

# Prognostic Value of Tertiary Lymphoid Structures in Stage I Nonsmall Cell Lung Cancer: Does Location Matter?

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## ABSTRACT

**BACKGROUND:** Emerging evidence indicates the importance of tertiary lymphoid structures (TLSs) in predicting the outcomes of nonsmall cell lung cancer (NSCLC) patients; however, their prognostic value and correlations with peripheral inflammatory prognostic indices in stage I patients have been less well studied.

**METHODS:** Stage I NSCLC patients were recruited retrospectively; the presence and location of TLSs (peritumoral [pTLSs] and intratumoral [iTLSs]) were determined via hematoxylin and eosin (H&E)-stained slides. Peripheral inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), prognostic nutritional index (PNI), and advanced lung cancer inflammation index (ALI), were obtained and compared among these subgroups. Disease-free survival (DFS) and overall survival (OS) were tested via Kaplan-Meier analysis, and risk factors for survival were determined via a Cox proportional hazards model.

**RESULTS:** A total of 24.73% and 92.73% of patients were positive for pTLSs and iTLSs, respectively. The absolute number of iTLSs was significantly greater than that of pTLSs ( $P < .001$ ). Low preoperative LMR and ALI were detected only in patients with pTLSs but not in those without. Only pTLS was found to be a risk factor for both DFS and OS, and it was independently associated with OS (HR = 3.93, 95% confidence interval [CI] = 1.16–13.37;  $P = .028$ ). Accordingly, patients with pTLSs had relatively poor DFS (log rank = 5.46,  $P = .019$ ) and OS (log rank = 10.48,  $P = .001$ ) rates.

**CONCLUSIONS:** Among the heterogeneous results concerning the prognostic value of pTLSs and iTLSs in stage I NSCLC, our results for the first time indicated that the presence of pTLSs may predict poor outcomes in these patients and no correlation of iTLSs with the outcomes was validated; however, additional studies with large sample size are needed in future.

**KEYWORDS:** Lung cancer, tertiary lymphoid structures, neutrophil-lymphocyte ratio, lymphocyte to monocyte ratio, advanced lung cancer inflammation index, survival

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## Introduction

Lung cancer is still the leading cause of cancer-related death in China, with a mortality rate as high as 54.57/100 000 in men and women,<sup>1</sup> and nonsmall cell lung cancer (NSCLC) is still the main pattern of this disease.<sup>2</sup> To date, early diagnosis and radical resection are still the most important approaches for improving long-term survival in victims. Luckily, an obvious shift toward stage I along with improved survival has been observed in recent years due to the increasing popularity of low-dose computed tomography screening.<sup>3</sup> However, notably, the 5-year overall survival (OS) rate is 68% to 92% for stage I cases<sup>4</sup> and the benefit of adjuvant treatments for these patients is still controversial.<sup>5–10</sup> Potential risk factors associated with long-term outcomes are still highly needed.

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Previously, many studies have indicated that additional prognostic information for NSCLC patients can be obtained from single hematoxylin and eosin (H&E)-stained slides. Examples include tumor-infiltrating lymphocytes,<sup>11</sup> tumor growth patterns,<sup>12</sup> and the tumor-to-stroma ratio.<sup>13,14</sup> Tertiary lymphoid structures (TLSs), which involve the ectopic expansion of lymphoid cells in tumors or adjacent normal areas, have been increasingly validated as prognostic factors in various malignancies,<sup>15–19</sup> including NSCLC.<sup>20,21</sup> However, the prognostic value of these structures in stage I patients is still under debate. For example, Dieu-Nosjean et al enrolled 74 early-stage patients (including 62 stage I patients) and found that although a high density of intratumoral TLSs (iTLSs) (marked by mature dendritic cells [DCs]) was correlated with favorable disease-specific survival (DFS) and OS, it was less likely to have prognostic value in the stage I subgroup.<sup>22</sup> Similarly,



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Fukuhara et al included 112 stage I patients and reported that TLSs (with no specific description of the TLS locations) were not associated with DFS.<sup>23</sup> In contrast, in a study that included 213 stage I patients, Rakaee et al reported that high peritumoral TLSs (pTLSs) were significantly correlated with superior OS.<sup>20</sup> On the basis of these data, the prognostic value of TLSs in stage I NSCLC patients, particularly the role of their locations, still needs further investigation.

Notably, numerous studies have indicated that a series of inflammatory prognostic indices collected from peripheral blood tests, including the neutrophil-to-lymphocyte ratio (NLR),<sup>24</sup> lymphocyte-to-monocyte ratio (LMR),<sup>25</sup> prognostic nutritional index (PNI),<sup>26</sup> and advanced lung cancer inflammation index (ALI),<sup>27</sup> also play important roles in predicting outcomes in patients with NSCLC. Among these factors, the correlations of the NLR, LMR and ALI with patient outcomes have been further validated in stage I patients.<sup>28–30</sup> Interestingly, heterogeneous immune cells, including DCs,<sup>22</sup> B cells,<sup>31</sup> CD4+ T cells,<sup>32</sup> CD8+ T cells,<sup>20</sup> and others, have been identified in TLSs in NSCLC<sup>33,34</sup>; however, some previous reports have indicated that systemic inflammatory cells are correlated with CD4+ immune cells in the tumor microenvironment (TME)<sup>35</sup> and that the NLR is inversely associated with tumoral and stromal CD8+ lymphocytes.<sup>36</sup> These data support a potential correlation of the aforementioned inflammatory prognostic indices with the presence of TLSs in NSCLC. Coincidentally, a low NLR was found to be associated with high TLSs in a previous study by Fukuhara et al, but they included 23.81% (35/147) patients who exceeded stage I.<sup>23</sup> The correlations of these indices with TLSs in stage I cases are still largely unknown.

On the basis of these data, we aimed to explore the prognostic value of TLSs in different locations by H&E in stage I NSCLC patients and determine their correlations with peripheral blood inflammatory prognostic indices.

## Methods

### *Data collection*

Stage I NSCLC patients who underwent thoracoscopic radical resection in Hainan Hospital of Chinese PLA General Hospital from December 2012 to June 2020 were enrolled. Patients were excluded if they met any of the following criteria: (1) were not naïve to neoadjuvant therapies; (2) had detectable remote lesions before surgery; (3) had *in situ* lesions; (4) did not have adenocarcinoma or squamous carcinoma; or (5) had follow-up problems. Clinical variables, including age, sex, type of resection, pathology, smoking/drinking history, comorbidities (cases with hypertension or type 2 diabetes), and body mass index, were collected from the archived digital records as described previously.<sup>37</sup> In addition, preoperative absolute counts of neutrophils, lymphocytes, monocytes, and albumin were also obtained from the digital system within 1 week before surgery via routine blood tests as described previously.<sup>38</sup>

Peripheral inflammatory indices, including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), prognostic nutritional index (PNI), and advanced lung cancer inflammation index (ALI), were calculated according to previous reports.<sup>39,40</sup> This study was approved by the ethics committee of Hainan Hospital of Chinese PLA General Hospital (ID: S2023-12), and mandatory written informed consent was not necessary because of its retrospective nature according to national regulations.

### *Identify TLSs in H&E-stained slides*

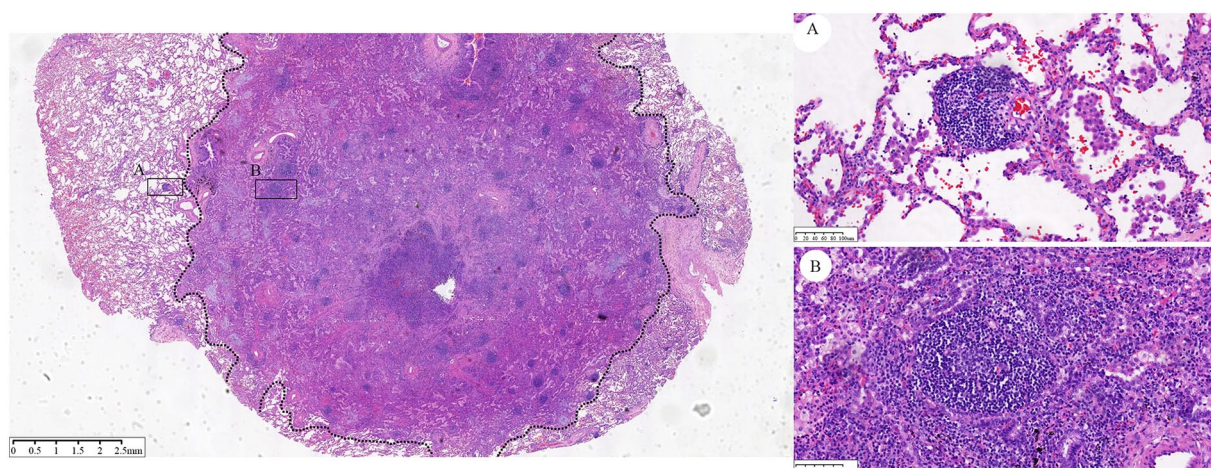
Slides (4 µm) were cut from the archived formalin-fixed, paraffin-embedded samples and processed via standard histological methods with H&E staining. We partly followed the methods of Rakaee et al<sup>20</sup> to identify TLSs; in brief, first, a representative slide with a maximum tumor-stromal interface was selected; second, areas with diffuse infiltration of lymphocytes and the absence of distinguishable lymphoid follicles or aggregate structures were ruled out; and third, irregularly shaped lymphoid expansion architectures were ignored. The TLSs were located by using a light microscope at a magnification of 10× (Leica DM2500, Leica Corporation) in the whole field, and then a magnification of 200× was used to determine the absolute number of TLSs by a senior pathologist who was blinded to the clinical features. pTLSs were identified from an invasive border without any malignant components, and iTLSs were identified from an invasive border to the tumor center with all malignant components (Figure 1). Inconclusive areas were consulted with another senior pathologist to reach a conclusive result.

### *Study endpoints*

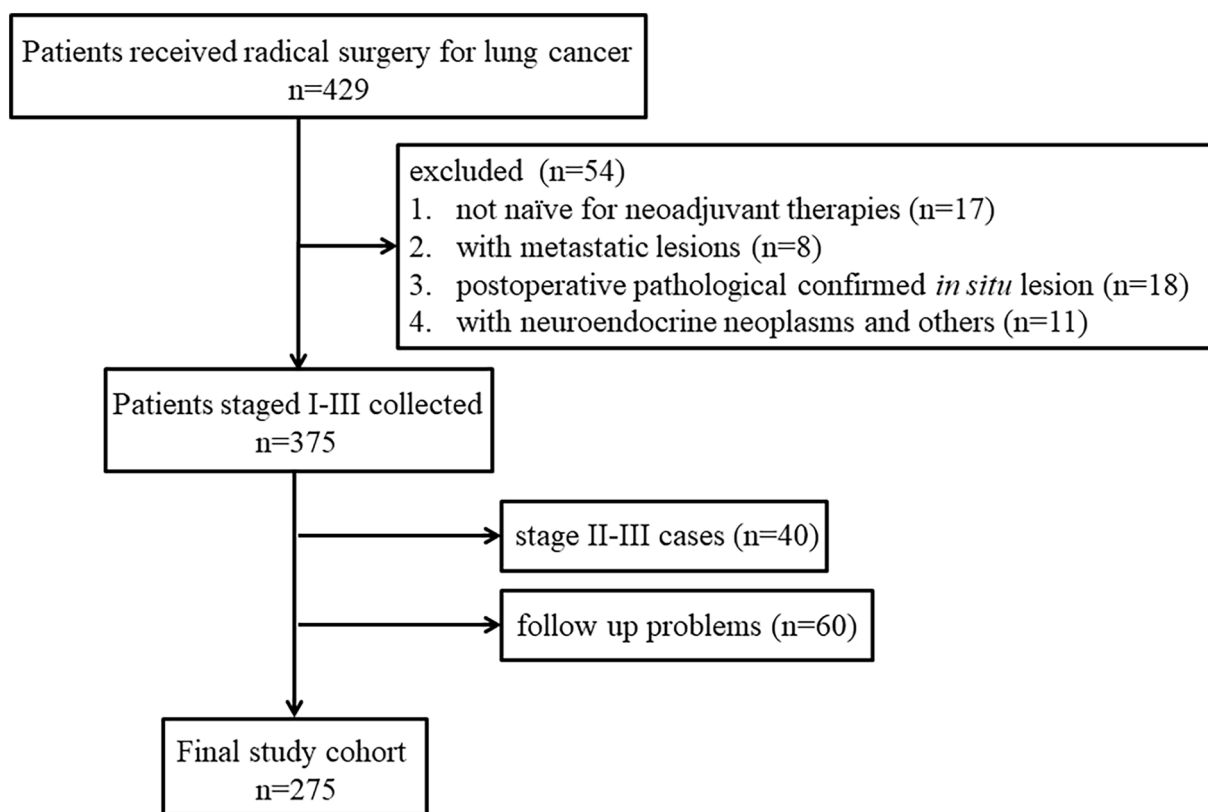
Disease-specific survival and OS were set as the endpoints in this study, and the follow-up procedure was conducted as described previously;<sup>37</sup> the latest follow-up was terminated in August 2023.

### *Statistical tests*

All of the data were analyzed via SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). Patients were divided into pTLS, iTLS-low or -high subgroups on the basis of the median absolute counts in the whole cohort. The differences in the distributions of the clinical variables among these subgroups were tested via Chi-square tests, and the differences in the NLR, LMR, PNI, and ALI among them were determined via independent-samples *t* tests or Mann-Whitney U tests when a Gaussian distribution was not met. Correlations between pTLSs and iTLSs were determined via the Pearson test. Differences in DFS and OS among these subgroups were analyzed by the Kaplan-Meier method. Risk factors for the



**Figure 1.** A representative image showing the presence of pTLSs and iTLSs. The left panel displays the whole tumor (10×). The right panel shows the enlarged fields of pTLSs (A) and iTLSs (B) (200×). The black dots indicate the invasive border of the tumor. pTLSs: peritumoral tertiary lymphoid structures; iTLSs: intratumoral tertiary lymphoid structures.



**Figure 2.** Patient enrollment.

outcome were calculated via the Cox proportional hazards model. A 2-sided  $P$  value  $< .05$  was considered statistically significant.

## Results

### Description of patient features

A total of 275 patients were included (Figure 2), with 148 women and 127 men. The median age of the patients was 57 years (years) (range: 23–84 years), and the median follow-up

was 51 months (m) (range: 9–121 m). At the end of the follow-up, 4 and 8 patients died in stages IA and IB, respectively. The median number of pTLSs was 0 (range: 0–50), and the median number of iTLSs was 6 (range: 0–46), with 20 patients having 0; thus, 24.73% (68/275) and 92.73% (255/275) of the patients were positive for pTLSs and iTLSs, respectively. The absolute number of iTLSs was significantly greater than that of pTLSs ( $P < .001$ ). As shown in Table 1, male patients with squamous carcinoma, a smoking history or comorbidities, and IB were more common in the high-pTLS subgroup.

**Table 1.** The differences of clinical variables amongst pTILs and iTILs low or high subgroups.

VARIABLES	CASES	PTILS			ITILS		
		LOW	HIGH	P	LOW	HIGH	P
<b>Age (years)</b>				.117			.267
<60	165	130	35		83	82	
≥60	110	77	33		47	63	
<b>Sex</b>				.003*			.276
Female	148	122	26		65	83	
Male	127	85	42		65	62	
<b>Type of resection</b>				.001*			.006*
Lobectomy or sublobectomy	202	142	60		85	117	
Wedge resection	73	65	8		45	28	
<b>Pathology</b>				<.001*			.064
Adenocarcinoma	264	206	58		128	136	
Squamous	11	1	10		2	9	
<b>Smoking history</b>				.014*			.888
Yes	66	42	24		32	34	
No	209	165	44		98	111	
<b>Drinking history</b>				.455			.519
Yes	89	64	25		45	44	
No	186	143	43		85	101	
<b>Comorbidities</b>				.007*			.042
Yes	73	46	27		27	46	
No	202	161	41		103	99	
<b>TNM stage</b>				.009*			.894
IA	197	157	40		94	103	
IB	78	50	28		36	42	

pTILs: peritumoral tertiary lymphoid structures; iTILs: intratumoral tertiary lymphoid structures.

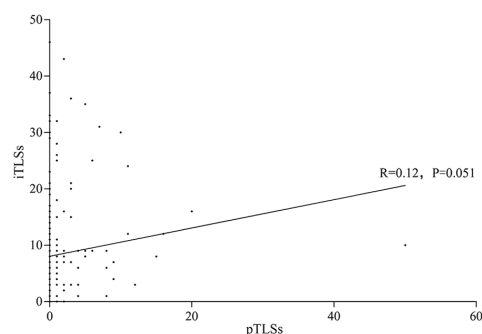
\*Significant statistical difference.

### Correlation between pTILs and iTILs

As shown in Figure 3, there was a positive correlation between the densities of the pTILs and iTILs ( $R=0.12$ ,  $P=.051$ ), but the correlation was not statistically significant.

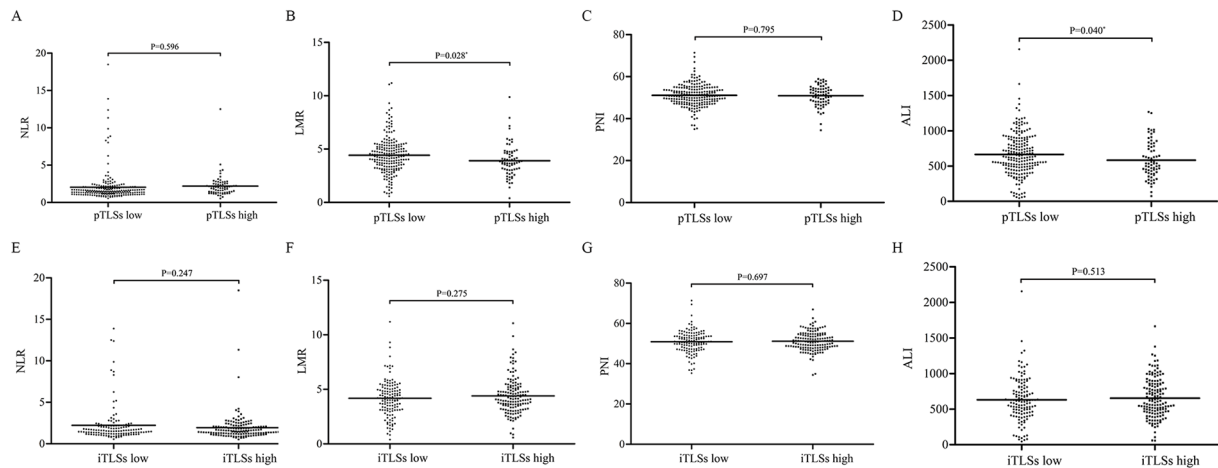
### Distribution of peripheral inflammatory prognostic indices among the absent or present pTIL subgroups

As shown in Figure 4, significant differences in the preoperative LMR and ALI were detected among patients with pTILs but not among those without. Patients with pTILs were more likely to have a relatively low LMR and ALI.

**Figure 3.** The correlation between pTILs and iTILs was checked via the Pearson test.

pTILs: peritumoral tertiary lymphoid structures; iTILs: intratumoral tertiary lymphoid structures.





**Figure 4.** Differences in inflammatory prognostic indices, including the NLR (A, E), LMR (B, F), PNI (C, G), and ALI (D, H), between the low- and high-TLS subgroups.

NLR: neutrophil-to-lymphocyte ratio; LMR: lymphocyte-to-monocyte ratio; PNI: prognostic nutritional index; ALI: advanced lung cancer inflammation index; pTLSs: peritumoral tertiary lymphoid structures; iTLSs: intratumoral tertiary lymphoid structures.

### *Survival differences among the absent or present pTLS subgroups*

As shown in Figure 5, significant survival differences were detected only among the absent or present pTLS subgroups, as patients with pTLSs had significantly worse DFS (log rank=5.46,  $P=.019$ ) and OS (log rank=10.48,  $P=.001$ ). No such differences were found in the iTLS low- or high-level subgroups.

### *Determination of risk factors for DFS and OS by univariate and multivariate tests*

Univariate tests revealed that TNM stage and pTLSs were common risk factors for both DFS and OS, whereas smoking history was a statistically significant risk factor for DFS and was a definite risk factor for OS (Table 2). Together, these factors were included in multivariate tests, and the results indicated that pTLSs were an independent risk factor only for OS (HR=3.93, 95% confidence interval [CI] = 1.16-13.37;  $P=.028$ ) (Table 3).

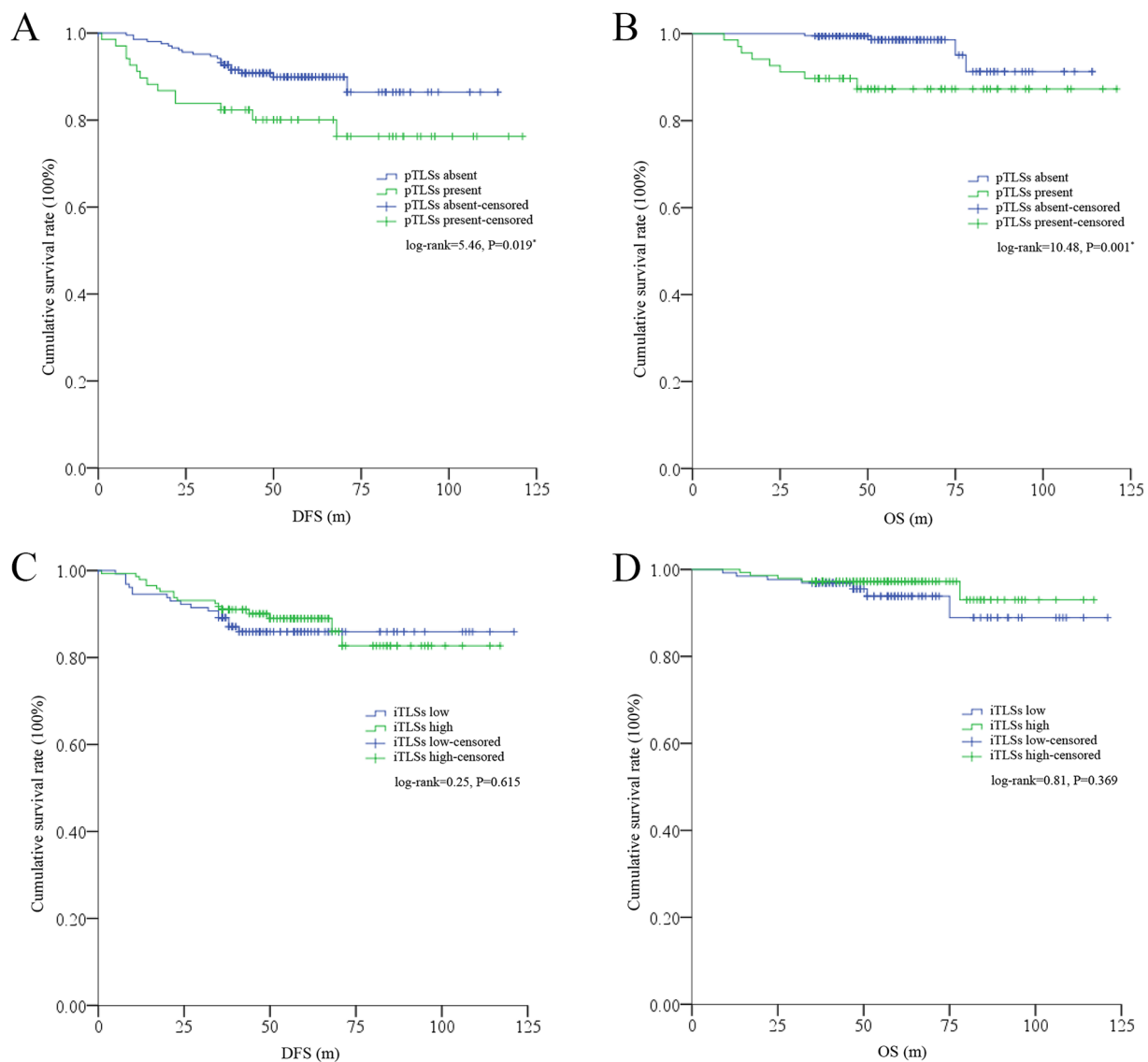
## **Discussion**

In this study, pTLSs, rather than iTLSs, were found to be significantly associated with survival in stage I NSCLC patients and were identified as independent risk factors for OS. Notably, patients with pTLSs had significantly worse DFS and OS than their counterparts did.

Previously, a group of studies indicated the presence or density of TLSs with different locations in NSCLC and explored their value in prognostication; however, the results were highly conflicting. For example, Rakae et al included 553 stage I-III patients and reported that a high frequency of TLSs (mainly iTLSs according to the description) was associated with improved DFS and OS<sup>20</sup>; similarly, Fukuhara et al enrolled 147 stage I-IV patients and reported that a high TLS density (also

a high iTLS density according to the description) was correlated with better DFS.<sup>23</sup> In line with these findings, Giatromanolaki et al included 103 stage I-IV patients and individually explored the prognostic values of pTLSs and iTLSs; their results indicated that high densities of both pTLSs and iTLSs were linked to better OS.<sup>21</sup> In contrast, Brunet et al included 114 metastatic patients who were enrolled in the BIP study (NCT02534649) and reported that the presence of TLSs (iTLSs + pTLSs) had no value in predicting progression-free survival (PFS).<sup>41</sup> Conventionally, these heterogeneous results may be explained by the fact that these studies included patients with mixed stages; surprisingly, these controversies are still present in the stage I scenario. For example, some reports have suggested that the presence of iTLSs is positively associated with outcomes.<sup>20,42</sup> However, the aforementioned studies did not support these results;<sup>22,23</sup> furthermore, He et al suggested that these structures were not associated with OS in a large sample study ( $n=326$ ).<sup>43</sup> In our study, we found no correlations between iTLSs and DFS or OS, which was partly in line with the findings of previous studies.<sup>22,23</sup> In addition, we found that the presence of pTLSs was significantly correlated with worse survival; interestingly, the role of pTLSs in survival has been supported by several other studies. For example, Sofopoulos et al included 112 breast cancer patients and reported that the presence of pTLSs was correlated with poor DFS and OS,<sup>44</sup> whereas Zhang et al enrolled 170 hepatocellular carcinoma patients and suggested that the presence of pTLSs predicts inferior DFS and OS.<sup>45</sup> These reports suggest that the function of pTLSs in cancer may differ from that of iTLSs.

In fact, some important questions remain unanswered regarding the cellular constitutions and functions of TLSs. Previous studies have indicated that the TLSs consists of a group of immune cells, including DCs,<sup>22</sup> B cells,<sup>31</sup> CD4+ T cells, CD8+ T cells,<sup>20,32</sup> T regulatory cells (Tregs),<sup>33</sup> mast cells,<sup>46</sup> and macrophages,<sup>47</sup> notably, these cells and their



**Figure 5.** Survival differences between the low- and high-TLS subgroups. (A) DFS differences between the low- and high-pTLS subgroups. (B) OS differences between the low- and high-pTLS subgroups. (C) DFS differences between the low- and high-iTLS subgroups. (D) OS differences between the low- and high-iTLS subgroups. DFS: disease-free survival; OS: overall survival; pTLSs: peritumoral tertiary lymphoid structures; iTLSs: intratumoral tertiary lymph.

secreted cytokines and chemokines may act as “double-edged swords” in the TME during the development of cancer. Correspondingly, the functions of TLSs may be controlled by the comprehensive coordination of these factors at different locations. In our study, the presence of pTLS was negatively correlated with survival, which can also be partly explained by these cells and cytokines. For example, previous studies have shown that mast cells can induce epithelial-to-mesenchymal transition and migration by regulating the IL-8/Wnt/ $\beta$ -catenin pathway or promoting cell proliferation in NSCLC.<sup>48,49</sup> Noticeably, these cells are multifaceted with distinct phenotypes in the tumor compared with adjacent normal areas,<sup>50</sup> and further, although it was not validated in NSCLC, these cells in adjacent normal mucosa but not in the invasive front can promote disease progression.<sup>51</sup> In addition, fibroblasts, which are the main source of several cytokines, such as IL-33,<sup>52</sup> are

critically involved in the formation of TLSs.<sup>53</sup> Interestingly, the level of IL-33 is significantly greater in adjacent normal tissues than in tumor areas in lung cancer<sup>54</sup>; moreover, IL-33 can fuel the growth and metastasis of cancer cells.<sup>55,56</sup> In addition, IL-33 has a functionally important role in recruiting neutrophils, which are the main leukocytes in NSCLC.<sup>57</sup> Notably, numerous reports have shown that neutrophils are key players in promoting cancer progression and metastasis.<sup>58,59</sup> Moreover, IL-33 can promote IL-10 secretion by macrophages,<sup>60</sup> which have broad functions in regulating immunosuppression,<sup>61</sup> promoting cell growth,<sup>62</sup> and enhancing treatment resistance in lung cancer.<sup>63</sup> Actually, the formation of pTLSs may greatly be manipulated by the TME in the invasive border. As some of the aforementioned immune cells; for example, the mast cells, were activated by the cancer cell-derived extracellular vesicles and the neutrophils were stimulated by the hypoxic TME.<sup>64,65</sup>

**Table 2.** Determination of the risk factors for DFS and OS by univariate analyses.

	UNIVARIATE			MULTIVARIATE		
	HR	95% CI	P	HR	95% CI	P
<b>Age (years)</b>						
<60	1			1		
≥60	1.20	0.61-2.36	.599	1.06	0.34-3.35	.916
<b>Sex</b>						
Female	1			1		
Male	1.44	0.73-2.84	.294	3.22	0.87-11.96	.080
<b>Type of resection</b>						
Lobectomy or sublobectomy	1			1		
Wedge resection	1.31	0.57-3.03	.526	2.45	0.74-8.14	.144
<b>Pathology</b>						
Adenocarcinoma	1			1		
Squamous	2.23	0.68-7.34	.188	1.78	0.22-13.92	.581
<b>Smoking history</b>						
Yes	1			1		
No	0.50	0.25-1.00	.050	0.22	0.07-0.69	.010*
<b>Drinking history</b>						
Yes	1			1		
No	0.92	0.46-1.87	.827	0.51	0.17-1.59	.248
<b>Comorbidities</b>						
Yes	1			1		
No	0.87	0.41-1.81	.703	0.54	0.12-2.48	.430
<b>TNM stage</b>						
IA	1			1		
IB	5.75	2.80-11.79	<.001*	4.88	1.47-16.21	.010*
<b>pTILs</b>						
Low	1			1		
High	2.22	1.22-4.41	.023*	5.85	1.75-19.59	.004*
<b>iTILs</b>						
Low	1			1		
High	0.84	0.43-1.65	.616	0.59	0.19-1.88	.375

\*Significant statistical difference.

On the basis of these data, it is likely that the presence of pTILs may act as “a lighthouse in the sea” in the TME, which can facilitate the migration and expansion of cancer cells. Although the density of iTILs was significantly greater than that of pTILs in our study, considering the complexity and

diversity of the immune and inflammatory context of the TME, their role in cancer could be to promote antitumor immunity, contribute to immunosuppression, or be just bystander sentinels in such a scenario.<sup>66</sup>For example, CD8+ T-cell secretion of IL-10 was found to be correlated with a

**Table 3.** Determination of the risk factors for DFS and OS by multivariate analyses.

	UNIVARIATE			MULTIVARIATE		
	HR	95% CI	P	HR	95% CI	P
<b>Smoking history</b>						
Yes				1		
No				0.23	0.07-0.75	.015*
<b>TNM stage</b>						
IA	1			1		
IB	5.51	2.66-11.41	<.001*	4.84	1.41-16.63	.012*
<b>pTILs</b>						
Low				1		
High				3.93	1.16-13.37	.028*

\*with significant statistical difference.

good prognosis in patients with lung cancer and was further validated in patients with stage I disease<sup>67,68</sup>; however, some other studies have suggested that T-cell-derived IL-10 can promote lung cancer cell growth and migration.<sup>62,69</sup> These results indicate that more studies concerning the role of iTLSs in NSCLC are needed in the future.

In recent years, peripheral inflammatory indices have been increasingly reported as easily accessible and reliable prognostic factors in NSCLC patients.<sup>24-27</sup> With respect to stage I patients, several indices, such as the NLR, LMR and ALI, have been extensively validated in previous studies as significant predictors of survival; generally, a high preoperative NLR and low LMR and ALI are conventionally associated with poor outcomes in these patients.<sup>30,70-75</sup> Interestingly, TLSs are clustered with CD4<sup>+</sup><sup>32</sup> and CD8<sup>+</sup> T cells,<sup>20</sup> and previous reports have indicated that peripheral systemic inflammatory cells or inflammatory prognostic indices such as the NLR can potentially be correlated with these cells in tumors.<sup>35,36</sup> Coincidentally, a low NLR was found to be associated with high iTLSs in the study of Fukuhara et al, which may partly support the prognostic value of iTLSs in these patients.<sup>23</sup> Notably, iTLSs in this study were not a significant predictor of DFS in stage I patients.<sup>23</sup> In our study, we did not find any differences in the NLR, LMR, PNI, or ALI among the iTLS low or high subgroups but we did detect significantly low LMR and ALI in patients with high pTLSs. Together with the role of these markers in the prognosis of stage I patients,<sup>30,74</sup> we believe that they may also contribute to the prognostic value of pTLSs in stage I patients.

Our study also presented several limitations. First, we collected data retrospectively from a single hospital with relatively small sample sizes, and some bias cannot be excluded; in particular, both the relatively low presence of pTLSs and the wide CIs of it in the multivariate Cox regression analysis indicated a low statistical power and the necessities of enlarged sample size.

Second, a group of previous studies has indicated that some other features of TLSs, particularly the maturity or specific immune cells that they contain, are important in determining their prognostic value in NSCLC. However, some of these results (mainly those of iTLSs), for example, the role of the maturation of TLSs in predicting the efficacy of chemotherapy, are still controversial.<sup>20-23,41-43</sup> This additional information may be helpful in our study, as we not only focused on iTLSs but also further addressed pTLSs, specifically in stage I patients. Third, some key genetic alterations, such as epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK), have a great impact on the presence and density of TLSs in NSCLC,<sup>76,77</sup> which could be more reasonable if stratification analysis could be performed on the basis of these characteristics of the patients in our study. Nonetheless, we advocate more detailed studies to validate our results in the future.

## Conclusion

Overall, among the heterogeneous results concerning the prognostic value of pTLSs and iTLSs in stage I NSCLC, our results for the first time indicated that the presence of pTLSs may predict poor outcomes in these patients. In addition, we found no such correlation of iTLSs with the outcomes; however, these results should be further validated with large sample size in future taken the aforementioned limitations into consideration.

## Author Contributions

HY designed the work. XTH, YQW, and LPH obtained the data. XTH and ZXX analyzed the data. XTH drafted the article. All authors read and approved the final manuscript.

## Availability of Data and Materials

The data sets generated or analyzed during the current study are available from the first author (Xue TH) on reasonable request.



## Ethics Approval and Consent to Participate

This study was approved by the ethics committee of our hospital (ID: S2023-12), and mandatory written informed consent was not necessary because of its retrospective nature according to national regulations.

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## REFERENCES

- Qi J, Li M, Wang L, et al. National and subnational trends in cancer burden in China, 2005-20: an analysis of national mortality surveillance data. *Lancet Public Health*. 2023;8:e943-e955.
- Cufer T, Knez L. Update on systemic therapy of advanced non-small-cell lung cancer. *Expert Rev Anticancer Ther*. 2014;14:1189-1203.
- Potter AL, Rosenstein AL, Kiang MV, et al. Association of computed tomography screening with lung cancer stage shift and survival in the United States: quasi-experimental study. *BMJ*. 2022;376:e069008.
- Goldstraw P, Chansky K, Crowley J, et al. The IASLC lung cancer staging project: proposals for revision of the TNM stage groupings in the forthcoming (eighth) edition of the TNM classification for lung cancer. *J Thorac Oncol*. 2016;11:39-51.
- Zhou K, Zhao Y, Liang L, et al. Adjuvant chemotherapy may improve long-term outcomes in stage IB non-small cell lung cancer patients with previous malignancies: a propensity score-matched analysis. *Front Oncol*. 2022;12:938195.
- Tsutani Y, Imai K, Ito H, et al. Adjuvant chemotherapy for high-risk pathologic stage I non-small cell lung cancer. *Ann Thorac Surg*. 2022;113:1608-1616.
- Zhang P, Duan J, Bai H, et al. Influence of adjuvant chemotherapy on survival for patients with stage IB and IIA non-small cell lung cancer. *Thorac Cancer*. 2021;12:30-39.
- Pisters K, Kris MG, Gaspar LE, Ismaila N. Adjuvant systemic therapy and adjuvant radiation therapy for stage I-IIIA completely resected non-small-cell lung cancer: ASCO guideline rapid recommendation update. *J Clin Oncol*. 2022;40:1127-1129.
- O'Brien M, Paz-Ares L, Marreaud S, et al. Pembrolizumab versus placebo as adjuvant therapy for completely resected stage IB-IIIA non-small-cell lung cancer (PEARLS/KEYNOTE-091): an interim analysis of a randomised, triple-blind, phase 3 trial. *Lancet Oncol*. 2022;23:1274-1286.
- Kenmotsu H, Sugawara S, Watanabe Y, et al. Adjuvant atezolizumab in Japanese patients with resected stage IB-IIIA non-small cell lung cancer (IMpower010). *Cancer Sci*. 2022;113:4327-4338.
- Rakaee M, Kilvaer TK, Dalen SM, et al. Evaluation of tumor-infiltrating lymphocytes using routine H&E slides predicts patient survival in resected non-small cell lung cancer. *Hum Pathol*. 2018;79:188-198.
- Yang X, Liu Y, Lian F, et al. Lepidic and micropapillary growth pattern and expression of Napsin A can stratify patients of stage I lung adenocarcinoma into different prognostic subgroup. *Int J Clin Exp Pathol*. 2014;7:1459-1468.
- Xi KX, Wen YS, Zhu CM, et al. Tumor-stroma ratio (TSR) in non-small cell lung cancer (NSCLC) patients after lung resection is a prognostic factor for survival. *J Thorac Dis*. 2017;9:4017-4026.
- Zhang X, Zhang Y, Sun Z, Ma H. Research on correlation between tumor-stroma ratio and prognosis in non-small cell lung cancer. *Minerva Med*. 2019;110:590-592.
- Figenschau SL, Fismen S, Fenton KA, Fenton C, Mortensen ES. Tertiary lymphoid structures are associated with higher tumor grade in primary operable breast cancer patients. *BMC Cancer*. 2015;15:101.
- Kroeger DR, Milne K, Nelson BH. Tumor-infiltrating plasma cells are associated with tertiary lymphoid structures, cytolytic T-cell responses, and superior prognosis in ovarian cancer. *Clin Cancer Res*. 2016;22:3005-3015.
- Schweiger T, Berghoff AS, Glogner C, et al. Tumor-infiltrating lymphocyte subsets and tertiary lymphoid structures in pulmonary metastases from colorectal cancer. *Clin Exp Metastasis*. 2016;33:727-739.
- Sakimura C, Tanaka H, Okuno T, et al. B cells in tertiary lymphoid structures are associated with favorable prognosis in gastric cancer. *J Surg Res*. 2017;215:74-82.
- Koti M, Xu AS, Ren KYM, et al. Tertiary lymphoid structures associate with tumour stage in urothelial bladder cancer. *Bladder Cancer*. 2017;3:259-267.
- Rakaee M, Kilvaer TK, Jamaly S, et al. Tertiary lymphoid structure score: a promising approach to refine the TNM staging in resected non-small cell lung cancer. *Br J Cancer*. 2021;124:1680-1689.
- Giatromanolaki A, Chatzipantelis P, Contrafouris CA, Koukourakis MI. Tertiary lymphoid structures, immune response, and prognostic relevance in non-small cell lung cancer. *Cancer Invest*. 2023;41:48-57.
- Dieu-Nosjean MC, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J Clin Oncol*. 2008;26:4410-4417.
- Fukuhara M, Muto S, Inomata S, et al. The clinical significance of tertiary lymphoid structure and its relationship with peripheral blood characteristics in patients with surgically resected non-small cell lung cancer: a single-center, retrospective study. *Cancer Immunol Immunother*. 2022;71:1129-1137.
- Yin Y, Wang J, Wang X, et al. Prognostic value of the neutrophil to lymphocyte ratio in lung cancer: a meta-analysis. *Clinics*. 2015;70:524-530.
- Mandaliya H, Jones M, Oldmeadow C, Nordman II. Prognostic biomarkers in stage IV non-small cell lung cancer (NSCLC): neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), platelet to lymphocyte ratio (PLR) and advanced lung cancer inflammation index (ALI). *Transl Lung Cancer Res*. 2019;8:886-894.
- Hu Y, Shen J, Liu R, et al. Prognostic value of pretreatment prognostic nutritional index in non-small cell lung cancer: a systematic review and meta-analysis. *Int J Biol Markers*. 2018;33:372-378.
- Zhang Y, Chen B. Prognostic value of the advanced lung cancer inflammation index in patients with lung cancer: a meta-analysis. *Dis Markers*. 2019;2019:2513026.
- Wang J, Kalhor N, Hu J, et al. Pretreatment neutrophil to lymphocyte ratio is associated with poor survival in patients with stage I-III non-small cell lung cancer. *PLoS ONE*. 2016;11:e0163397.
- Xia H, Sun Z, Deng L, Zhu D, Wang D. Prognostic significance of the preoperative lymphocyte to monocyte ratio in patients with stage I non-small cell lung cancer undergoing complete resection. *Cancer Invest*. 2016;34:378-384.
- Kobayashi S, Karube Y, Inoue T, et al. Advanced lung cancer inflammation index predicts outcomes of patients with pathological stage IA lung adenocarcinoma following surgical resection. *Ann Thorac Cardiovasc Surg*. 2019;25:87-94.
- Germain C, Gnjatich S, Tamzalit F, et al. Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am J Respir Crit Care Med*. 2014;189:832-844.
- de Chaisemartin L, Goc J, Damotte D, et al. Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. *Cancer Res*. 2011;71:6391-6399.
- Germain C, Devi-Marulkar P, Knockaert S, et al. Tertiary lymphoid structure-B cells narrow regulatory T cells impact in lung cancer patients. *Front Immunol*. 2021;12:62776.
- Zhao H, Wang H, Zhao Y, Sun Q, Ren X. Tumor-resident T cells, associated with tertiary lymphoid structure maturity, improve survival in patients with stage III lung adenocarcinoma. *Front Immunol*. 2022;13:877689.
- Choi Y, Kim JW, Nam KH, et al. Systemic inflammation is associated with the density of immune cells in the tumor microenvironment of gastric cancer. *Gastric Cancer*. 2017;20:602-611.
- Shirasawa M, Yoshida T, Horinouchi H, et al. Prognostic impact of peripheral blood neutrophil to lymphocyte ratio in advanced-stage pulmonary large cell neuroendocrine carcinoma and its association with the immune-related tumour microenvironment. *Br J Cancer*. 2021;124:925-932.
- Xu H, Zhao G, Lin J, Ye Q, Xiang J, Yan B. A combined preoperative red cell distribution width and carcinoembryonic antigen score contribute to prognosis prediction in stage I lung adenocarcinoma. *World J Surg Oncol*. 2023;21:56.
- Huang X, Huan Y, Liu L, Ye Q, Guo J, Yan B. Preoperative low absolute lymphocyte count to fibrinogen ratio correlated with poor survival in nonmetastatic colorectal cancer. *World J Surg Oncol*. 2022;20:309.
- Hailun Xie, Huang S, Yuan G, Tang S, Gan J. Prognostic significance of preoperative fibrinogen-to-prealbumin ratio in patients with stage I-III colorectal cancer undergoing surgical resection: a retrospective cohort study. *BioMed Res Int*. 2021;2021:3905353.
- Derman BA, Macklis JN, Azeem MS, et al. Relationships between longitudinal neutrophil to lymphocyte ratios, body weight changes, and overall survival in patients with non-small cell lung cancer. *BMC Cancer*. 2017;17:141.
- Brunet M, Crombé A, Cousin S, et al. Mature tertiary lymphoid structure is a specific biomarker of cancer immunotherapy and does not predict outcome to chemotherapy in non-small-cell lung cancer. *Ann Oncol*. 2022;33:1084-1085.
- Xu X, Gao Y, Duan S, et al. Clinical implications and molecular features of tertiary lymphoid structures in stage I lung adenocarcinoma. *Cancer Med*. 2023;12:9547-9558.
- He M, He Q, Cai X, et al. Intratumoral tertiary lymphoid structure (TLS) maturation is influenced by draining lymph nodes of lung cancer. *J Immunother Cancer*. 2023;11:e005539.
- Sofopoulos M, Fortis SP, Vaxevanis CK, et al. The prognostic significance of peritumoral tertiary lymphoid structures in breast cancer. *Cancer Immunol Immunother*. 2019;68:1733-1745.
- Zhang T, Lei X, Jia W, et al. Peritumoral tertiary lymphoid structures are associated with infiltrating neutrophils and inferior prognosis in hepatocellular carcinoma. *Cancer Med*. 2023;12:3068-3078.
- Li J, Zhang L, Xing H, et al. The absence of intra-tumoral tertiary lymphoid structures is associated with a worse prognosis and mTOR signaling activation in

- hepatocellular carcinoma with liver transplantation: a multicenter retrospective study. *Adv Sci*. 2024;11:e2309348.
47. Sun X, Liu W, Sun L, et al. Maturation and abundance of tertiary lymphoid structures are associated with the efficacy of neoadjuvant chemioimmunotherapy in resectable non-small cell lung cancer. *J Immunother Cancer*. 2022;10:e005531.
  48. Qu J, Cheng T, Liu L, et al. Mast cells induce epithelial-to-mesenchymal transition and migration in non-small cell lung cancer through IL-8/Wnt/ $\beta$ -catenin pathway. *J Cancer*. 2019;10:5567.
  49. Stoyanov E, Uddin M, Mankuta D, Dubinett SM, Levi-Schaffer F. Mast cells and histamine enhance the proliferation of non-small cell lung cancer cells. *Lung Cancer*. 2012;75:38-44.
  50. Leveque E, Rouch A, Syrykh C, et al. Phenotypic and histological distribution analysis identify mast cell heterogeneity in non-small cell lung cancer. *Cancers*. 2022;14:1394.
  51. Xia Q, Ding Y, Wu XJ, et al. Mast cells in adjacent normal colon mucosa rather than those in invasive margin are related to progression of colon cancer. *Chin J Cancer Res*. 2011;23:276-282.
  52. Amuso VM, Haas MR, Cooper PO, et al. Fibroblast-mediated macrophage recruitment supports acute wound healing [published online ahead of print November 22, 2024]. *J Invest Dermatol*. 2024.
  53. Barone F, Gardner DH, Nayar S, Steinthal N, Buckley CD, Luther SA. Stromal fibroblasts in tertiary lymphoid structures: a novel target in chronic inflammation. *Front Immunol*. 2016;7:477.
  54. Yang M, Feng Y, Yue C, et al. Lower expression level of IL-33 is associated with poor prognosis of pulmonary adenocarcinoma. *PLoS ONE*. 2018;13:e0193428.
  55. Wang C, Chen Z, Bu X, et al. IL-33 signaling fuels outgrowth and metastasis of human lung cancer. *Biochem Biophys Res Commun*. 2016;479:461-468.
  56. Jiang Y, Liao H, Zhang X, et al. IL-33 synergistically promotes the proliferation of lung cancer cells in vitro by inducing antibacterial peptide LL-37 and proinflammatory cytokines in macrophages. *Immunobiology*. 2020;225:152025.
  57. McCarthy PC, Phair IR, Greger C, et al. IL-33 regulates cytokine production and neutrophil recruitment via the p38 MAPK-activated kinases MK2/3. *Immunol Cell Biol*. 2019;97:54-71.
  58. Swierczak A, Mouchemore KA, Hamilton JA, Anderson RL. Neutrophils: important contributors to tumor progression and metastasis. *Cancer Metastasis Rev*. 2015;34:735-751.
  59. Mollinedo F. Neutrophil degranulation, plasticity, and cancer metastasis. *Trends Immunol*. 2019;40:228-242.
  60. Zhang HF, Wu MX, Lin YQ, et al. IL-33 promotes IL-10 production in macrophages: a role for IL-33 in macrophage foam cell formation. *Exp Mol Med*. 2017;49:e388.
  61. Yanagawa H, Takeuchi E, Suzuki Y, Ohmoto Y, Bando H, Sone S. Presence and potent immunosuppressive role of interleukin-10 in malignant pleural effusion due to lung cancer. *Cancer Lett*. 1999;136:27-32.
  62. Sharma S, Stolina M, Lin Y, et al. T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. *J Immunol*. 1999;163:5020-5028.
  63. Vahl JM, Friedrich J, Mittler S, et al. Interleukin-10-regulated tumour tolerance in non-small cell lung cancer. *Br J Cancer*. 2017;117:1644-1655.
  64. Salamon P, Mekori YA, Shefler I. Lung cancer-derived extracellular vesicles: a possible mediator of mast cell activation in the tumor microenvironment. *Cancer Immunol Immunother*. 2020;69:373-381.
  65. Zhang C, Tang B, Hu J, et al. Neutrophils correlate with hypoxia microenvironment and promote progression of non-small-cell lung cancer. *Bioengineered*. 2021;12:8872-8884.
  66. Colbeck EJ, Ager A, Gallimore A, Jones GW. Tertiary lymphoid structures in cancer: drivers of antitumor immunity, immunosuppression, or bystander sentinels in disease? *Front Immunol*. 2017;8:1830.
  67. Miotto D, Lo Cascio N, Stendardo M, et al. CD8+ T cells expressing IL-10 are associated with a favourable prognosis in lung cancer. *Lung Cancer*. 2010;69:355-360.
  68. Soria JC, Moon C, Kemp BL, et al. Lack of interleukin-10 expression could predict poor outcome in patients with stage I non-small cell lung cancer. *Clin Cancer Res*. 2003;9:1785-1791.
  69. Wang Y, Yi J, Chen X, Zhang Y, Xu M, Yang Z. The regulation of cancer cell migration by lung cancer cell-derived exosomes through TGF- $\beta$  and IL-10. *Oncol Lett*. 2016;11:1527-1530.
  70. Takahashi Y, Horio H, Hato T, et al. Prognostic significance of preoperative neutrophil-lymphocyte ratios in patients with stage I non-small cell lung cancer after complete resection. *Ann Surg Oncol*. 2015;22(suppl 3):S1324-S1331.
  71. Liu W, Zhang T, Li L, Zou J, Xu C. Assessing the prognostic value of the neutrophil-to-lymphocyte ratio in stage I non-small-cell lung cancer with complete resection. *Can Respir J*. 2022;2022:6837872.
  72. Cata JP, Gutierrez C, Mehran RJ, et al. Preoperative anemia, blood transfusion, and neutrophil-to-lymphocyte ratio in patients with stage I non-small cell lung cancer. *Cancer Cell Microenviron*. 2016;3:e1116.
  73. Mizuguchi S, Izumi N, Tsukioka T, Komatsu H, Nishiyama N. Neutrophil-lymphocyte ratio predicts recurrence in patients with resected stage 1 non-small cell lung cancer. *J Cardiothorac Surg*. 2018;13:78.
  74. Chen Y, Wang W, Zhang X, et al. Prognostic significance of combined preoperative platelet-to-lymphocyte ratio and lymphocyte-to-monocyte ratio in patients undergoing surgery with stage IB non-small-cell lung cancer. *Cancer Manag Res*. 2018;10:5411-5422.
  75. Jin F, Han A, Shi F, Kong L, Yu J. The postoperative neutrophil-to-lymphocyte ratio and changes in this ratio predict survival after the complete resection of stage I non-small cell lung cancer. *Oncol Targets Ther*. 2016;9:6529-6537.
  76. Xie M, Gao J, Ma X, et al. The radiological characteristics, tertiary lymphoid structures, and survival status associated with EGFR mutation in patients with subsolid nodules like stage I-II LUAD. *BMC Cancer*. 2024;24:372.
  77. Zou Y, Zhao J, Huang F, Xiang X, Xia Y. Decreased tertiary lymphoid structures in lung adenocarcinomas with ALK rearrangements. *J Clin Med*. 2022;11:5935.