deaths [1]. Recently, because of immunotherapy, there has been a paradigm shift in lung cancer treatment. However, there is no established method for predicting the effects of immunotherapy. Analysis of the cancer immune microenvironment is important in considering cancer immunotherapy [2]. Reportedly, studies involving various tumors have demonstrated that the proportions of tumor-infiltrating lymphocytes (TIL) are related to patient prognosis [3–6]. Many studies have attempted to establish the prognostic significance of TIL in lung cancer [7–11]; however, there is no established measurement method for TIL. Recently, the International Immuno-Oncology Biomarker Working Group proposed a standardized methodology to assess TIL in solid tumors using hematoxylin and eosin (H&E)-stained sections [12–14]. This method was recommended in the WHO classification of breast tumors [15]. Furthermore, in several recent studies, the usefulness of the methodology has been described [9,16,17]; therefore, we also examined TIL using this method.

The number of neutrophils in peripheral blood is related to patient outcome [18]; however, studies using pathological specimen preparation are scarce [19–22]. In various cancers, including non-small cell lung cancer (NSCLC), the neutrophil-to-lymphocyte ratio has been widely studied as a prognostic factor [23–25]. Although lymphocytes are known to be essential in tumor defense and are associated with a favorable prognosis, neutrophil recruitment into the tumor stroma can lead to tumorigenic effects through the inhibition of apoptosis and promotion of metastasis and angiogenesis [26].

Accumulating bodies of evidence have demonstrated the role of tumor-infiltrating neutrophils (TIN) on carcinogenesis and tumor progression. However, the effects of TIN on patient prognosis is multifarious. For example, Ilie et al. indicated that a high $CD66b^+$ neutrophil-to-$CD8^+$ lymphocyte ratio is a novel independent prognostic factor for a high rate of disease recurrence and poor overall survival (OS) in patients with resectable NSCLC [20]. In contrast, Carus et al. reported that the densities of tumor nest containing $CD66b^+$ neutrophils and $CD163^+$ macrophages were not significantly correlated with recurrence-free probability (RFP) or OS [21].

In this study, we analyzed Japanese patients with therapy-naive lung adenocarcinoma and squamous cell carcinoma who underwent surgery to investigate the correlation between TIL, TIN, and clinicopathological factors in lung cancer.

2. Materials and methods

2.1. Patients

This retrospective study was approved by the Institutional Review Board of Kagawa University. We reviewed patients with therapy-naive lung adenocarcinoma and squamous cell carcinoma who underwent lobectomy or more and limited resection at Kagawa University between April 1, 1999, and December 31, 2016. Patients with multifocal invasive carcinoma, surgical margins positive, and stage IIIB-IV NSCLC were excluded from the study. The final cohort comprised 1191 patients.

Clinical data were collected from a prospectively maintained lung carcinoma database. Disease recurrence was confirmed by clinical, radiological, and pathological assessments. The TNM stage was assigned based on the 8th edition of the American Joint Committee on Cancer TNM Staging Manual [27].

2.2. Histologic evaluation

Two pathologists unaware of the clinical outcomes of the patients reviewed the H&E-stained slides independently. An Olympus BX53 upright microscope (Olympus Corporation, Japan) with a standard 22 mm diameter eyepiece was used to examine the slides. Tumors were classified according to the 2015 WHO classification of lung carcinomas [28]. The presence of lymphatic or vascular invasion was defined by at least one cell cluster in the lymphatic vessel or vein. All slides prepared at the time of diagnosis were reviewed, and one representative slide with the largest tumor area, less necrosis, and histological type consistent with the final diagnosis was selected and evaluated. We applied the methodology proposed by the International Immuno-Oncology Biomarker Working Group for TIL assessment in lung cancer [12,13]. Briefly, the entire slide was scanned at low power fields (×4 to ×10 magnification), and the area of the infiltrating lymphocyte ratio in the tumor stroma as a percentage was evaluated. The TIL, including tertiary lymphoid structures, was also evaluated. The level of TIL was calculated and compared to the percentage of stroma in the tumor area and was measured as a percentage in 10% increments. In the case of TIL levels of less than 10%, 1% or 5% criteria were applied (Fig. 1). For the counting of TIN, the whole tumor was screened at low power fields (×10 magnification), and the mean number of infiltrating neutrophils was measured in

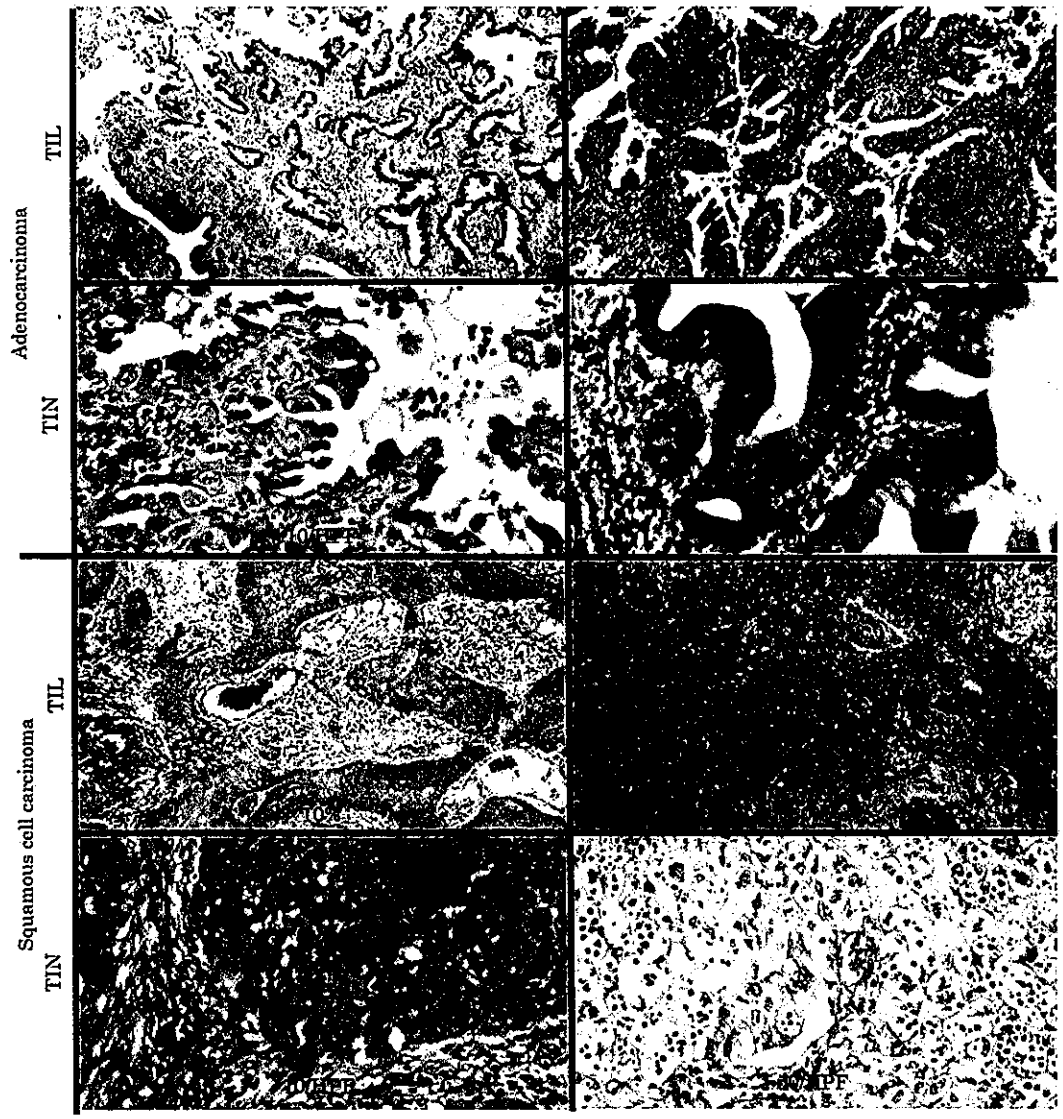

Fig. 1 Representative histopathological image of tumor-infiltrating lymphocytes (TIL) (hematoxylin and eosin (H&E) slides ×10) and tumor-infiltrating neutrophils (TIN) (H&E slides ×40). The upper row represents adenocarcinoma images, and the lower row represents squamous cell carcinoma images.

three hot spots at high power field (×40 magnification; one high power field is equivalent to 0.237 mm^2). A hot spot represents a field of vision with many neutrophils. Necrosis and abscess (cluster of more than 10 neutrophils), blood vessels, and intra-alveolar neutrophils were excluded from the measurement (Fig. 1).

2.3. Statistical analysis

The associations between variables were analyzed using the *chi*-squared test for categorical variables. RFP was defined as the time from surgical resection to the date of disease recurrence. Among the RFP analyses, the deaths before recurrence were treated as censored. OS was defined as the time from surgical resection to the date of death or the last follow-up. RFP and OS were estimated using the Kaplan–Meier (KM) method, and nonparametric group comparisons were performed using the log-rank test. Multivariate analyses were performed using the Cox proportional hazards regression model. Multivariate models were built to include factors that were significant in univariate analysis. Associations between pathological factors were checked. In the case of a strong association between different factors, only one factor was included in the model. All statistical tests were two-sided, and a 5% significance level was used. Statistical analyses were conducted using IBM SPSS Statistics for Windows (version 23.0; IBM Corporation, Armonk, NY, USA).

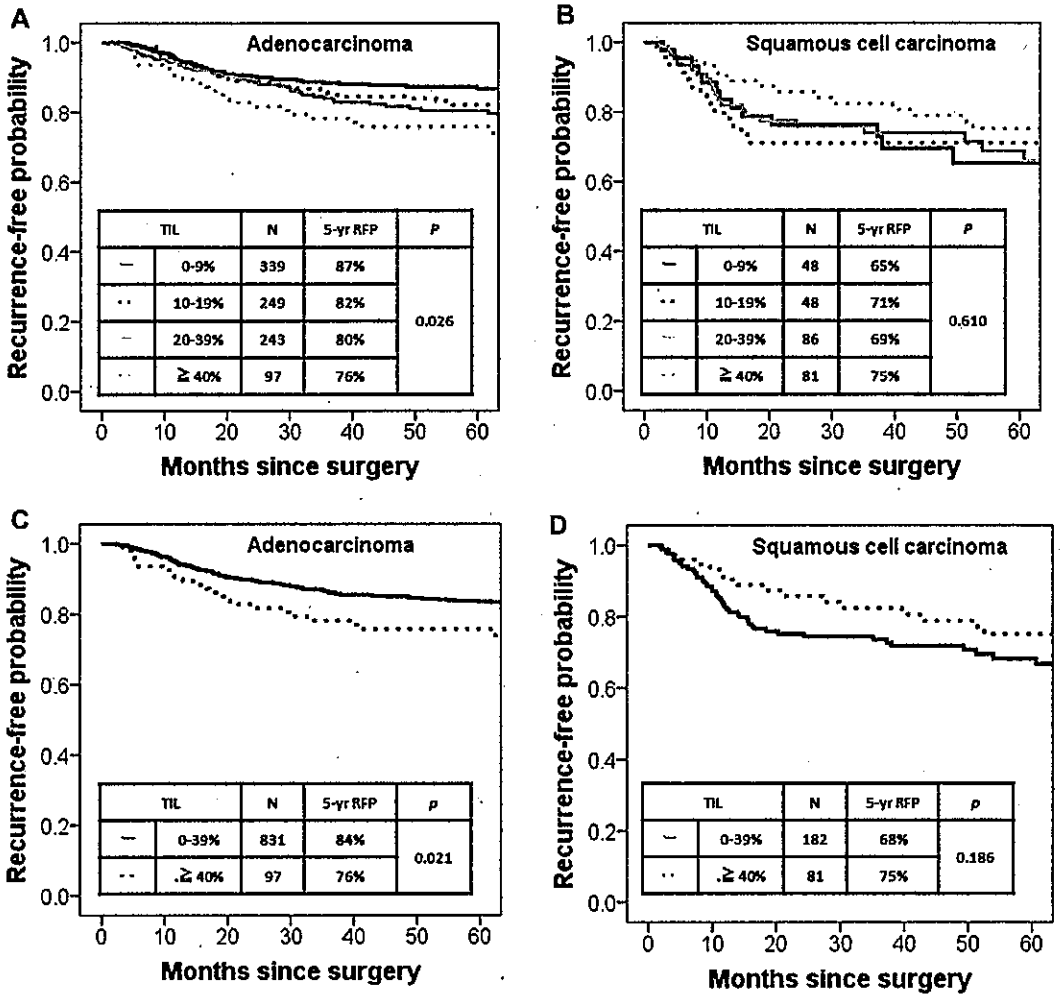

Fig. 2 Tumor-infiltrating lymphocytes (TIL) counts and recurrence-free probability (RFP) according to histological subtypes. The cut-off was determined by dividing the patients based on the TIL counts into four groups (0%–9%, 10%–19%, 20%–39%, ≥40%), and the 5-year RFP was estimated in adenocarcinoma (A), and squamous cell carcinoma (B), using Kaplan–Meier method. The 5-year RFP for patients with (C), adenocarcinoma and (D), squamous cell carcinoma with low (0%–39%) and high TIL counts. A p-value of 0.05 is considered significant.

3. Results

3.1. Clinicopathological characteristics of patients

The median age of the 1191 patients was 69 years (range, 26–92 years). More than half of the patients were men (n = 699). A total of 928 patients had adenocarcinoma, and 263 patients had squamous cell carcinoma. The pathologic stage 0-I was predominant in most patients (n = 943). A total of 886 patients underwent a lobectomy or more, and 305 underwent limited resection (segmentectomy or wedge resection). During the study period, 226 patients experienced recurrence, and 260 died. The median follow-up period for patients alive at the last follow-up was 60 months (mean ± SD, 63 ± 39 months).

3.2. Association between the TIL counts and patient outcome

Patients with adenocarcinoma had significantly lower TIL levels (mean ± SD, 16 ± 15%; median, 10%) than those with squamous cell carcinoma (mean ± SD, 26 ± 18%; median 20%) ($p < 0.001$). To determine the cut-off of TIL, we divided the patients into four groups of 0–9%, 10–19%, 20–39%, and 40% or more, and examined 5-year RFP for adenocarcinoma and squamous cell carcinoma using the KM method (Fig. 2A and B). When the cut-off was set to 40%, the 5-year RFP for patients with adenocarcinoma with high TIL count was lower than that for those with low TIL count (5-year RFP, high TIL count: 76% and low TIL count: 84%; p = 0.021) (Fig. 2C).

Patients with squamous cell carcinoma, there is no correlation between TIL counts and 5-year RFP (5-year RFP, high TIL count: 75% and low TIL count: 68%; p = 0.186) (Fig. 2D). The statistical correlation was not significant for the relationship between TIL counts and OS for both adenocarcinoma and squamous cell carcinoma.

3.3. Association between the TIN counts and patient outcome

The median TIN counts were 0/high power field (HPF) (mean ± SD, 5 ± 17/HPF) and 4/HPF (mean ± SD, 20 ± 34/HPF) for patients with adenocarcinoma and squamous cell carcinoma, respectively. These results indicated a lower neutrophil infiltration in patients with adenocarcinoma than that in patients with squamous cell carcinoma ($p < 0.001$).

To determine the cut-off value of TIN, the patients were divided into four groups based on their TIN counts: 0–4/HPF, 5–9/HPF, 10–19/HPF, 20 or more/HPF, and examined the 5-year RFP for patients with adenocarcinoma and squamous cell carcinoma using the KM method (Fig. 3A and B). When the cut-off was set to 10/HPF, the 5-year RFP for patients with adenocarcinoma with high TIN counts was

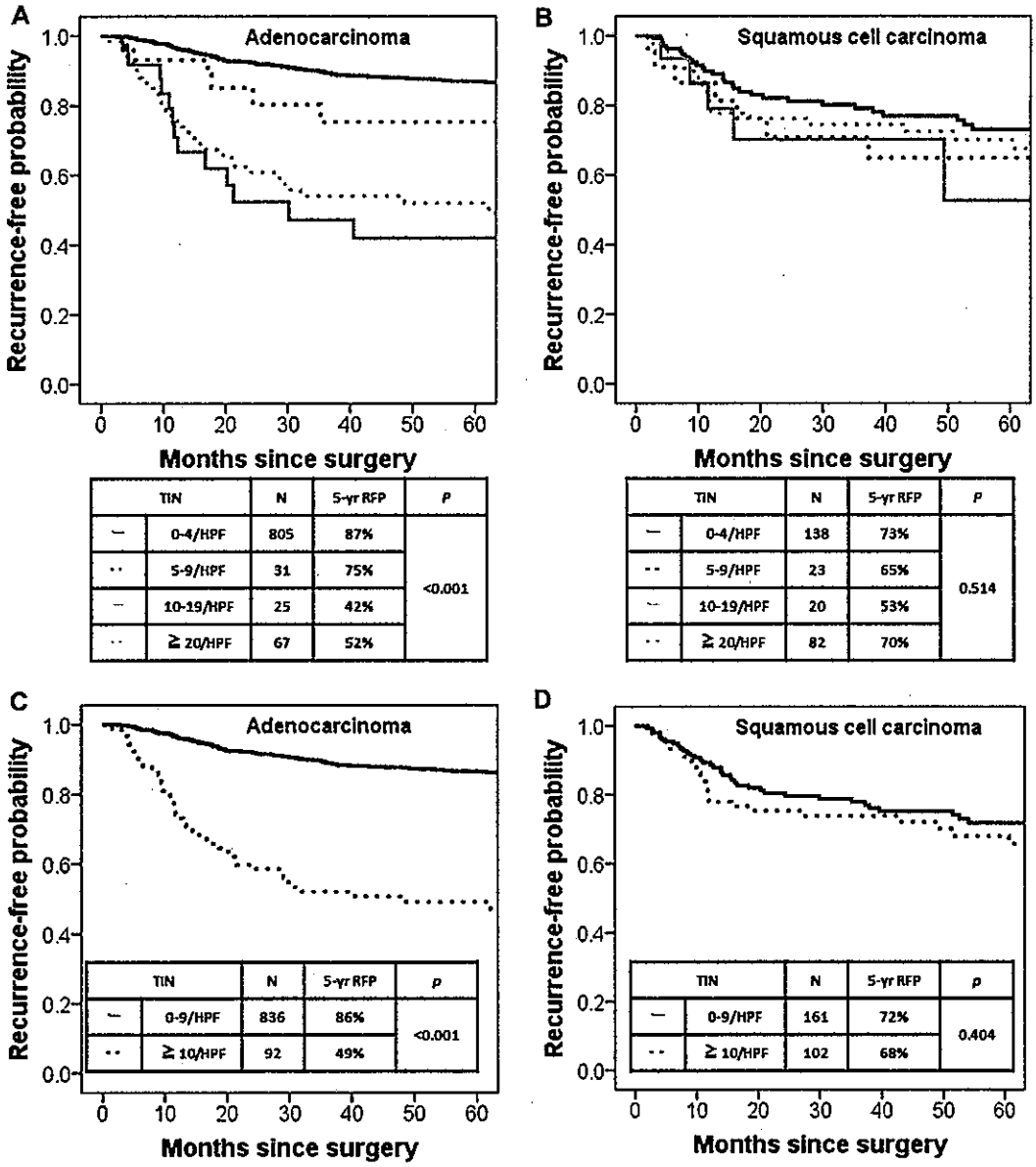

Fig. 3 Tumor-infiltrating neutrophils (TIN) counts and recurrence-free probability (RFP) according to histological subtypes. The cut-off was determined by dividing the patients based on the TIN counts into four groups (0–4/HPF, 5–9/HPF, 10–19/HPF, ≥20/HPF), and the 5-year RFP was estimated in adenocarcinoma (A), and squamous cell carcinoma (B), using Kaplan–Meier method (C) The 5-year RFP for patients with (C) adenocarcinoma and (D) squamous cell carcinoma with low (0–39/HPF) and high (≥10/HPF) TIN counts. A p-value of 0.05 is considered significant.

lower than that for those with low TIN counts (5-year RFP, high TIN count: 49% and low TIN count: 86%; $p < 0.001$) (Fig. 3C). The statistical correlation was not significant for the association between TIN counts and 5-year RFP in squamous cell carcinoma (Fig. 3D). OS for patients with adenocarcinoma with high TIN counts was lower than that for those with low TIN counts (OS, high TIN counts: 63% and low TIN counts: 88%; $p < 0.001$). The statistical correlation was not significant for the association between TIN counts and OS in squamous cell carcinoma.

3.4. Association between the TIL counts, TIN counts, and clinicopathologic features

In patients with adenocarcinoma, TIL counts were associated with sex ($p = 0.007$), pathological stage ($p = 0.001$), lymphovascular invasion ($p < 0.001$), histological subtypes ($p < 0.001$), TIN counts ($p = 0.003$), abscess ($p < 0.001$), and necrosis ($p = 0.003$). TIN counts were correlated with sex ($p < 0.001$), pathological stage ($p < 0.001$), lymphovascular invasion ($p < 0.001$), histological subtypes ($p < 0.001$), TIL counts ($p = 0.003$), abscess ($p < 0.001$), necrosis ($p < 0.001$), and spread through air spaces (STAS) ($p < 0.001$) (Table 1).

In squamous cell carcinoma, TIL counts were related to TIN counts ($p = 0.019$) and abscesses ($p = 0.004$). TIN counts were correlated with TIL counts ($p = 0.019$) and abscesses ($p < 0.001$) (Table 2).

3.5. Multivariate analysis of the patient outcome

In multivariate analysis for patients with adenocarcinoma, pathological stage (hazard ratio (HR) = 2.22, $p < 0.001$), histological subtype (HR = 0.18, $p < 0.001$), lymphovascular invasion (HR = 2.02, $p = 0.001$), TIN counts (HR = 1.94, $p < 0.001$), and STAS (HR = 2.83,

Table 1 Associations between tumor-infiltrating lymphocytes, neutrophils and clinicopathological factors in adenocarcinoma.

	TIL (cut off 40%)				*p*	TIN (cut off 10/HPF)				*p*	Total	
	low	(%)	high	(%)		low	(%)	high	(%)		N	(%)
Age, years					0.854					0.121		
≦65	325	(90)	37	(10)		333	(92)	29	(8)		362	(39)
>65	506	(89)	60	(11)		503	(89)	63	(11)		566	(61)
Sex					**0.007**					**<0.001**		
Female	429	(92)	36	(8)		440	(95)	25	(5)		465	(50)
Male	402	(87)	61	(13)		396	(86)	67	(14)		463	(50)
Pathological stage					**0.001**					**<0.001**		
Stage 0-	714	(91)	71	(9)		732	(93)	53	(7)		785	(85)
Stage -A	117	(82)	26	(18)		104	(73)	39	(27)		143	(15)
Lymphovascular invasion					**<0.001**					**<0.001**		
Absent	600	(93)	46	(7)		618	(96)	28	(4)		646	(70)
Present	231	(82)	51	(18)		218	(77)	64	(23)		282	(30)
Histological subtypes					**<0.001**					**<0.001**		
Acinar/Papillary	341	(88)	48	(12)		344	(88)	45	(12)		389	(42)
AIS/MIA/Lepidic	368	(95)	20	(5)		386	(99)	2	(1)		388	(42)
Solid/Micropapillary	68	(74)	24	(26)		65	(71)	27	(29)		92	(10)
Mucinous/Colloid	54	(92)	5	(8)		41	(70)	18	(30)		59	(6)
TIN(cut off 10/HPF)					**0.003**							
Low	757	(91)	79	(9)							836	(90)
High	74	(80)	18	(20)							92	(10)
TIL (cut off 40%)										**0.003**		
Low						757	(91)	74	(9)		831	(90)
High						79	(81)	18	(19)		97	(10)
Abscess					**<0.001**					**<0.001**		
Absent	741	(92)	65	(8)		778	(97)	28	(3)		806	(87)
Present	90	(74)	32	(26)		58	(48)	64	(52)		122	(13)
Necrosis					**0.003**					**<0.001**		
Absent	750	(91)	78	(9)		771	(93)	57	(7)		828	(89)
Present	81	(81)	19	(19)		65	(65)	35	(35)		100	(11)
STAS					0.075					**<0.001**		
Absent	587	(91)	60	(9)		614	(95)	33	(5)		647	(70)
Present	244	(87)	37	(13)		222	(79)	59	(21)		281	(30)

Significant *p*-values are shown in bold.

AIS, adenocarcinoma in situ; MIA, minimally invasive adenocarcinoma; STSA, spread through air spaces; TIL, tumor infiltrating lymphocytes; TIN, tumor infiltrating neutrophils.

Table 2 Associations between tumor-infiltrating lymphocytes, neutrophils, and clinicopathological factors in squamous cell carcinoma.

	TIL (cut off 40%)				*p*	TIN (cut off 10/HPF)				*p*	Total	
	low	(%)	high	(%)		low	(%)	high	(%)		N	(%)
Age, years					0.872					0.299		
≦65	30	(68)	14	(32)		30	(68)	14	(32)		44	(17)
>65	152	(70)	67	(30)		131	(60)	88	(40)		219	(83)
Sex					0.058					0.292		
Female	23	(85)	4	(15)		14	(52)	13	(48)		27	(10)
Male	159	(67)	77	(33)		147	(62)	89	(38)		236	(90)
Pathological stage					0.145					0.336		
Stage	104	(66)	54	(34)		93	(59)	65	(41)		158	(60)
Stage -A	78	(74)	27	(26)		68	(65)	37	(35)		105	(40)
Histological subtype					0.641					0.092		
Keratinizing	124	(71)	51	(29)		113	(65)	62	(35)		175	(67)
Non-keratinizing	46	(65)	25	(35)		36	(51)	35	(49)		71	(27)
Basaloid	12	(71)	5	(29)		12	(71)	5	(29)		17	(6)
Lymphovascular invasion					0.475					0.753		
Absent	79	(67)	39	(33)		71	(60)	47	(40)		118	(45)
Present	103	(71)	42	(29)		90	(62)	55	(38)		145	(55)
TIN (cut off 10/HPF)					**0.019**							
Low	120	(75)	41	(25)								
High	62	(61)	40	(39)								
TIL (cut off 40%)										**0.019**		
Low						120	(66)	62	(34)		182	(69)
High						41	(51)	40	(49)		81	(31)
Abscess					**0.004**					**<0.001**		
Absent	79	(80)	20	(20)		89	(90)	10	(10)		99	(38)
Present	103	(63)	61	(37)		72	(44)	92	(56)		164	(62)
Necrosis					0.776					0.053		
Absent	73	(68)	34	(32)		58	(54)	49	(46)		107	(41)
Present	109	(70)	47	(30)		103	(66)	53	(34)		156	(59)
STAS					0.082					0.274		
Absent	110	(66)	58	(34)		107	(64)	61	(36)		168	(64)
Present	72	(76)	23	(24)		54	(57)	41	(43)		95	(36)

Significant p-values are shown in bold.
STSA, spread through air spaces; TIL, tumor infiltrating lymphocytes; TIN, tumor infiltrating neutrophils.

$p < 0.001$) were correlated with 5-year RFP. Age (HR = 2.37, $p < 0.001$), sex (HR = 1.51, $p = 0.023$), pathological stage (HR = 1.92, $p = 0.001$), lymphovascular invasion (HR = 2.24, $p < 0.001$), TIN counts (HR = 1.75, $p = 0.006$), and STAS (HR = 2.14, $p < 0.001$) were correlated with 5-year OS (Table 3). In multivariate analysis for squamous cell carcinoma, TIL counts and TIN counts were not related to patient outcomes.

4. Discussion

In this study, we performed a series of H&E-stained slide analyses of TIL and TIN counts to identify their prognostic significance. We examined a large cohort of patients with therapy-naive lung adenocarcinoma and squamous cell carcinoma who had undergone a lobectomy or more and limited resection at a single institution. In patients with adenocarcinoma, high TIN counts were correlated with worse 5-year RFP and OS, whereas high TIL counts were correlated with worse 5-year RFP but not with OS. On the contrary, in patients with squamous cell carcinoma, TIL and TIN counts were not statistically correlated with patient outcomes.

Several reports suggest that TIL counts are related to the outcome of patients with lung cancer. For instance, Horne et al. analyzed 273 cases of stage 1A NSCLC using H&E staining and reported that high levels of TIL are associated with improved recurrence-free survival [7]. Kilic et al. also used H&E staining to investigate 219 cases of stage 1A-1B NSCLC and reported that a high density of TIL was associated with lower disease recurrence and improved 5-year disease-free survival in patients with tumors ≥5 cm in diameter [8]. However, these reports used different methods for estimating TIL counts. In this study, we employed the method proposed by the International

Table 3 Multivariate analysis of recurrence-free probability and overall survival in adenocarcinoma.

Variables		HR	95% CI	p
Recurrence-free probability				
Sex	Male vs Female	0.85	0.61–1.18	0.339
Pathological stage	-A vs 0-	2.22	1.58–3.12	**<0.001**
Histological subtype	AIS/MIA/Lepidic vs Acinar/Papillary	0.18	0.07–0.45	**<0.001**
	Solid/Micropapillary vs Acinar/Papillary	1.40	0.97–2.04	0.075
	Mucinous/Colloid vs Acinar/Papillary	1.01	0.54–1.90	0.98
Lymphovascular invasion	Present vs absent	2.02	1.31–3.11	**0.001**
TIL (cut off 40%)	High vs low	0.97	0.63–1.51	0.905
TIN (cut off 10/HPF)	High vs low	1.94	1.34–2.80	**<0.001**
STAS	Present vs absent	2.83	1.90–4.23	**<0.001**
Overall survival				
Age	>66 vs ≦65	2.37	1.63–3.43	**<0.001**
Sex	Male vs Female	1.51	1.06–2.14	**0.023**
Pathological stage	-A vs 0-	1.92	1.31–2.80	**0.001**
Histological subtype	AIS/MIA/Lepidic vs Acinar/Papillary	0.82	0.46–1.47	0.506
	Solid/Micropapillary vs Acinar/Papillary	1.15	0.76–1.74	0.511
	Mucinous/Colloid vs Acinar/Papillary	0.78	0.38–1.61	0.504
Lymphovascular invasion	Present vs absent	2.24	1.43–3.52	**<0.001**
TIL (cut off 40%)	High vs. low	0.90	0.56–1.46	0.67
TIN (cut off 10/HPF)	High vs. low	1.75	1.18–2.61	**0.006**
STAS	Present vs. absent	2.14	1.43–3.20	**<0.001**

Significant p-values are shown in bold.
AIS, adenocarcinoma in situ; CI, confidence interval; HR, hazard ratio; MIA, minimally invasive adenocarcinoma; STSA, spread through air spaces; TIL, tumor-infiltrating lymphocytes; TIN, tumor-infiltrating neutrophils.

Immuno-Oncology Biomarker Working Group, which is relatively simple and easy. Furthermore, using an internationally standardized method allows a better comparison of the results reported in several studies. In our cohort, low TIL counts were correlated with a good 5-year RFP in patients with adenocarcinoma. However, using the same method, Kim et al. [9] reported that high TIL levels were a good independent prognostic factor for progression-free survival (PFS) in a total cohort of 146 patients with lung adenocarcinoma. The disparities between our study and that of Kim et al. could be due to the difference in cell fractionation of TIL and interaction with other immune cells. Although our study suggests that TIL is a useful index for predicting the prognosis and the effect of immunotherapy, more case studies are necessary to evaluate TIL counts and patient outcomes in lung carcinoma, with standardized guidelines for counting TIL.

In the last two decades, researchers have demonstrated that inflammatory immune cells are essential players in cancer-related inflammation. While chronic inflammation has a significant effect on cancer, the effect of acute inflammation on tumor progression has not been well studied. Neutrophils are among the first immune cells to be recruited to damaged tissue; therefore, they are recognized as key players during acute inflammation [26]. Teramukai et al. reported that elevated neutrophil counts in peripheral blood were associated with short overall and PFS in 388 chemo-naïve patients with stage IIIB or IV NSCLC (18), but there are few reports using pathological specimen preparation. In this study, evaluation of the TIN counts using H&E-stained slides and their association with clinicopathological factors revealed that high TIN counts were correlated with worse 5-year RFP and OS in patients with adenocarcinoma.

Fridlender et al. indicated that tumor-associated neutrophils (TAN) also have differential states, such as tumor-associated macrophages (TAM) [29]. Since there are no definitive markers yet, they suggested a classification scheme for TAN similar to that of TAM, an anti-tumorigenic (N1) phenotype versus a pro-tumorigenic (N2) phenotype. N1 TAN expressing immune-activating chemokines and cytokines and lower levels of arginase are capable of killing tumor cells. However, most TAN appear to have an N2 phenotype and thus contribute to tumor growth and immunosuppression. Therefore, depletion of N2 neutrophils is expected to inhibit tumor growth. Neutrophils infiltrate the lungs in response to the secretion of IL-7 by γδ T cells, and support the survival and proliferation of disseminated cancer cells by suppressing effector $CD8^+$ T cells. These results demonstrate that the protumor and anti-tumor functions of TAN depend on the immune microenvironment [26]. Considering these

findings, we hypothesize that neutrophils with N2 phenotype infiltrate lung adenocarcinoma, leading to a worse prognosis. The development of markers to identify these two populations is expected to clarify the role of neutrophils in cancer immunity.

Our study has some limitations. First, interobserver variation may occur while counting TIL and TIN. It is important to use a standardized measurement method such as the International Immuno-Oncology Biomarker Working Group proposed method used in this report. However, digital image analyses (30) using image analysis software or AI should be considered in future studies to achieve reproducibility. Second, we only used standard H&E staining instead of immunohistochemistry (IHC) to evaluate TIL and TIN counts. H&E-stained slides are very useful for their ease of protocol and low cost, but additional examination by IHC may be necessary to evaluate the cancer immune microenvironment.

In conclusion, in this single-centered study, the evaluation of TIL and TIN counts in lung cancer based on a series of H&E-stained slide analyses demonstrated that high TIN counts are unfavorable prognostic markers for resected lung adenocarcinoma, wherein the TIL counts are not correlated with patient outcome. On the contrary, in resected lung squamous cell carcinoma, the study revealed no significant association of the TIL and TIN counts with patient prognosis. These findings suggested that the immune response to cancer cells may differ depending on the histological type. Therefore, it is necessary to examine the cancer immune microenvironment for each histological subtype. Furthermore, understanding how neutrophils are programmed to perform protumor activities is necessary for the future design of targeted immunotherapies.

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