The prognostic value of tumor-infiltrating lymphocytes in non-small cell lung cancer patients who received neoadjuvant chemotherapy followed by surgery

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Abstract

Background. The relationship between tumor-infiltrating lymphocyte (TIL) levels and the prognosis of patients with non-small cell lung cancer (NSCLC) who receive neoadjuvant chemotherapy followed by surgery is a problem that requires more research.

Objectives. To evaluate the prognostic value of TIL levels in patients with NSCLC who received neoadjuvant chemotherapy followed by surgery.

Materials and methods. Patients with NSCLC who received neoadjuvant chemotherapy followed by surgery in our hospital from December 2014 to December 2020 were selected for a retrospective analysis. Sections were stained with hematoxylin and eosin (H&E) to evaluate TIL levels in surgically-resected tumor tissues. Patients were divided into TIL– (low-level infiltration) and TIL+ (medium- to high-level infiltration) groups according to the recommended TIL evaluation criteria. Univariate (Kaplan–Meier) and multivariate (Cox) survival analyses were used to analyze the impact of clinicopathological features and TIL levels on prognosis.

Results. The study involved 137 patients, including 45 who were TIL– and 92 who were TIL+. The median overall survival (OS) and disease-free survival (DFS) of the TIL+ group were higher than those of the TIL– group. The univariate analysis showed that smoking, clinical and pathological stages, and TIL levels, were the factors influencing OS and DFS. The multivariate analysis showed that smoking (OS, hazard ratio (HR): 1.881, 95% confidence interval (95% CI): 1.135–3.115, $p = 0.014$; DFS, HR: 1.820, 95% CI: 1.181–2.804, $p = 0.007$) and clinical stage III (DFS, HR: 2.316, 95% CI: 1.350–3.972, $p = 0.002$) were adverse factors affecting the prognosis of patients with NSCLC who received neoadjuvant chemotherapy followed by surgery. At the same time, TIL+ status was an independent factor for a good prognosis in OS (HR: 0.547, 95% CI: 0.335–0.894, $p = 0.016$) and DFS (HR: 0.445, 95% CI: 0.284–0.698, $p = 0.001$).

Conclusions. Medium to high levels of TILs were associated with a good prognosis in NSCLC patients who received neoadjuvant chemotherapy followed by surgery. The levels of TILs are of prognostic value in this population of patients.

Key words: non-small cell lung cancer, overall survival, neoadjuvant chemotherapy, disease-free survival, tumor-infiltrating lymphocyte

Background

Lung cancer is one of the most common malignant tumors in the world and the leading cause of cancer-related death.[1] Non-small cell lung cancer (NSCLC) is the main form of lung cancer. If there are no contraindications and the prognosis is good, tumors in patients with stage I or II disease can be removed surgically.[2] However, due to the low popularity of lung cancer screening, most patients with NSCLC are diagnosed with locally advanced or metastatic disease and lose the opportunity for radical surgery.[3]

Some studies have shown that preoperative neoadjuvant chemotherapy can reduce the staging of some stage III NSCLC patients with surgical indications, which increases the possibility of complete resection of tumor tissue.[4–6] Chemotherapy changes the tumor immune microenvironment. Cancer patients who receive preoperative neoadjuvant chemotherapy usually have a high level of T cells, which participate in the antitumor processes through a specific immune response. Indeed, the immune response has been shown to play an important role in the invasion, progression and metastasis of cancer.[7] Previous studies have shown that the level of tumor-infiltrating lymphocytes (TILs), such as $CD8^+$ T cells, is considered to be an expression of an immune response. Such responses are related to survival rate and response to treatment in patients with a variety of solid tumors, and have an impact on the overall survival (OS) and disease-free survival (DFS) of these patients.[8–11] Recent studies have demonstrated that patients with NSCLC who underwent early surgery had high levels of TILs in tumors, which were associated with an improvement in relapse-free survival rate and a decreased risk of systemic recurrence.[8] Additionally, Gataa et al. reported an association between TILs and the prognosis of patients with advanced NSCLC who were receiving immunotherapy.[12]

Objectives

This study aimed to evaluate the correlation between TIL levels and the prognosis of patients with NSCLC who received neoadjuvant chemotherapy followed by surgery.

Materials and methods

Patients

The clinical and pathological data of patients with NSCLC who underwent surgery after neoadjuvant chemotherapy in the Second Affiliated Hospital of Guizhou Medical University (Guizhou, China) from December 2014 to December 2020 were retrospectively analyzed. The following inclusion criteria were used: 1) patients diagnosed with NSCLC through pathological biopsy before neoadjuvant therapy; 2) patients who had stage I–III NSCLC according to the American Joint Committee on Cancer Eighth Edition TNM Staging Manual Classifications for T2b and T3 NSCLC[13]; 3) patients whose operations were R0 resectioned; and 4) patients whose resected specimens were well preserved and could be stained with hematoxylin and eosin (H&E). The following exclusion criteria were used: 1) patients who received neoadjuvant chemotherapy combined with immunotherapy; 2) patients who received neoadjuvant immunotherapy.

This research complied with the Declaration of Helsinki. The approval was granted by the Ethics Committee of the Second Affiliated Hospital of Guizhou Medical University (approval No. 2020-Ethical Review-03). The risks, benefits and goals of the study were briefly explained to each participant. In addition, all of the individuals gave signed informed consent. A total of 164 patients were included in the study, though 10 samples could not be used. Additionally, there were 8 cases of R1 resection, 4 cases of mixed large and small cell types, and 5 cases lost on follow-up. Therefore, 137 cases were included in the analysis. Age of the patients ranged from 33 to 79 years, with a median age of 62 years. The last follow-up was in January 2022, with a median follow-up of 32 months, a median DFS of 23 months and an unmet median OS. There were 45 patients assessed as TIL– (low-level infiltration) and 92 patients assessed as TIL+ (medium- and high-level infiltration).

Tumor-infiltrating lymphocyte assessment

Two pathologists evaluated the TIL level of hematoxylin and eosin (H&E)-stained postoperative tumor tissue sections. The levels of TIL assessed on each slide were used to divide patients into TIL– and TIL+ groups. It has been reported that low-level infiltration is indicated by a scattered distribution of lymphocytes in the stroma, and high-level infiltration by the presence of a large number of stromal lymphocytes and lymphocytes infiltrating between tumor cells.[8] Medium-level infiltration is indicated by a small number of stromal lymphocytes in the absence of tumor nest infiltration.

Response assessment

The primary endpoints included OS and DFS. Overall survival was defined as the time from diagnosis until death from any cause, and DFS was defined as the time from radical surgery to disease recurrence or death from any cause.

Statistical analyses

The data were statistically analyzed using IBM SPSS v. 22.0 software (IBM Corp., Armonk, USA). The general data were analyzed using descriptive analysis, and the baseline data of patients with various levels of TIL were compared using the χ^2 test. The Kaplan–Meier curves and log-rank tests

were used for the survival analysis. Multivariate Cox proportional hazards (PH) regression modeling was used to analyze the risk factors associated with survival. In Cox regression analysis, the univariate Cox analysis was initially performed on the variables, and then the variables with $p < 0.05$ were further analyzed with multifactor Cox analysis. The survival plot output (log of negative log), in which the abscissa is the log of time and the ordinate is the log of the survival function, was visually inspected. The judgment method was as follows: if the survival curves were roughly parallel, then the PH condition of Cox regression was considered valid; if the curves were not parallel and there was a crossover phenomenon, the PH condition was considered not valid. It was found that the survival curves of all variables were roughly parallel. The evaluation indicators were hazard ratio (HR) and 95% confidence interval (95% CI). The value of $p < 0.05$ was considered statistically significant.

Results

There was a relationship between levels of TILs and clinicopathological features in patients with NSCLC.

Table 1 lists the baseline characteristics of the 137 patients. Forty-five patients were classified as TIL– and the remaining 92 patients were defined as TIL+. Compared with TIL–, more TIL+ patients had a lower pathological stage.

Univariate analysis of OS and DFS in patients with NSCLC

The univariate analysis showed that lower clinical and pathological stages, being TIL+ and never smoking were associated with better OS and DFS, as shown in Table 2 and Fig. 1.

Multivariate analysis of OS and DFS in patients with NSCLC

Figure 2 presents the results of multivariate analysis on the relationship between clinicopathological variables and OS and DFS in patients with NSCLC. In the multivariate survival analysis, being a current or ex-smoker was associated with poor OS and DFS, and clinical stage III was associated with poor DFS. However, TIL+ was a potentially positive prognostic factor for OS and DFS.

Discussion

Previous studies have confirmed that neoadjuvant chemotherapy is beneficial to patients with NSCLC (stage IIIA). To some extent, neoadjuvant chemotherapy can change the tumor immune microenvironment and the local infiltration of tumor cells to effectively achieve pathological decline, improve R0 resection rate, inhibit disease progression, improve patient survival rate, and improve prognosis.[14,15] Mounting evidence shows that there are dynamic and complex interactions between TILs and other immune and tumor cells, and that these interactions closely controls tumor progression.[16]

Tumor-infiltrating lymphocyte level is a prognostic factor in patients with cancer. Indeed, the correlation between TIL levels and prognosis has been demonstrated in various cancers.[8–11] A large-scale study conducted by Feng et al. showed that TIL+ patients had better postoperative survival results compared with TIL– patients with pathological stage IIIA (N2) NSCLC who underwent complete cancer resection (5-year OS of 35.6% compared to 34%).[17] However, there are few reports on the relationship between TILs and the prognosis of patients with NSCLC after neoadjuvant chemotherapy. Therefore, the current

Table 1. Relationship between TIL levels and clinicopathologic features of NSCLC patients evaluated using the χ^2 test

Characteristics		Number of patients	TIL–, n (%)	TIL+, n (%)	χ^2	p-value
All patients		137	45 (32.8)	92 (67.2)	–	–
Gender	male female	86 51	24 (27.9) 21 (41.2)	62 (72.1) 30 (58.8)	2.537	0.111
Age [years]	<60 ≥60	54 83	17 (31.5) 28 (33.7)	37 (68.5) 55 (66.3)	0.075	0.784
Smoking status	never smoking current or ex-smoker	59 78	23 (39.0) 22 (28.2)	36 (61.0) 56 (71.8)	1.756	0.185
Pathological pattern	adenocarcinoma squamous carcinoma	78 59	24 (30.8) 21 (35.6)	54 (69.2) 38 (64.4)	0.352	0.553
Clinical stages	I–II III	40 97	12 (30.0) 33 (34.0)	28 (70.0) 64 (66.0)	0.206	0.649
Pathological stages	I–II III	82 55	20 (24.4) 25 (45.5)	62 (77.6) 30 (54.5)	6.574	0.010
Regimens	PP GP TP	62 44 31	17 (27.4) 17 (38.6) 11 (35.5)	45 (72.6) 27 (61.4) 20 (64.5)	1.594	0.451

NSCLC – non-small cell lung cancer; PP – pemetrexed + platinum; TP – paclitaxel + platinum; GP – gemcitabine + platinum; TIL – tumor-infiltrating lymphocyte.

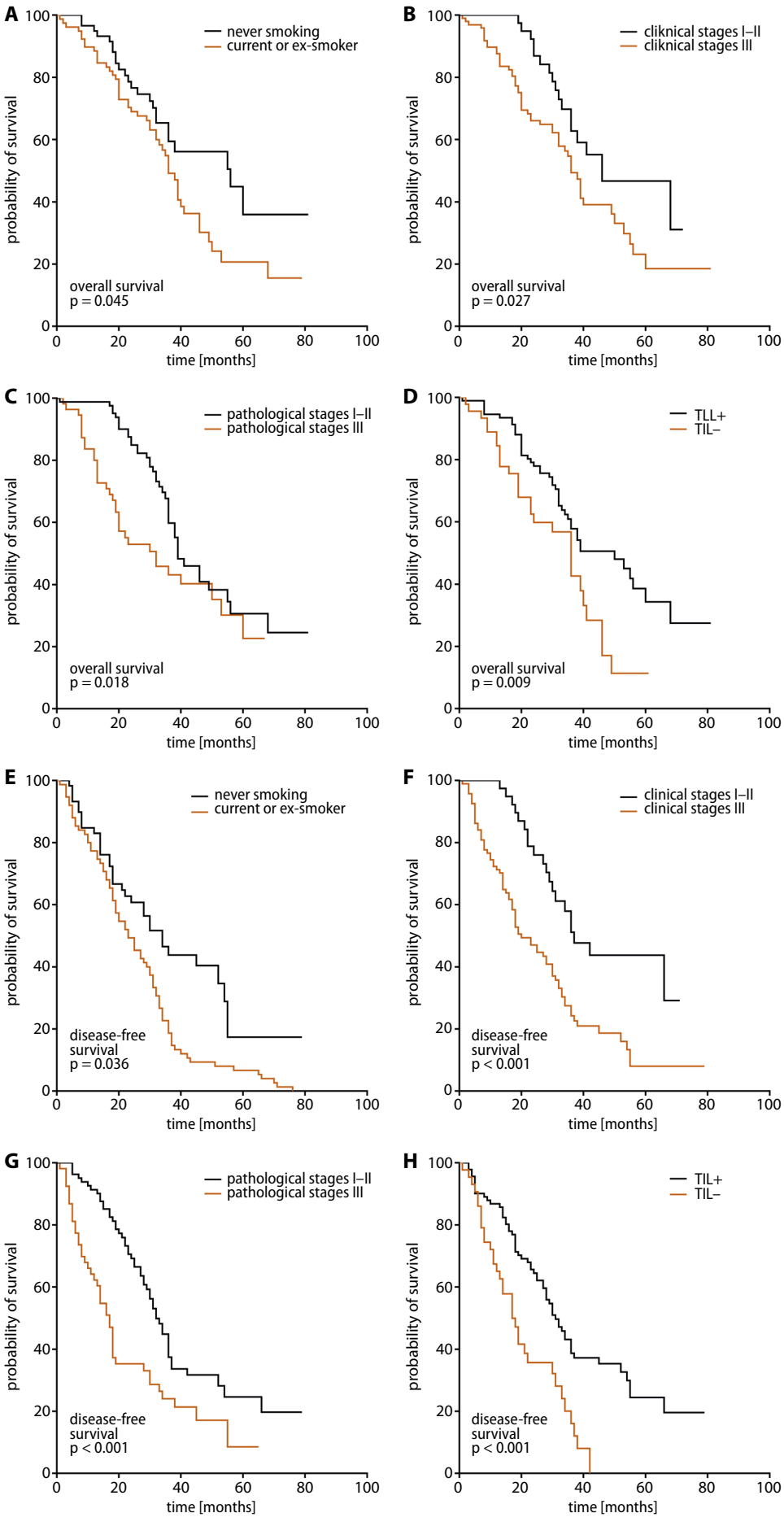

Fig. 1. Kaplan–Meier analysis of overall survival and disease-free survival in patients with non-small cell lung cancer

Table 2. Univariate analysis of OS and DFS in patients with NSCLC using the log-rank test

Characteristics		OS				DFS			
		MST [months]	5-year [%]	χ^2	p-value	MST [months]	5-year [%]	χ^2	p-value
Gender	male female	39 38	28.6 23.2	0.215	0.643	27 28	22.2 10.8	0.211	0.646
Age [years]	<60 ≥60	50 36	30.5 26.8	1.550	0.213	29 27	21.8 15.0	1.059	0.303
Smoking status	never smoking current or ex-smoker	56 36	35.9 20.7	4.018	0.045	34 23	17.3 16.4	4.378	0.036
Pathological pattern	adenocarcinoma squamous carcinoma	38 39	30.9 26.0	0.003	0.952	25 30	17.3 18.7	0.419	0.517
Clinical stages	I–II III	46 36	46.7 18.5	4.864	0.027	37 19	43.7 7.75	15.098	<0.001
Pathological stages	I–II III	39 32	30.6 22.6	5.587	0.018	32 16	24.3 8.24	13.389	<0.001
Regimens	PP GP TP	36 50 39	32.7 30.9 29.6	0.924	0.630	23 25 32	12.3 25.3 20.2	1.778	0.411
TIL levels	TIL– TIL+	36 50	11.4 34.3	6.747	0.009	17 31	0.00 24.2	15.414	<0.001

NSCLC – non-small cell lung cancer; OS – overall survival; DFS – disease-free survival; MST – median survival time; TIL – tumor-infiltrating lymphocyte.

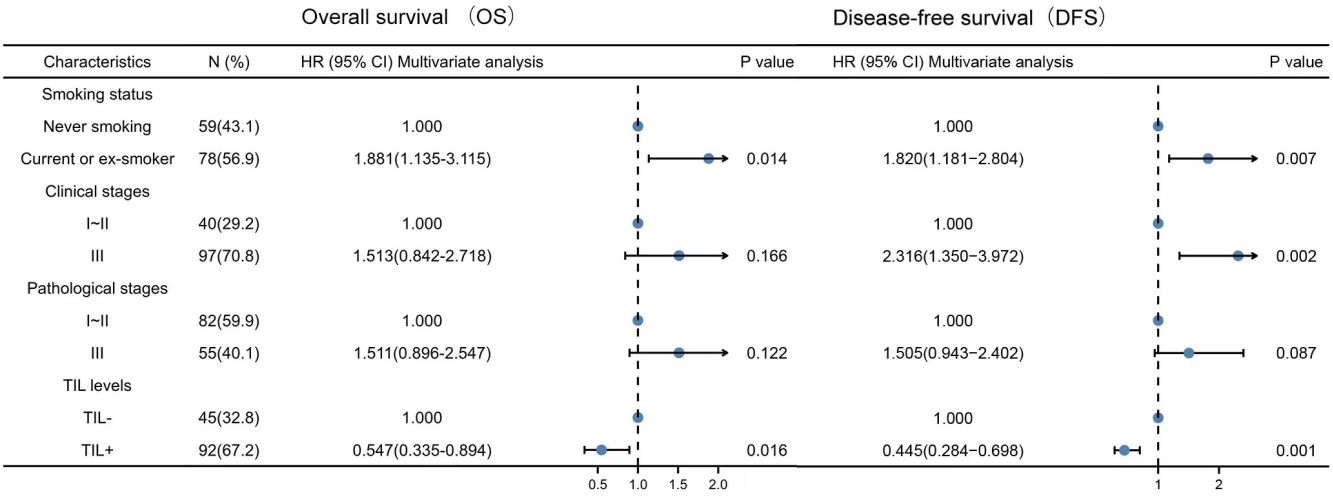

Fig. 2. Multivariate Cox regression analysis represented by a forest plot

TIL – tumor-infiltrating lymphocyte; HR – hazard ratio; 95% CI – 95% confidence interval.

study evaluated TIL levels in the tumor stroma of patients with NSCLC who were diagnosed and treated in a single medical institution to determine its effectiveness as a prognostic factor for patients with NSCLC undergoing surgery after neoadjuvant chemotherapy.

An intense spectrum of TIL staining was observed in the tumor samples of patients with NSCLC who had received surgery after neoadjuvant chemotherapy. More than 60% of the samples were considered TIL+, suggesting that high levels of TILs may be more common in patients with NSCLC who received surgery after neoadjuvant chemotherapy. However, the proportion of TIL+ patients in our cohort was higher than that found in NSCLC studies conducted by Horne et al., who used the same TIL evaluation method.[8] This could be attributed to the fact that neoadjuvant chemotherapy improves the tumor immune microenvironment, thereby increasing the density of TILs. Nonetheless, despite the possibility that the proportion of TIL+ patients and the neoadjuvant chemotherapy may be related, it is important to note that there was no significant relationship between the TIL+ ratio and the neoadjuvant chemotherapy regimen. Studies have shown that there is a lower proportion of TIL+ patients in the cancer population with higher stages of the disease.[16]

The results of this study showed that the proportion of TIL+ patients in pathological stage I–II (77.6%) was significantly higher than it was for pathological stage III (54.5%), while TIL+ status was not related to gender, age stratification, smoking status, or clinical stage, as has been reported by others.[16–18] A study on NSCLC reported

that TILs had a higher infiltration frequency in squamous cell carcinoma due to the heterogeneity of tumor types.[19] Notably, we observed that TIL+ results were not related to the histological type of NSCLC. This may be due to the large number of adenocarcinomas included in the study or the different genetic backgrounds of the patients. However, these findings should be considered and validated in future TIL studies.

Our data showed that a TIL+ status improved the survival rate of patients with NSCLC who received surgery after neoadjuvant chemotherapy. This indicates that patients with high TIL levels may have a strong immune response to the tumor, resulting in a better prognosis. A study that evaluated the TIL levels in tumor tissues surgically removed from 537 patients with stage I–III NSCLC found that the TIL level in tumor tissues was closely related to OS (HR: 0.51, 95% CI: 0.32–0.82) and DFS (HR: 0.34, 95% CI: 0.19–0.64), and a higher TIL level was an independent indicator of a good prognosis.[20] A similar result was obtained from the multivariate analysis in our study. In addition, higher levels of TIL in NSCLC are related to a better prognosis, which was confirmed in another comparative study.[21] However, in our study, variables in the multivariate analysis were derived from the univariate analysis. Therefore, whether gender, pathological type or other variables play a role in prognosis in multivariate analysis is still unknown and should be given attention in future studies.

The antitumor mechanisms of TILs described by recent studies may include the following: 1) lymphocytes mediate cell lysis and apoptosis by inhibiting the proliferation of regulatory T cells, increasing interferon gamma (IFN-γ) secretion and reducing the secretion of transforming growth factor beta (TGF-β), interleukin 10 (IL-10) and IL-17. This pathway can kill target cells by releasing particles or through a direct cell contact[22]; 2) lymphocytes bind to suicide-related factors on target cells, which mediates apoptosis[23]; 3) perforin or granzyme-mediated apoptosis.[24] Although this study demonstrated that high levels of TILs improved the prognosis of patients with NSCLC who underwent surgery after neoadjuvant chemotherapy, the challenges in the analysis of prognoses,[25,26] such as the activation status of TILs and their location and density in the tumor, still remain. Previous studies found that $CD8^+$ T cells were more likely to play an antitumor role in tumors than in the matrix.[26] Also, when $CD8^+$ T cells are in a state of inhibition, focusing only on the number of cells may result in different conclusions.

In addition to the TIL level, the multivariate analysis suggests that a history of smoking and a higher clinical stage may indicate a poor prognosis for patients with NSCLC who received surgery after neoadjuvant chemotherapy. Recent studies have also shown that NSCLC patients with higher smoking rates and those at advanced stages of disease have lower OS.[27] The results of a large prospective cohort study by Fujiyoshi et al. showed that for TIL– cancers, the correlation between smoking status and colorectal cancer mortality was stronger when diagnosing colorectal cancer, indicating that there may be an interaction between smoking and the lymphocyte response in the colorectal cancer microenvironment.[28] However, whether this relationship exists in NSCLC patients who received surgery after neoadjuvant chemotherapy remains unclear and further studies are required.

Limitations

There were certain limitations to the present study. First of all, this was a single-center study. Similar studies should be conducted in the future to further verify the accuracy of the findings. In addition, because this was a retrospective study of outcomes related to a subjective assessment of pathological variables, we cannot be fully confident about the effects of TILs in patients with early-stage NSCLC.

Conclusions

This study confirmed that TILs in surgical specimens from NSCLC patients treated with neoadjuvant chemotherapy may be associated with prognosis. Therefore, low levels of TILs in tumor tissue sections after an operation indicate a poor prognosis, and the number of postoperative adjuvant chemotherapy should be strengthened, to improve the therapeutic effect and prolong patients survival.

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