Feasibility and reliability of evaluate PD-L1 expression determination using small biopsy specimens in non-small cell lung cancer

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Funding information
National Natural Science Foundation of China

Abstract
Background: Programmed cell death ligand-1 (PD-L1) is a useful biomarker in non-small cell lung cancer (NSCLC) patients who would probably benefit from immunotherapy. In most patients with advanced stage NSCLC, only small biopsy specimens were available for the evaluation of PD-L1 expression. In this study, we evaluated the feasibility and reliability of PD-L1 testing on small biopsy samples.

Methods: Small specimens of advanced NSCLC patients obtained via endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA), endobronchial biopsy (EBB), or computed tomography (CT)-guided core-needle biopsy were collected. Tumor cell count and tissue sufficiency for PD-L1 immunohistochemistry (IHC) were evaluated and compared. The clinical course of patients who received immunotherapy in the study population was also examined.

Results: Tissue acquisitions for PD-L1 testing in three groups were all above 90%, with no statistically significant differences. The PD-L1 expression levels were concordant in most patients with more than one sample (8/11). In the EBB group, PD-L1-positive patients had higher objective response rate (ORR) (53.2% vs. 26.9%, p = 0.048) and longer progression-free survival (PFS) (312 vs. 179 days, p = 0.035) than PD-L1 negative patients. In the core needle biopsy group, patients with positive PD-L1 expression also trended to have higher ORR and longer PFS. However, in the EBUS-TBNA group, both ORR and PFS were similar between patients with positive or negative PD-L1 expression.

Conclusions: This study showed that EBUS-TBNA, EBB, and core needle biopsy provides adequate samples for PD-L1 testing. The predictive value of PD-L1 expression on different small samples still warrants further studies.

KEYWORDS
Core needle biopsy, EBB, EBUS-TBNA, immune checkpoint inhibitor, PD-L1 expression

INTRODUCTION

The use of immune checkpoint inhibitors (ICIs) yields high response rates against many malignancies and has become the standard-of-care treatment for advanced non-small cell lung cancer (NSCLC). Programmed death ligand 1 (PD-L1) expression on cancer cells is useful as both a predictor and a biomarker for selecting patients who would probably benefit from immunotherapy. For example, pembrolizumab has been approved as a first-line treatment for metastatic NSCLC with >50% positivity of PD-L1 staining. Guidelines recommend performing PD-L1 tests on surgical or core needle biopsy specimens. However, in advanced NSCLC, small biopsy specimens from endobronchial biopsy
(EBB) or endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) are often the only available material from cancer tissue for histological analysis. The utility of EBB and EBUS-TBNA for sample acquisition for molecular analysis of epidermal growth factor receptor and anaplastic lymphoma kinase is well documented. However, the adequacy of PD-L1 expression determination after comprehensive genetic profiling with next-generation sequencing (NGS) on small biopsy specimens acquired through bronchoscopy has not been systematically evaluated to date. Furthermore, PD-L1 expression has been found to vary between different regions within the same tumor, and previous studies found that PD-L1 expression in lymph nodes may not be a biomarker for efficacy. The predictive value of PD-L1 expression on small samples has also not well established. In this study, our primary goal was to determine the success rate of assessing PD-L1 expression after NGS testing of samples obtained with EBUS-TBNA, EBB, and core needle biopsy in patients with advanced NSCLC. A secondary aim was to evaluate the predictive value of PD-L1 expression tested in small samples.

**METHODS**

**Patients**

We searched the database for patients who were diagnosed with NSCLC in Peking Union Medical College Hospital between January 2018 and September 2020. Patients whose tissue specimens were obtained through EBB, EBUS-TBNA, or CT-guided core needle biopsy were included. Patients with EGFR positive gene mutations and ALK rearrangements were not excluded. Demographic information, periprocedural data, the type of biopsy procedure, biopsy sites, histological diagnosis, and NGS results were retrospectively reviewed.

**Sampling and histological procedures**

Bronchoscopy and EBUS-TBNA were performed by experienced pulmonologists and CT-guided core needle biopsy was performed by interventional thoracic radiologists following standard protocols. As per routine standard of practice, after obtaining informed consent, linear EBUS bronchoscopy (BF-UC260F, Olympus) and biopsy of mediastinal or hilar lymph nodes were performed under topical anesthesia using a 21 G needle (ViZiShot 2, Olympus America Inc). For central tumors, EBB was performed using forceps under direct vision. For peripheral tumors, CT-guided core needle biopsy was performed after local anesthesia with 1% xylocaine. Procedures were guided by 64-slice spiral CT scanner and 18-gauge biopsy needles were used. Tissues were subjected to hematoxylin and eosin (HE) staining and IHC staining for histological evaluation. NSG was performed in all the nonsquamous and some of the squamous lung cancer specimens. PD-L1 staining was performed on archived samples.

PD-L1 immunostaining was performed using the Dako 22C3 antibody clone (mouse monoclonal primary anti-PD-L1 antibody, prediluted, clone 22C3, Dako) according to a previously described protocol. The level of PD-L1 expression was based on a tumor proportion score (TPS) which was defined as the percentage of viable tumor cells showing partial or complete membrane labeling regardless of intensity or completeness. Nontumor elements such as infiltrating immune cells, normal cells, necrotic cells, and debris, were excluded from interpretation. The PD-L1 expression was scored from 0% to 100%. The score was interpreted as “negative” when PD-L1 expression was <1%, “low” when PD-L1 expression was ≥1% to <50%, and “high” when PD-L1 expression was ≥50%.

**Ethical considerations**

This retrospective study was reviewed and approved by the Center for Ethics of Peking Union Medical College Hospital.

**Statistical analysis**

Statistical analysis was performed using SPSS 16.0 (SPSS Inc.). Continuous variables were summarized as the mean (± standard deviation) and were compared using the Student’s t-test (GraphPad Software Inc). Categorical variables were expressed as a frequency (percentage) and were compared using the χ2 test or Fisher’s exact test (GraphPad Software Inc). A nonparametric test was used to compare tumor cell numbers between groups. Survival curves were estimated with the Kaplan–Meier method. Statistical significance level was defined as a two-sided p value < 0.05.

**RESULTS**

**Clinicopathological characteristics**

A total of 245 specimens were analyzed from NSCLC patients. The median age was 62 years (range: 33–79 years), with most patients being male (82.9%), at stage IV (92.7%) of the disease, and with a history of smoking (72.7%). Fifty-nine specimens (24.1%) were obtained with EBUS-TBNA procedures, 109 (44.5%) with endobronchial biopsies, and 77 (31.4%) with CT-guided procedures. For pathological subtypes, 113 (50.6%) patients had a diagnosis of adenocarcinoma, 124 (46.1%) of squamous cell carcinoma, and 8 (3.3%) of other diagnoses (adenosquamous or not otherwise specified [NOS]). Age, gender, and smoking history were similar between the groups. Adenocarcinoma was the main histological type in core needle biopsy specimens whereas squamous cell carcinoma was more frequent in EBB specimens. NGS of driver mutations including EGFR, ALK ROS1, RET, and MET had been performed in 179 specimens before IHC of PD-L1. Driver gene
mutations other than EGFR and ALK tested positive in seven specimens (including one BRAF, two MET-14 skipping two ROS-1 and two RET rearrangements). The patient characteristics are shown in Table 1.

**Tissue acquisition and PD-L1 assessment in samples acquired by different biopsy techniques**

Most specimens of the three groups contained sufficient tumor cells (more than 100 tumor cells) for TPS evaluation. The failure rate among patients diagnosed with EBUS-TBNA, EBB, and core needle biopsy were 6.8%, 9.2%, and 6.5%, respectively, with no statistically significant difference among groups. The ratio of high, low, and negative PD-L1 expression was also similar among groups. Table 2 shows the number of tumor cells and the levels of PD-L1 expression of the specimens.

Eleven patients had more than one sample obtained via different biopsy techniques. The PD-L1 expression in different samples was also compared. The results showed that PD-L1 expression was concordant in eight patients. The PD-L1 expression of one patient was negative in the core-needle biopsy sample but positive in the EBUS-TBNA sample. Two other patients had different PD-L1 expression levels between samples. These paired results are shown in Table 3.

**Response to immunotherapy**

A total of 176 patients received immunotherapy. In first-line therapy, ICIs were given with platinum-based doublet combination chemotherapy. The most used platinum-doublet combinations in patients with non-squamous histological type were pemetrexed plus carboplatin or cisplatin. The most used combination chemotherapies in patients with squamous histological type were albumin-bound paclitaxel or paclitaxel plus carboplatin. In the second and above lines therapy, ICIs were used as monotherapy. The objective response rate in ICIs combination therapy and monotherapy were 47.9% and 20%, respectively. The median PFS in first- and second-line therapy was 312 days and 179 days, respectively (Figure 1).

The predictive value of PD-L1 expression tested on different specimens was also compared. The ORR in positive and negative PD-L1 expression tested on core needle biopsy, EBB, and EBUS-TBNA were 47.6% versus 29.4% ($p = 0.252$), 53.2% versus 26.9% ($p = 0.048$), and 33.3% versus 36.4% ($p = 1.000$), respectively. The PFS in positive and negative PD-L1 expression patients in different groups were 354 versus 195 days ($p = 0.410$), 312 versus 179 days ($p = 0.035$), and 213 versus 192 days ($p = 0.594$), respectively. The survival curves of patients are shown in Figure 2.

**DISCUSSION**

Use of ICIs and PD-L1 expression monitoring have improved survival in NSCLC patients. In clinical trials, core-needle biopsy and surgery specimens have been used to determine PD-L1 expression. In patients with end stage disease, however, EBB and EBUS-TBNA are widely used as the initial diagnostic procedure. Moreover, biopsy under bronchoscopy is often the only way to obtain specimens for the diagnosis of NSCLC in certain circumstances. Therefore, we conducted this study to assess the reliability of small samples obtained with bronchoscopy and core-needle biopsy for PD-L1 testing.
In this study, we demonstrate that the small specimens from the three biopsy methods were sufficient for PD-L1 testing after cytohistological subtyping and genetic testing which is consistent with previous reports.12 The satisfactory rates were all above 90%. Although the cellularity of EBB samples tended to be lower than those of EBUS and core-needle biopsy samples, the differences were not statistically significant.

Previous studies showed that PD-L1 testing on small biopsies may be highly discordant from testing preformed on larger specimens because of the heterogeneity of PD-L1 expression.13,14 The discrepancy in PD-L1 expression between metastatic lymph node and primary tumor has also been reported.15–17 However, some subsequent studies using EBUS-TBNA samples showed good concordance with primary tumor, especially when using dichotomized PD-L1 cut-offs of 1% and 50%.16,18 In our study, 11 patients had more than one specimen using different sampling methods. PD-L1 expression was highly concordant between samples using dichotomized cutoffs, with only three patients having inconsistent results. The results are consistent with those of previous studies.14

To investigate the clinical value of PD-L1 testing on small samples, we also studied the response to ICIs in patients with positive expression of PD-L1 on samples obtained by different methods. We found that patients with positive PD-L1 tested on EBB specimens had higher ORR and longer PFS than patients with negative PD-L1 expression. The results were comparable with previous research.9,19 In patients whose PD-L1 was tested on core needle biopsy specimens the ORR and PFS were trended to be higher in the PD-L1 positive group than in the PD-L1-negative group but owing to the limited cases, the differences were not significant.

| Patient | Histology     | Sampling method | PD-L1 (%) | Sampling method | PD-L1 (%) |
|---------|---------------|-----------------|-----------|-----------------|-----------|
| 1       | Squamous      | EBB             | Negative (0) | EBUS            | Negative (0) |
| 2       | Adenocarcinoma| Core-needle     | Negative (0) | EBUS            | Low (20%)  |
| 3       | Squamous      | Core-needle     | Low (10%)  | EBB             | Low (10%)  |
| 4       | Squamous      | EBB             | Negative (0) | EBUS            | Negative (0) |
| 5       | Squamous      | EBB             | Low (20%)  | EBUS            | High (80%) |
| 6       | Squamous      | EBB             | Low (35%)  | EBUS            | High (60%) |
| 7       | Adenocarcinoma| Core-needle     | High (50%) | EBB             | High (70%) |
| 8       | Adenocarcinoma| EBB             | Negative (0) | EBB             | Negative (0) |
| 9       | Adenocarcinoma| Core-needle     | Negative (0) | EBB             | Negative (0) |
| 10      | Adenocarcinoma| Core-needle     | Negative (0) | EBUS            | Negative (0) |
| 11      | Adenocarcinoma| Core-needle     | Low (10%)  | EBUS            | Low (20%)  |

**TABLE 3** Programmed death ligand 1 expression of patient in different samples

![Figure 1](image_url)  
**Figure 1** Progression-free survival (PFS) of patients. (a) PFS in all the patients receiving first-line immunotherapy. (b) PFS in all the patients receiving second-line and above immunotherapy.
In this study we also evaluated the predictive value of PD-L1 expression on EBUS-TBNA samples. Unlike core-needle and EBB specimens, EBUS-TBNA specimens were mainly from metastasis lymph nodes instead of the original tumor. Some studies doubt the predictive value of PD-L1 expression on metastasis lymph nodes, but the results are inconsistent. In our study, the ORR and PFS of patients with different PD-L1 expression levels tested on EBUS-TBNA specimens were similar. Despite the limited number of cases, the predictive value of EBUS-TBNA sample still needs to be evaluated further.

Our study has some limitations. First, it was performed at a single institution and the number of cases was limited. Second, because we did not collect specimens from patients with positive EGFR and ALK driver gene mutations, the specimen sufficiency for PD-L1 testing in these patients is unknown. Moreover, in this study, all the patients were at an advanced stage and surgical materials were not available. Therefore, the assessment of concordance between small samples and in surgical materials were not compared. The findings of this study should thus be interpreted with caution. Further studies on larger prospective cohorts are warranted to evaluate the reliability of PD-L1 expression on small samples.

In conclusion, this study showed that both EBUS-TBNA, EBB, and core needle biopsy specimens were adequate to determine the status of PD-L1 expression in NSCLC after detection of driver mutations by NGS. Positive PD-L1 expression on EBUS-TBNA sample might not predict better efficacy and longer PFS of immunotherapy and still warrants further studies.

ACKNOWLEDGMENTS
This work was supported by the National Natural Science Foundation of China (Grant No. 81702292, and No. 82003309).

CONFLICT OF INTEREST
The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

REFERENCES
1. Garon EB, Rizvi NA, Hui R, Leight N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372:2018–28. https://doi.org/10.1056/NEJMoal501824
2. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Caóski T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375:1823–33.
3. NCCN Guidelines Insights: Non-Small Cell Lung Cancer, Version 2.2021. J Natl Compr Canc Netw. 2021;19:254–66. https://doi.org/10.6004/jnccn.2021.0013
4. Wahidi MM, Herth F, Yasufuku K, Shepherd RW, Yarmus L, Chawla M, et al. Technical aspects of endobronchial ultrasound-guided transbronchial needle aspiration: CHEST guideline and expert panel report. Chest. 2016;149:816–35.
5. Turner SR, Buonocore D, Desmeules P, Rekhtman N, Dogan S, Lin O, et al. Feasibility of endobronchial ultrasound transbronchial needle aspiration for massively parallel next-generation sequencing in thoracic cancer patients. Lung Cancer. 2018;119:85–90.
6. Kage H, Kohsaka S, Shinozaki-Ushiku A, Hiraishi Y, Sato J, Nagayama K, et al. Small lung tumor biopsy samples are feasible for high quality targeted next generation sequencing. Cancer Sci. 2019;110:2652–7. https://doi.org/10.1111/cas.14112
7. Yang H, Hall SRR, Yao F. The value of PD-L1 expression in metastatic lymph nodes of advanced non-small cell lung cancer. Chest. 2020;158:1785–7. https://doi.org/10.1016/j.chest.2020.06.077
8. Hong L, Negaro MV, Dibaj SS, Chen R, Reuben A, Bohac JM, et al. Programmed death-ligand 1 heterogeneity and its impact on benefit from immune checkpoint inhibitors in NSCLC. J Thorac Oncol. 2020;15:1449–59.
9. Ilie M, Khambata-Ford S, Copie-Bergman C, Huang L, Jusco J, Hofman V, et al. Use of the 22C3 anti-PD-L1 antibody to determine PD-L1 expression in multiple automated immunohistochemistry platforms. PLoS One. 2017;12:e0183023.
10. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med. 2018;378:2078–92.
11. Gandhi L, Garassino MC. Pembrolizumab plus chemotherapy in lung cancer. N Engl J Med. 2018;379:e18.
12. Kim I, Kim A, Lee CH, Lee G, Kim A, Jo EI, et al. Reliability of PD-L1 assays using small tissue samples compared with surgical specimens. Medicine. 2019;98:e14972. https://doi.org/10.1097/MD.0000000000014972
13. Ilie M, Long-mira E, Bence C, et al. Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of
NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies. Ann Oncol. 2016;27:147–53.

14. Heymann JJ, Bulman WA, Swinarski D, Pagan CA, Crapanzano JP, Haghhighi M, et al. PD-L1 expression in non-small cell lung carcinoma: comparison among cytology, small biopsy, and surgical resection specimens. Cancer Cytopathol. 2017;125:896–907. https://doi.org/10.1002/cncy.21937

15. Sakakibara R, Inamura K, Tambo Y, Ninomiya H, Kitazono S, Yanagitani N, et al. EBUS-TBNA as a promising method for the evaluation of tumor PD-L1 expression in lung cancer. Clin Lung Cancer. 2017;18:527–34. e521.

16. Kim S, Koh J, Kwon D, Keam B, Go H, Kim YA, et al. Comparative analysis of PD-L1 expression between primary and metastatic pulmonary adenocarcinomas. Eur J Cancer. 2017;75:141–9.

17. Uruga H, Bozkurtlar E, Huynh TG, Muzikansky A, Goto Y, Gomez-Caraballo M, et al. Programmed cell death ligand (PD-L1) expression in stage II and III lung adenocarcinomas and nodal metastases. J Thorac Oncol. 2017;12:458–66.

18. Sakata KK, Midhun DE, Mullon JJ, Kern RM, Nelson DR, Edell ES, et al. Comparison of programmed death ligand-1 immunohistochemical staining between endobronchial ultrasound transbronchial needle aspiration and resected lung cancer specimens. Chest. 2018;154:827–37.

19. Gadgeel S, Rodríguez-Abreu D, Speranza G, Esteban E, Felip E, Dómine M, et al. Updated analysis from KEYNOTE-189: pembrolizumab or placebo plus pemetrexed and platinum for previously untreated metastatic nonsquamous non-small-cell lung cancer. J Clin Oncol. 2020;38:1505–17. https://doi.org/10.1200/JCO.19.03136

20. Mineura K, Hamaji M, Yoshizawa A, Nakajima N, Kayawake H, Tanaka S, et al. Diagnostic yield of endobronchial ultrasound-guided transbronchial needle aspiration to assess tumor-programmed cell death ligand-1 expression in mediastinal lymph nodes metastasized from non-small cell lung cancer. Surg Today. 2020;50:1049–55. https://doi.org/10.1007/s00595-020-01989-6

21. Perrotta F, Nankivell M, Adzie B, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for PD-L1 testing in non-small cell lung cancer. Chest. 2020;158:1230–9. https://doi.org/10.1016/j.chest.2020.04.059

How to cite this article: Chen M, Xu Y, Zhao J, Li J, Liu X, Zhong W, et al. Feasibility and reliability of evaluate PD-L1 expression determination using small biopsy specimens in non-small cell lung cancer. Thorac Cancer. 2021;12:2339–44. https://doi.org/10.1111/1759-7714.14075