# The solid subtype, according to the IASLC/ATS/ERS classification of lung adenocarcinoma, as a predictive marker for PD-L1 expression

**Dachuan Zhang**
The Third Affiliated Hospital of Soochow University
**Xie Gao**
The Third Affiliated Hospital of Soochow University
**Yongqiang Shi**
The Third Affiliated Hospital of Soochow University
**Zhantao Yan**
The Third Affiliated Hospital of Soochow University
**Wenting He**
The Third Affiliated Hospital of Soochow University
**Qing Li** ( 6603752@qq.com )
The Third Affiliated Hospital of Soochow University

**Research Article**

**Keywords:** non-small cell lung cancer, adenocarcinoma, PD-L1, TPS

**Posted Date:** February 18th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-199151/v1

# Abstract

**Background:** PD-L1 expression in tumor cells can predict the efficacy of PD-1/PD-L1 inhibitors and prognosis in patients. However, the correlation between the PD-L1 expression and the novel lung adenocarcinoma classification are obscure.

**Methods:** 126 lung adenocarcinoma cases were reviewed in the Third Affiliated Hospital of Soochow University from Jan. to Dec. 2019. PD-L1 (DAKO 22C3) was used to test the PD-L1 expression in lung cancer tissue.

**Result:** TPS was used to interpret the PD-L1 expression. The negative, low positive and high positive of PD-L1 were 72 cases (57.14%), 39 cases (30.95%) and 15 cases (11.90%). PD-L1 TPS in solid structure was significantly higher than that in acinar structure, lepidic structure and papillary structure (P<0.001, respectively). The results of $c^2$ test showed the PD-L1 expression had the significant difference with gender ($P$ = 0.005), age ($P$ = 0.030), smoking history ($P$ = 0.024), lymph node metastasis ($P$ <0.001), TNM stage ($P$ = 0.001), acinar structure ($P$ = 0.003) and solid structure ($P$ < 0.001). Multi-factor linear regression results suggested that solid structure, TNM stage and smoking history were associated with PD-L1 expression ($P$ < 0.05). The solid structure showed more capability to PD-L1 expression (β = 0.428).

**Conclusion:** PD-L1 expression was heterogeneity in lung adenocarcinoma. The solid structure, TNM stage and smoking history were correlation to up-regulation of PD-L1 expression, and solid structure was the most importance factor.


# Background

Due to the highest incidence and mortality rate in kinds of malignant tumor, lung cancer is a major public health problem(1,2). Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, with lung adenocarcinoma (ADC) being most frequent(3-5). According to the new classification criteria of ADC released by International association for the study of lung cancer (IASLC), American thoracic society (AST) and European respiratory society (ERS) in 2011, lung ADC can be classified into acinar subtype, lepidic subtype, papillary subtype, micropapillary subtype, and solid subtype based on morphological characteristics(6). The recently-emerged targeted therapies and immunotherapies have good therapeutic effects on NSCLC. Among them, the immune checkpoint inhibitors represented by PD-1/PD-L1 have achieved significant therapeutic efficacy in clinical practice. According to KEYNOTE-024 study, PD-L1 expression in tumor cells can predict the efficacy of PD-1/PD-L1 inhibitors and prognosis in patients(7). At present, there is limited study on the correlation between lung cancer, especially the new pathological classification, and the expression of PD-L1. To this end, in the present study, we aimed to explore the expression of PD-L1 in lung cancer tissues and to investigate its relationship with the new classification of lung ADC.

# Materials And Methods

1. **Inclusion and exclusion criteria**

Inclusion criteria: 1. Puncture tissue or surgically excised tissue, which was pathologically diagnosed as invasive ADC; 2. Radiotherapy or chemotherapy was not performed prior to surgery; 3. Patients were older than 18 years. Exclusion criteria: 1. Combined with other primary tumors; 2. Recurrent tumors; 3. Pathological diagnosis of special types, including small cell lung cancer, adenosquamous carcinoma or unspecified lung cancer; 4. Adenocarcinoma in situ.

2. **Clinical data**

A total of 126 cases of pathologically confirmed lung ADC were collected from the Third Affiliated Hospital of Soochow University from January to December, 2019, including 32 cases of lung puncture and 94 cases of lung tumor resection. Patients were pathologically diagnosed according to the new classification criteria of lung ADC (2010), and the 8th UICC lung cancer TNM staging was used to determine TNM stage. Of the 126 patients, 52 were males and 74 were females, with age ranging from 38 to 79 years. There were 95 cases of lymph node examination, 24 lymph node metastatic cases. In terms of TNM staging, 60 cases were of stage I, 12 cases were of stage II, 16 cases were of stage III and 6 cases were of stage IV. Of the 126 ADC patients, 95 cases had acinar structures, 31 cases had lepidic structures, 49 cases had papillary structures, 21 cases had micropapillary structures and 38 cases had solid structures (Table 1).

3. **Immunohistochemistry staining**

All samples were fixed with neutral formaldehyde solution and embedded into paraffin. The paraffin-embedded sections were serially cut into 4 μm-thick slices for PD-L1 staining (DAKO PD-L1 22C3) on Autostainer Link 48 staining platform. The interpretation of PD-L1 was performed by two pathologists using a double-blind method. Tumor cells with PD-L1 staining on the cell membrane were positive cells. Tumor proportion score (TPS) was used to determine PL-1 staining: PD-L1 negative (TPS <1%), PD-L1 low-expression (1%≤TPS<50%), and PD-L1 high-expression (TPS≥50%).

4. **Statistical analysis**

Statistical analyses were carried out using the IBM SPSS statistical software V24.0 (IBM, Armonk, NY). $\chi^2$ test, one-way anova and multivariate linear regression model were used as appropriate. All statistical analyses were two-sided test and the statistical significance was defined as $P<0.05$.

# Results

1. **Comparison of PD-L1 expression between the five subtypes of the novel lung adenocarcinoma classification**

The details of PD-L1 expression in the five subtypes of the novel lung adenocarcinoma classification are shown in Table 1. The result of Mann-Whiteny U test suggested that the patients with the acinar or lepidic

structure frequently had low TPS of PD-L1 ($P$=0.019; $P$=0.009, Fig.1 A, B). The PD-L1 TPS of patients with papillary or micropapillary structure was no different from that of patients without those structure ($P$=0.169; $P$=0.201, Fig.1 C, D). However, there was a significant association between solid structure and high TPS of PD-L1 ($P$<0.001, Fig.1 E). The result of one-way anova showed that PD-L1 TPS in solid structure was significantly higher than that in acinar structure, lepidic structure and papillary structure ($P$<0.001, respectively, Fig.1 F).

2. **The correlation of PD-L1 expression with clinicopathological parameters and new classification of ADC**

IHC was used to detect PD-L1 expression in 126 cases of ADC tissues, revealing negative in 72 cases (57.14%), low expression in 39 cases (30.95%) and high expression in 15 cases (11.90%). The expression of PD-L1 was significantly correlated with gender, age, smoking history, lymph node metastasis, TNM stage, acinar structure and solid structure of patients ($P$ < 0.05Table 2). The high expression of PD-L1 was instructive in decision-making of clinical drug regimen for ADC patients. Therefore, patients were further divided into PD-L1 non/low expression (111 cases) and PD-L1 high expression groups (15 cases). As a result, high expression of PD-L1 was correlated with gender, smoking history, lymph node metastasis, TNM stage, acinar structure and solid structure of patients ($P$ < 0.05Table 2).

3. **Influencing factors of PD-L1 expression**

In this study, we aimed to investigate the following pathological parameters, including patient's age, gender, smoking history, lymph node metastasis, TNM stage, and lung morphological subtypes. First, a univariate linear regression equation was constructed to screen the independent variables (Table 3). Subsequently, the patient's age, gender, smoking history, lymph node metastasis, TNM stage, acinar structure and solid structure were included in the multivariate linear analysis in a stepwise method. The final multivariate linear regression equation was as follows: the expression of PD-L1 = -19.735 + 23.826 * solid structure + 20.866 * TNM stage + 18.836 * smoking history. The equation suggested that under the same conditions of other pathological parameters, PD-L1 expression was increased by 23.826 times if solid structures appeared in ADC tissues (B = 23.826, $P$ < 0.001, Table 3, Fig.2), PD-L1 expression was increased by  times when the lung ADC progressed from TNM stage I-II to TNM stage III-IV (B = 20.866, $P$ = 0.001, Table 3), PD-L1 expression was increased by 18.836 times in the case of smoking history (B = 18.836, $P$ = 0.001, Table 3). The comparison of the standardized regression coefficients of the three parameters indicated that the effect of solid structure (β = 0.428) was stronger than TNM stage (β = 0.345) and smoking history (β = 0.275) on PD-L1 expression.

# Discussion

In addition to traditional surgical resection and adjuvant chemoradiotherapy, immunotherapy has become an important novel approach for tumor treatment. PD-1 / PD-L1 inhibitors, as representative drugs, have significant therapeutic effects on various tumors(8). PD-L1 expression is heterogeneity in different types of cancer, along with the uniformed detection techniques and scoring standards(9,10). In

this study, DAKO PD-L1 22C3 was used to detect PD-L1 expression(11), The positive rate of PD-L1 in ADC tumor tissue was 42.86% (54/126), and the high expression rate was 11.90% (15/126). The expression of PD-L1 also varied between different gender and age, suggesting that more attention should be paid to PD-L1 detection and the possibility of clinical medication on women and patients no more than 60 years. PD-L1 is highly expressed in the tissues of patients with advanced lung cancer (with lymph node metastasis, TNM III-IV), which is consistent with previous studies(12,13) and also provides an objective basis for the application of PD-L1 inhibitors in patients with advanced cancer. In addition, the high expression of PD-L1 in ADC tissues of smoking patients suggests that smoking patients are more likely to benefit from PD-L1 inhibitors, which might be associated with the immunosuppressive state in the tumor microenvironment caused by smoking.

The 2011 new classification of lung ADC has recommended a detailed description of invasive ADCs based on different histological subtypes(6). In this study, we analyzed the correlation between PD-L1 expression and five common histological subtypes of lung ADC (acinous subtype, lepidic subtype, papillary subtype, micropapillary subtype, and solid subtype). Previous studies have revealed the significant correlation between solid subtypes and poor prognosis of patients(14,15). In this study, we found that the appearance of solid structures in cancer tissues was significantly associated with up-regulated expression of PD-L1 expression(16), suggesting that the solid structure, as a histological subtype correlated with poor prognosis, may be related to the immunosuppressive state in ADC tumor immune microenvironment. And these patients might benefit more from PD-L1 inhibitors. Multiple linear regression equations evaluating the influencing factors of PD-L1 expression suggested that solid structure, TNM stage and smoking history were significantly correlated with up-regulated expression of PD-L1, and solid structure was the most significant factor affecting PD-L1 expression. Here, we investigated the correlation between ADC histological subtypes and the expression of PD-L1. The percentage of ADC histological subtypes need to be calculated in further studies.

# Conclusion

The novel lung adenocarcinoma classification showed a superior correlation with patient's prognosis. The patient's selection for PD-L1 immunological therapy based on the PD-L1 expression. However, PD-L1 expression was heterogeneity in ADC. There was a significant association between solid structure and higher expression of PD-L1. The solid subtype is an important predictor of PD-L1 expression. The patients with solid subtype might benefit more from PD-L1 inhibitors.

# Declarations

### Ethical approval and consent to participate

This study protocol was approved by the Ethics Committee of Soochow University and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from patients before enrollment in this study.

### Consent for publication

Not applicable.

### Availability of data and materials

All data generated or analyzed during this study are included in this published article.


### Funding

This study was supported in part by the Young Talent Development Plan of Changzhou Health Commission (CZQM2020040).


### Competing interests

These authors declare that they have no competing interests.

### Authors' Contributions

DC Zhang and WT He participated in the design of the study and performed the statistical analysis. YQ Shi, ZT Yan, and X Gao participated in the Immunohistochemical staining. DC Zhang and Q Li drafted the manuscript. All authors read and approved the final manuscript.


### Acknowledgements

Not applicable.


## References


1.Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA Cancer J Clin,2018,68(6):394-424.

2.Wei KR, Yu X, Zheng RS, Peng XB, Zhang SW, Ji MF, et al. Incidence and mortality of liver cancer in China, 2010[J]. Chin J Cancer,2014,33(8):388-394.

3.Schwaederle MC, Patel SP, Husain H, Ikeda M, Lanman RB, Banks KC, et al. Utility of Genomic Assessment of Blood-Derived Circulating Tumor DNA (ctDNA) in Patients with Advanced Lung Adenocarcinoma[J]. Clin Cancer Res,2017,23(17):5101-5111.

4.Hsu FT, Liu HS, Ali AAA, Tsai PH, Kao YC, Lu CF, et al. Assessing the selective therapeutic efficacy of superparamagnetic erlotinib nanoparticles in lung cancer by using quantitative magnetic resonance imaging and a nuclear factor kappa-B reporter gene system[J]. Nanomedicine,2018,14(3):1019-1031.

5.Ooki A, Dinalankara W, Marchionni L, Tsay JJ, Goparaju C, Maleki Z, et al. Epigenetically regulated PAX6 drives cancer cells toward a stem-like state via GLI-SOX2 signaling axis in lung adenocarcinoma[J]. Oncogene,2018,37(45):5967-5981.

6.Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/american thoracic society/european respiratory society international multidisciplinary classification of lung adenocarcinoma[J]. J Thorac Oncol,2011,6(2):244-285.

7.Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Updated Analysis of KEYNOTE-024: Pembrolizumab Versus Platinum-Based Chemotherapy for Advanced Non–Small-Cell Lung Cancer With PD-L1 Tumor Proportion Score of 50% or Greater[J]. Journal of Clinical Oncology,2019,37(7):537-546.

8.Chen DS, Mellman I. Elements of cancer immunity and the cancer–immune set point[J]. Nature,2017,541(7637):321-330.

9.Ancevski Hunter K, Socinski MA, Villaruz LC. PD-L1 Testing in Guiding Patient Selection for PD-1/PD-L1 Inhibitor Therapy in Lung Cancer[J]. Molecular Diagnosis & Therapy,2017,22(1):1-10.

10.Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, et al. PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project[J]. Journal of Thoracic Oncology,2017,12(2):208-222.

11.Neuman T, London M, Kania-Almog J, Litvin A, Zohar Y, Fridel L, et al. A Harmonization Study for the Use of 22C3 PD-L1 Immunohistochemical Staining on Ventana's Platform[J]. J Thorac Oncol,2016,11(11):1863-1868.

12.Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial[J]. Lancet,2019,393(10183):1819-1830.

13.Pawelczyk K, Piotrowska A, Ciesielska U, Jablonska K, Gletzel-Plucinska N, Grzegrzolka J, et al. Role of PD-L1 Expression in Non-Small Cell Lung Cancer and Their Prognostic Significance according to Clinicopathological Factors and Diagnostic Markers[J]. Int J Mol Sci,2019,20(4):

14.Yoshiya T, Mimae T, Tsutani Y, Tsubokawa N, Sasada S, Miyata Y, et al. Prognostic Role of Subtype Classification in Small-Sized Pathologic N0 Invasive Lung Adenocarcinoma[J]. Ann Thorac Surg,2016,102(5):1668-1673.

15.Xu Y, Zhu C, Qian W, Zheng M. Comprehensive study of mutational and clinicopathologic characteristics of adenocarcinoma with lepidic pattern in surgical resected lung adenocarcinoma[J]. J

Cancer Res Clin Oncol,2017,143(1):181-186.

16.Shimoji M, Shimizu S, Sato K, Suda K, Kobayashi Y, Tomizawa K, et al. Clinical and pathologic features of lung cancer expressing programmed cell death ligand 1 (PD-L1)[J]. Lung Cancer,2016,98(69-75.

# Abbreviations

NSCLC, Non-small cell lung cancer; ADC, lung adenocarcinoma; IASLC, international association for the study of lung cancer; AST, American thoracic society ERS, European respiratory society; PD-1/PD-L1, programmed cell death protein 1/ Programmed cell death 1 ligand 1; TPS, Tumor proportion score.

# References

Table 1. Comparison of PD-L1 expression between the five subtypes of the novel lung adenocarcinoma classification

| Subgroup | Petients (126 cases) | | PD-L1 TPS | | | |
|---|---|---|---|---|---|---|
| | Number | % | Min. | Max. | Mean | SD |
| Acinar structure | 95 | 75.40 | 0 | 90 | 9.10 | 19.53 |
| Lepidic structure | 31 | 24.60 | 0 | 60 | 4.26 | 12.73 |
| Papillary structure | 49 | 38.89 | 0 | 90 | 11.14 | 11.14 |
| Micropapillary structure | 21 | 16.67 | 0 | 70 | 15.76 | 24.06 |
| Solid structure | 38 | 30.16 | 0 | 90 | 34.46 | 33.65 |

Table 2. The correlation of PD-L1 expression with clinicopathological parameters and new classification of ADC

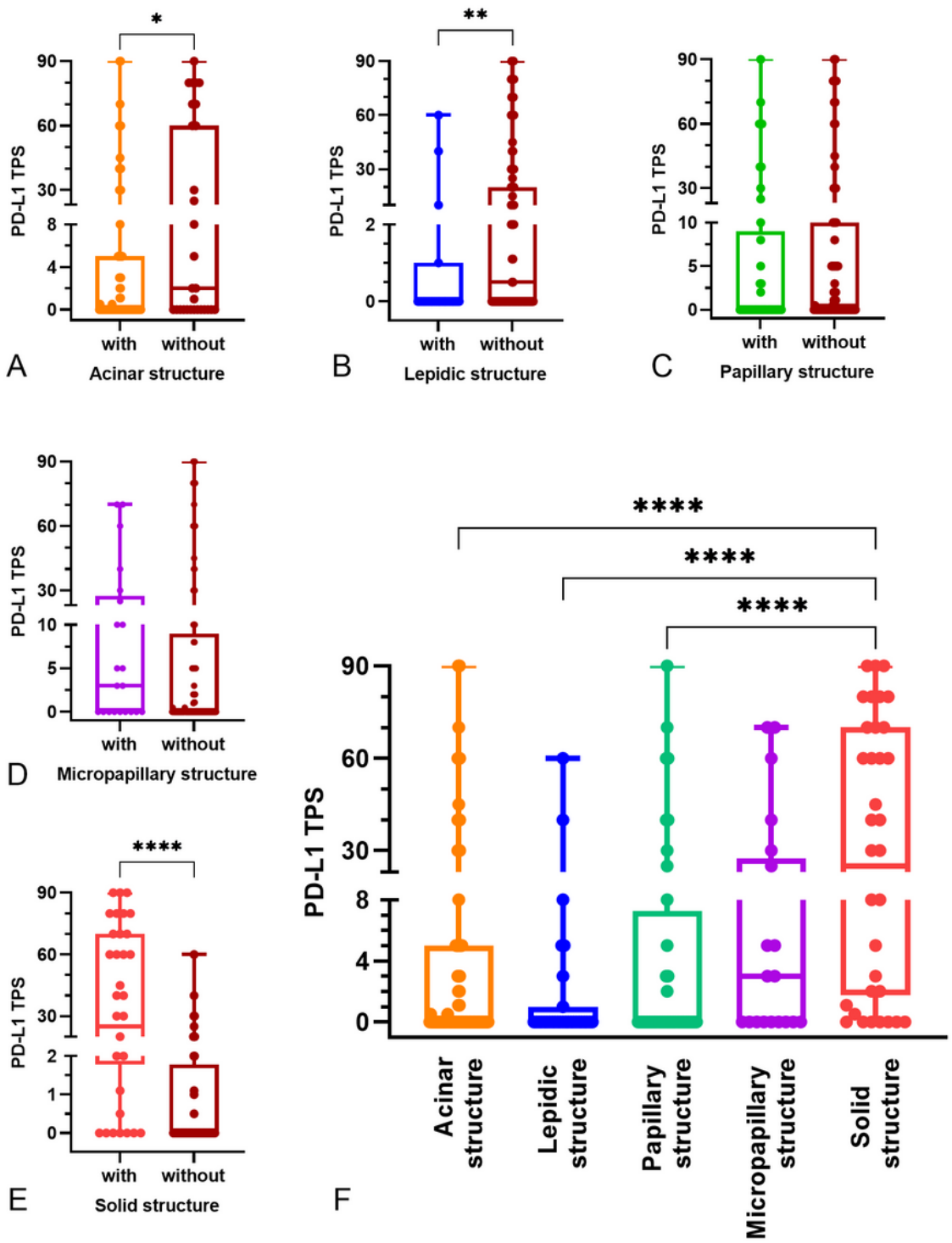
| Clinical parameters | PD-L1 | | | $\chi^2$ | *P* value | PD-L1 | | $\chi^2$ | *P* value |
|---|---|---|---|---|---|---|---|---|---|
| | - | 1+ | 2+ | | | -/1+ | 2+ | | |
| Gender | | | | | | | | | |
| Female | 50 | 20 | 4 | 10.665 | **0.005** | 70 | 4 | 7.222 | **0.007** |
| Male | 22 | 19 | 11 | | | 41 | 11 | | |
| Age (years) | | | | | | | | | |
| ≤60 | 35 | 11 | 3 | 6.989 | **0.030** | 46 | 3 | 2.556 | 0.110 |
| >60 | 37 | 28 | 12 | | | 65 | 12 | | |
| Smoking | | | | | | | | | |
| No | 63 | 34 | 9 | 7.424 | **0.024** | 97 | 9 | 7.422 | **0.006** |
| Yes | 9 | 5 | 6 | | | 14 | 6 | | |
| LN metastasis | | | | | | | | | |
| No | 46 | 20 | 5 | 15.283 | **<0.001** | 66 | 5 | 10.497 | **0.001** |
| Yes | 6 | 10 | 8 | | | 16 | 8 | | |
| TNM stage | | | | | | | | | |
| I-II | 45 | 22 | 5 | 13.422 | **0.001** | 67 | 5 | 12.238 | **<0.001** |
| III-IV | 7 | 7 | 8 | | | 14 | 8 | | |
| Acinar structure | | | | | | | | | |
| Without | 13 | 9 | 9 | 11.845 | **0.003** | 22 | 9 | 11.501 | **0.001** |
| With | 59 | 30 | 6 | | | 89 | 6 | | |
| Lepidic structure | | | | | | | | | |
| Without | 49 | 32 | 14 | 5.624 | 0.060 | 81 | 14 | 2.953 | 0.086 |
| With | 23 | 7 | 1 | | | 30 | 1 | | |
| Papillary structure | | | | | | | | | |
| Without | 40 | 27 | 10 | 2.212 | 0.331 | 67 | 10 | 0.221 | 0.638 |
| With | 32 | 12 | 5 | | | 44 | 5 | | |
| Micropapillary structure | | | | | | | | | |
| Without | 63 | 30 | 12 | 2.174 | 0.337 | 93 | 12 | 0.136 | 0.712 |
| With | 9 | 9 | 3 | | | 18 | 3 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Solid structure | | | | | | | | | |
| Without | 64 | 23 | 1 | 43.010 | **<0.001** | 87 | 1 | 32.263 | **<0.001** |
| With | 8 | 16 | 14 | | | 24 | 14 | | |

Table 3. Influencing factors of PD-L1 expression

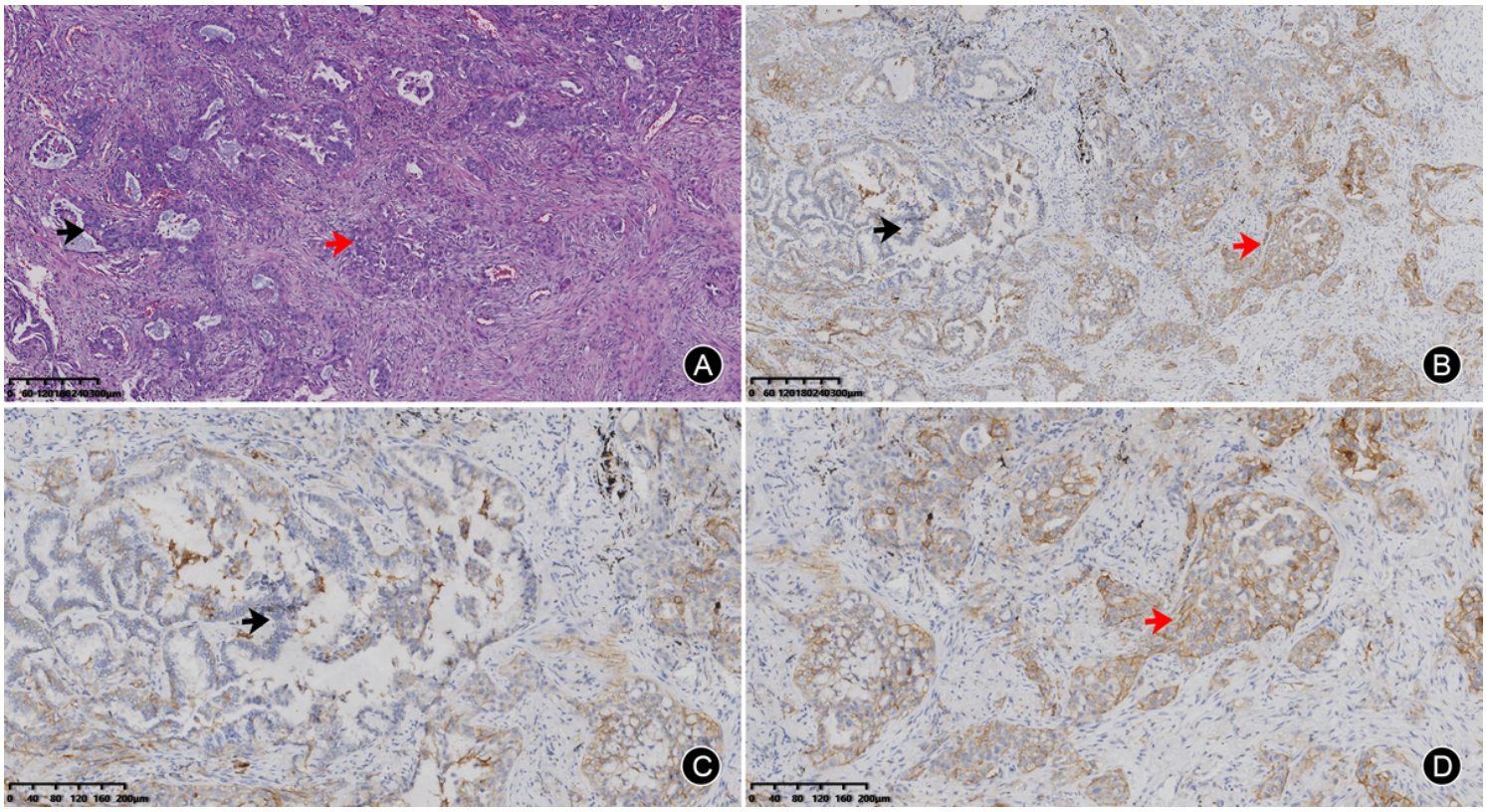
| Clinical parameters | Single-factor linear regression. | | | Multi-factor linear regression | | | |
|---|---|---|---|---|---|---|---|
| | B | SE | *P* value | B | SE | β | *P* value |
| Age (≤60/>60) | 9.476 | 4.419 | **0.034** | 4.542 | 4.179 | 0.087 | 0.280 |
| Gender (Female/Male) | 12.967 | 4.302 | **0.003** | -1.882 | 5.203 | -0.036 | 0.718 |
| Smoking (No/Yes) | 19.519 | 5.742 | **0.001** | 18.836 | 6.682 | 0.275 | **0.006** |
| LN metastasis (No/Yes) | 23.089 | 5.624 | **<0.001** | -4.485 | 6.652 | -0.076 | 0.502 |
| TNM stage (I-II/III-IV) | 25.941 | 5.724 | **<0.001** | 20.966 | 6.280 | 0.345 | **0.001** |
| Acinar structure (Without/With) | -15.677 | 4.896 | **0.002** | -4.570 | 4.978 | -0.080 | 0.361 |
| Lepidic structure (Without/With) | -11.534 | 4.988 | **0.022** | 3.316 | 5.542 | 0.051 | 0.551 |
| Papillary structure (Without/With) | -2.964 | 4.493 | 0.511 | - | - | - | - |
| Micropapillary structure (Without/With) | 3.370 | 5.879 | 0.568 | - | - | - | - |
| Solid structure (Without/With) | 30.797 | 3.899 | **<0.001** | 23.826 | 5.391 | 0.428 | **<0.001** |

# Figures




## Figure 1

The comparison of PD-L1 expression between the five subtypes of the novel lung adenocarcinoma classification. The box charts were used to show the difference of PD-L1 TPS between the patients with and without acinar structure (A), lepidic structure (B), papillary structure (C), micropapillary structure (D) and solid structure (E). The result of the comparison between groups showed that PD-L1 TPS in solid structure was significantly higher than that in acinar structure, lepidic structure and papillary structure (F).




## Figure 2

PD-L1 expression in the lung ADC. The solid subtype (red arrow) and papillary subtype (black arrow) presented in the lung ADC (A). The immunohistochemical staining for PD-L1 (B). The papillary subtype (black arrow) was the PD-L1 negative (C), but the solid subtype (red arrow) was the PD-L1 positive (D).