Original Article

The detection value of PD-L1 expression in biopsy specimens and surgical resection specimens in non-small cell lung cancer: a meta-analysis

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Background: The detection value of different types of specimens for programmed death ligand-1 (PD-L1) expression remains controversial. As such, the purpose of this meta-analysis was to compare the detection value of biopsy specimens and surgical resection specimens for PD-L1 expression in patients with non-small cell lung cancer (NSCLC).

Methods: PubMed and Web of Science were searched prior to December 2020 to identify studies that compared the detection value of biopsy specimens and surgical resection specimens for PD-L1 expression in NSCLC. Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 scale was used to evaluate the quality of the literature included. The detection value of different types of specimens for PD-L1 expression was then assessed. Besides, the relative risk (RR) with 95% CI were pooled using Review Manager 5.3 software and Stata 14.0 software.

Results: The meta-analysis involved 12 articles and included 877 patients. There was no significant difference in the detection rate of PD-L1 at the 1% cutoff between biopsy specimens and surgical resection specimens (RR =0.89, 95% CI: 0.70–1.12, P=0.33). However, there was a significant difference between two groups when the cutoff is 50% (RR =0.69, 95% CI: 0.58–0.83, P<0.01). In addition, a subgroup analysis of the type of biopsy specimens and the PD-L1 qualitative immunohistochemistry (IHC) assays showed that the detection rate of PD-L1 in small biopsies and using the SP142 antibody were lower than in surgical specimens and using other antibodies for both the 1% and 50% cut-offs (P<0.01).

Conclusions: Current evidence suggests that caution must be taken when using biopsy specimens from patients with advanced NSCLC to evaluate PD-L1 status eligible for immunotherapy, additional biopsy specimens sampling may be needed to minimize the risk of tumor misclassification. In addition, PD-L1 qualitative IHC assays and the type of biopsy specimens related to PD-L1 expression detection.

Keywords: Biopsy; specimen; programmed cell death ligand-1 (PD-L1); non-small cell lung cancer (NSCLC)

Introduction

Lung cancer is the second most common malignancy and the most common cause of cancer-related mortality worldwide, among which non-small cell lung cancer (NSCLC) accounts for more than 85% of cases (1,2). Patients with NSCLC often have locally advanced tumor or distant metastases at the time of diagnosis. For these patients, surgical treatment can no longer achieve radical treatment. In recent years, immunotherapy, such as Programmed death-1 (PD-1) and its Programmed death ligand-1 (PD-L1), which hinder tumor proliferation by blocking inhibitory pathways is important progress in oncology and a new direction for tumor therapy (3). At present, several clinical studies have confirmed that blocking the interaction between PD-1/PD-L1 with monoclonal antibodies can effectively alleviate the progression of advanced NSCLC and significantly improve the survival time of patients (4-7).

PD-L1 immunohistochemistry (IHC) is used to select patients for immunotherapy. Currently, a variety of PD-1 inhibitory monoclonal antibodies are available for the treatment of NSCLC patients. Pembrolizumab is approved by the Food and Drug Administration (FDA) for first-line treatment in patients with tumors showing ≥50% tumoral staining for PD-L1 (8). With the development of clinical trials, Pembrolizumab has also received FDA approval for second-line treatment in patients with advanced NSCLC who have received one or more previous treatment regimen and who have a tumor proportion score of at least 1%. KEYNOTE-010 showed that Pembrolizumab extended overall survival in patients with PD-L1 positive tumor cells (PD-L1 ≥1% Cutoff) (6). In addition, Pembrolizumab significantly prolonged progression-free and overall survival in patients with advanced NSCLC and PD-L1 expression at least 50% of tumor cells based on the result from the KEYNOTE-001 and KEYNOTE-024 trial (8,9).

However, the use of immune checkpoint inhibitors may potentially cause severe immune-related adverse events such as pneumonitis and endocrine diseases, and the expression level of PD-L1 in tumor cells is closely related to the degree of PD-1 blocking response in patients. Therefore, accurate detection of PD-L1 expression in tumor cells is an important link to guide the use of immune checkpoint inhibitors and predict the prognosis of patients (10-12). Recent clinical trials have found that the expression level of PD-L1 in tumor cells has a certain predictive value for NSCLC patients who can benefit from immunotherapy. However, the studies of Taube *et al.* and Rittmeyer *et al.* both reported that NSCLC patients

with negative PD-L1 expression also can benefit from immunotherapy (12,13). There may be a variety of reasons for this phenomenon: the biological complexity of newly discovered tumor-immune interactions, the heterogeneity of PD-L1 expression (14,15). Despite these limitations, the most recent National Comprehensive Cancer Network guidelines also recommend that PD-L1 expression should be measured in all NSCLC patients who intend to undergo immunotherapy.

In 2016, Ilie *et al.* compared the detection value of PD-L1 expression in tumor cells from biopsy specimens and surgical resection specimens of 160 NSCLC patients, and proposed for the first time that the different detection value of these two specimens might be a potential problem for the application of PD-L1 immunotherapy (16). Subsequently, Li *et al.* once again proved that the PD-L1 expression levels of tumor cells in biopsy specimens and surgical resection specimens were inconsistent, suggesting that the sensitivity of PD-L1 detection using biopsy specimens was lower and some NSCLC patients who could benefit from immunotherapy might be wrongly excluded (17). However, the studies of Munari *et al.* and Hernandez *et al.* both showed that biopsy specimens can replace surgical resection specimens for the detection of PD-L1 expression in tumor cells of NSCLC patients (18,19). Given the two controversial views mentioned above, the purpose of this meta-analysis was to evaluated and analyzed the accuracy of PD-L1 expression level detection in biopsy specimens and surgical resection specimens of NSCLC patients from the perspective of evidence-based medicine.

We present the following article in accordance with the PRISMA reporting checklist (available at https://dx.doi.org/10.21037/jtd-21-543) (20).

Methods

Search strategy

A systematic literature search was conducted in PubMed and Web of Science databases from inception to December 2020. The Mesh terms included *biopsy*; *cytologic*; *specimen*; *programmed cell death ligand-1 (PD-L1)*; and *lung cancer*.

Study selection

The criteria for inclusion were as follows: (I) patients who were diagnosed with NSCLC and accepted PD-L1 IHC; (II) diagnostic studies comparing biopsy specimens versus surgical resection specimens; (III) both biopsy specimens
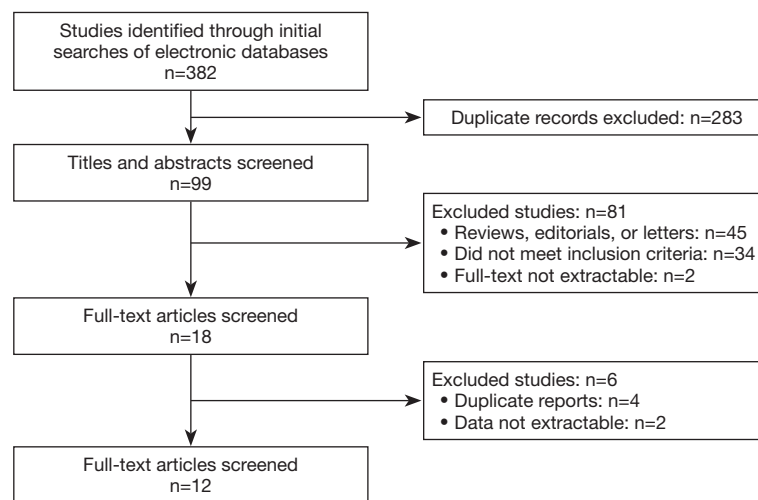
Figure 1 Flow diagram of study selection.

and surgical resection specimens should be available from the same patient; (IV) studies whose full texts were available and language was English. Studies were excluded based on the following criteria: publications with incomplete, useless data; review articles, meta-analysis, editorials, comments, letters, case reports, and animal experiments.

The selection of literatures was according to the process of reviewing titles, abstracts, and full texts. After preliminary screening of titles and abstracts, two independent researchers (J. D. and J. W.) assessed the full text to determine their conformity with the criteria. A consensus was achieved after discussion in case of disagreement.

Data extraction and quality assessment

Data from the included studies were extracted and summarized independently by two investigators (J. D. and J. W.) and then input into a Microsoft Excel (Microsoft Corporation, Redmond, WA, USA, 2019) database. The data extraction included the following: first author, time of publication; numbers of patients, median age, pathology or histology of cancer, PD-1/PD-L1 antibody, type of IHC specimens, and research type. Discrepancies in data extraction were resolved by consensus with a senior thoracic surgeon with professional-clinical knowledge (C. C.).

The quality of the included studies was independently evaluated according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 evaluation standard, which mainly consists of four parts: case selection, trial to be evaluated, gold standard, case flow, and progress (21).

All components were assessed for risk of bias, with the first three components also assessed for clinical suitability.

Statistical analysis

All mentioned analysis resorted to Review Manager 5.4 (Cochrane Collaboration, Oxford, UK) and Stata 14.0 (StataCorp, College Station, TX, USA). Relative risk (RR) with 95% confidence intervals (95% CI) were calculated for dichotomous variables. Heterogeneity in the meta-analysis was evaluated with I^2 test and Statistical heterogeneity was considered as I^2 statistic greater than 50% (22). The fixed-effects model was used if the heterogeneity was acceptable (P>0.10, or P≤0.10 but I^2 ≤50%); Otherwise, the random-effects model was used. Publication bias was checked by the funnel plots model and confirmed with Begg's and Egger's tests (23). The statistically significant P value was set at <0.05.

Results

Characteristics and quality assessment

A total of 564 potential articles were retrieved from PubMed and Web of Science databases up to December 2020. Finally, a total of 12 original studies (16-19,24-31) involved 877 patients comparing the detection value of biopsy specimens and surgical resection specimens for PD-L1 expression in NSCLC were obtained (*Figure 1*).

The baseline characteristics of the identified studies were summarized in *Table 1*. Except for the studies for which no

Table 1 Basic characteristic of included studies

Study (published year)	Country	N	Average age [range]a	M/F	Pathological stage				Histology (SCC/ADC/NOS)	PD-L1 IHC assays	PD-L1 ≥1%		PD-L1 ≥50%		Surgical resection specimens	Type of biopsy specimens	Biopsy modality	Study design
					I	II	III	IV			Biopsy specimens, n	Surgical resection specimens, n	Biopsy specimens, n	Surgical resection specimens, n				
Munari et al. (2018)	Italy	55	71 [51–83]	39/16	37	NR	NR	NR	6/45/4	SP263	NR	NR	10	15	Primary tumor	Cytologic specimens	FNA	R
Hernandez et al. (2019)	USA	38	68 [36–91]	NR	NR	NR	NR	NR	2/47/3	22C3	22	27	15	19	Primary tumor	Cytologic specimens	FNA	R
Xu et al. (2018)	USA	52	NR	NR	NR	NR	NR	NR	29/23/0	22C3	35	29	NR	NR	Primary tumor	Cytologic specimens	EBUS-TBNA/CT-TBNA	R
Russell-Goldman et al. (2018)	USA	46	67.5 [42–90]	13/33	NR	NR	NR	NR	3/36/7	Other	49	28	13	14	Primary tumor	Cytologic specimens	FNA	R
Ilie et al. (2016)	France	160	64 [41–85]	111/49	27	40	93	0	33/127/0	SP142	41	118	29	61	Primary tumor	Histological specimens	TBLB/CT-TBNA	R
Li et al. (2017)	China	190	56 [34–78]	134/56	53	43	78	16	74/96/0	22C3	63	70	21	26	Primary tumor	Tissue microarrays	NR	R
Sakata et al. (2018)	USA	61	68 [33–84]	30/31	2	17	41	1	21/39/1	22C3	21	29	10	15	Primary tumor	Histological specimens	EBUS-TBNA	R
Gradecki et al. (2018)	USA	51	65.5 [37–86]	NR	NR	NR	NR	NR	21/25/5	SP142	24	30	8	8	Primary tumor	Histological specimens	NR	R
Elfving et al. (2019)	Sweden	58	61 [40–82]	26/32	NR	NR	NR	NR	17/37/4	SP263	52	56	18	22	Primary tumor	Histological specimens/ tissue microarrays	NR	R
Kim et al. (2019)	Korea	46	69 [64–73]	35/11	18	18	10	0	24/20/2	22C3/SP142/ SP263	52	56	29	35	Primary tumor	Histological specimens	EBUS-TBNA/CT-TBNA	R
Kitazono et al. (2015)	Japan	79	68 [38–83]	50/28	38	18	23	0	23/44/11	Other	30	28	8	17	Primary tumor	Histological specimens	NR	R
Sakakibara et al. (2017)	Japan	41	63 [38–80]	30/11	NR	NR	NR	NR	16/24/1	Other	20	12	NR	NR	Primary tumor/lymph node	Histological specimens	EBUS-TBNA/TBLB	R

a, values for age were presented as average (minimum–maximum). M, male; F, female; PD-L1, programmed cell death ligand-1; SCC, squamous cell carcinoma; ADC, adenocarcinoma; NOS, not otherwise specified; R, retrospective study; FNA, fine-needle aspiration; EBUS-TBNA, endobronchial ultrasound-transbronchial needle aspiration; CT-TBNA, CT-guided transbronchial needle aspiration biopsy; TBLB, transbronchial lung biopsy; NR, not report.
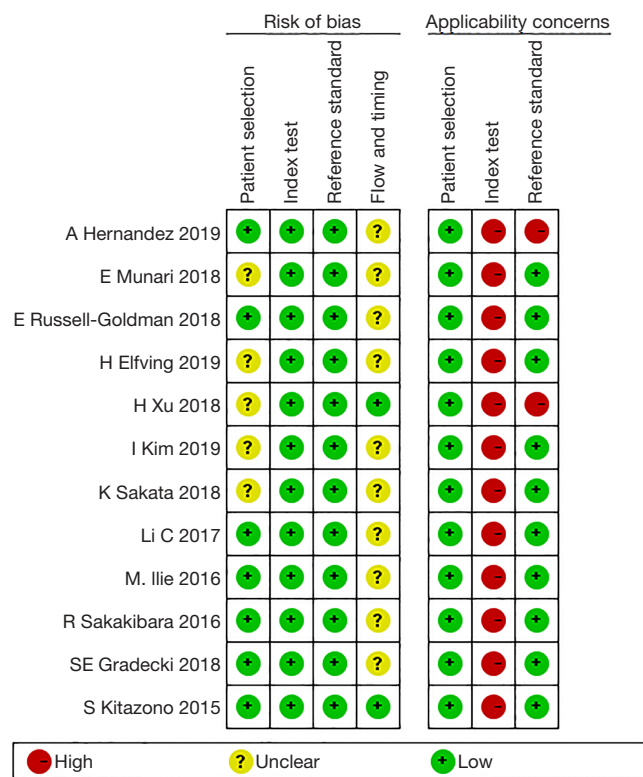
Figure 2 The risk of bias of included studies.

age data were available, only one study had an average age of less than 60 years old and immunostaining was conducted on tumor specimens mainly using the 22C3 or the SP142 or SP263 antibody clones. Only five original studies reported the complete staging information. Surgical resection specimens of all studies were primary tumors except for the study of Sakakibara *et al.* Modalities for biopsy specimens include fine-needle aspiration (FNA), endobronchial ultrasound-transbronchial needle aspiration (EBUS-TBNA), CT-guided transbronchial needle aspiration biopsy (CT-TBNA), and transbronchial lung biopsy (TBLB). In addition, all researches were retrospective cohort studies and published between 2015 and 2019. Overall, the quality of all the original studies was similar based on the QUADAS-2 evaluation standard (*Figure 2*).

PD-L1 ≥1% Cutoff

At a PD-L1 IHC staining cutoff of ≥1%, 11 original studies were evaluated and heterogeneity was identified in these included researches (P<0.01, I^2=85%). The meta-analysis indicated that there was not a significant difference in the

detection rate of PD-L1 at the 1% cutoff between biopsy specimens and surgical resection specimens (RR =0.89, 95% CI: 0.70–1.12, P=0.33; *Figure 3*).

PD-L1 ≥50% Cutoff

In regard to the PD-L1 IHC staining cutoff of ≥50%, 10 original studies were included in the analysis. Compared to surgical resection specimens, biopsy specimens experienced significantly higher detection value for PD-L1 expression (RR =0.69, 95% CI: 0.58–0.83, P<0.01), without evidence of heterogeneity (P=0.56, I^2=0%; *Figure 4*).

Subgroup analysis

Subgroup analysis was performed for the PD-L1 qualitative IHC assays and the type of biopsy specimens (*Table 2*). When compared with other PD-L1 qualitative IHC assays, the expression of PD-L1 in tumor cells detected by SP142 antibody was significantly reduced (1% cutoff, RR =0.48, 95% CI: 0.39–0.59, P<0.01; 50% cutoff, RR =0.53, 95% CI: 0.38–0.75, P<0.01). In regard to the type of specimens used in detection for PD-L1 expression, the value of histological specimens is lower than that of surgical resection specimens (1% cutoff, RR =0.75, 95% CI: 0.67–0.84, P<0.01; 50% cutoff, RR =0.64, 95% CI: 0.51–0.80, P<0.01).

Publication bias

Funnel plots of the original study results showed that multiple points were evenly distributed on both sides of the longitudinal axis, so there was no obvious publication bias, which was further identified by the Egger's test (1% cutoff, P=0.580, *Figure 5*; 50% cutoff, P=0.509, *Figure 6*).

Discussion

This meta-analysis of 12 original studies including 877 patients indicated that there was no significant difference in the detection rate of PD-L1 at the 1% cutoff between biopsy specimens and surgical resection specimens. However, there was a significant difference between the two groups when the cutoff is 50%. In addition, a subgroup analysis of the type of biopsy specimens and the PD-L1 qualitative IHC assays showed that the detection rate of PD-L1 in small biopsies and using the SP142 antibody were lower than in surgical specimens and using other antibodies for both the 1% and 50% cut-offs.
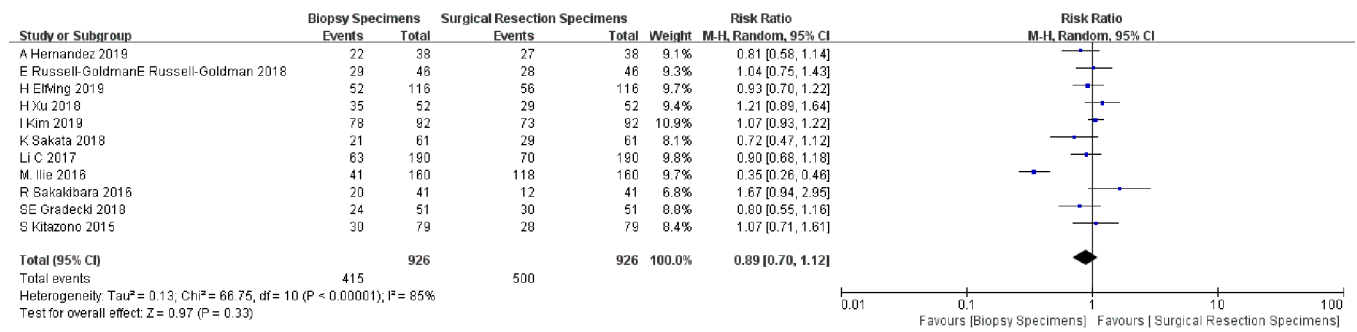
Figure 3 Meta-analysis: the detection rate of PD-L1 at the 1% cutoff between biopsy specimens and surgical resection specimens. PD-L1, programmed cell death ligand-1.
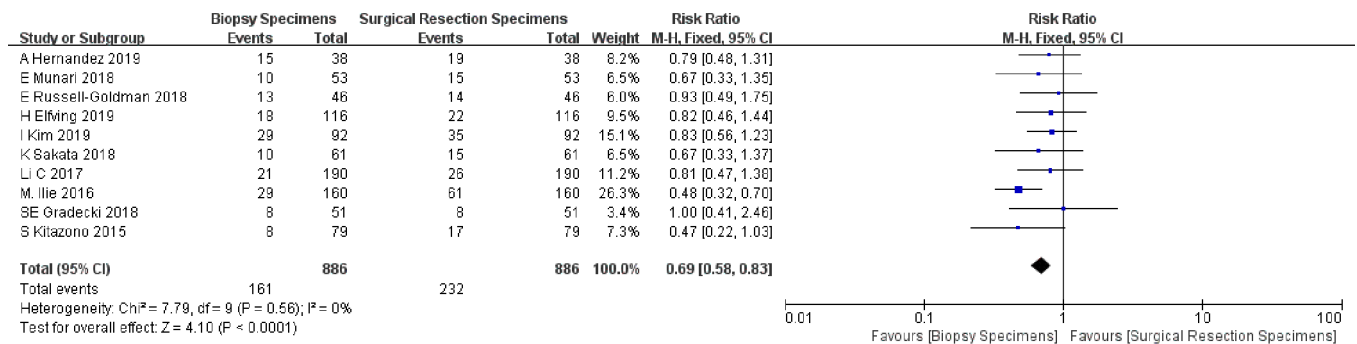

Figure 4 Meta-analysis: the detection rate of PD-L1 at the 50% cutoff between biopsy specimens and surgical resection specimens. PD-L1, programmed cell death ligand-1.

Table 2 Subgroup analyses for PD-L1 ≥1% Cutoff and PD-L1 ≥50% Cutoff

| Subgroups	Combined relative risk (95% CI), No. of studies	
	PD-L1 ≥1% Cutoff	PD-L1 ≥50% Cutoff
PD-L1 IHC assays	0.78 (0.71–0.86), 10	0.70 (0.58–0.84),10
SP263	0.98 (0.83–1.15), 2	0.79 (0.56–1.10), 3
22C3	0.93 (0.80–1.07), 5	0.79 (0.59–1.06), 4
SP142	0.48 (0.39–0.59), 3	0.53 (0.38–0.75), 3
Type of biopsy specimens	0.83 (0.76–0.91), 12	0.69 (0.58–0.83), 11
Histological specimens	0.75 (0.67–0.84), 7	0.64 (0.51–0.80), 6
Tissue microarrays	0.92 (0.73–1.15), 2	0.78 (0.50–1.23), 2
Cytologic specimens	1.02 (0.85–1.23), 3	0.79 (0.56–1.12), 3

PD-L1, programmed cell death ligand-1.
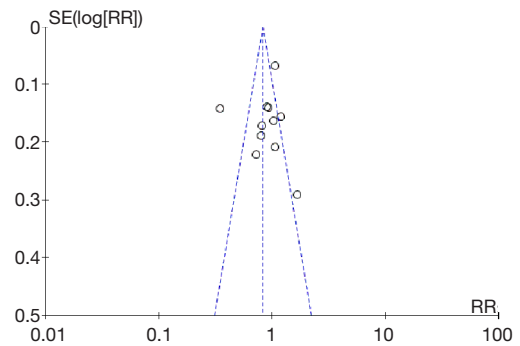
Figure 5 The funnel plot and publication bias tests for the detection rate of PD-L1 at the 1% cutoff between biopsy specimens and surgical resection specimens. PD-L1, programmed cell death ligand-1.
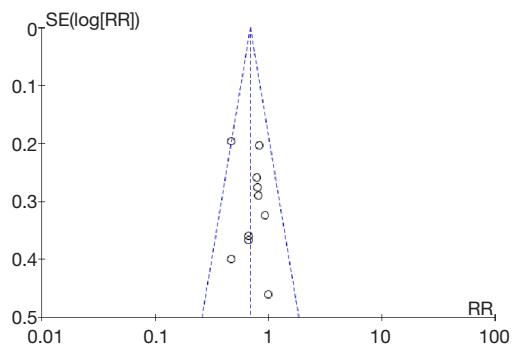
Figure 6 The funnel plot and publication bias tests for the detection rate of PD-L1 at the 50% cutoff between biopsy specimens and surgical resection specimens. PD-L1, programmed cell death ligand-1.

The treatment landscape for patients with NSCLC has dramatically changed after the discovery of immune checkpoint inhibitors, including PD-1 and PD-L1 inhibitors. The results of PD-L1 expression detection are of great significance for clinicians to formulate treatment plans and predict therapeutic efficacy, especially for clinical medication guidance of Pembrolizumab. Based on the current studies, Pembrolizumab is recommended for first-line treatment in advanced NSCLC patients with high PD-L1 expression (tumors showing ≥50% tumoral staining for PD-L1) and for second-line treatment in patients with advanced NSCLC who have received one or more previous treatment regimen and who have a tumor proportion score of at least 1% (6,8). The main outcome of the meta-analysis showed that when the threshold value was 1% or 50%, the detection value of the surgically resected specimen was higher than that of the biopsy specimen, and there was a significant difference in the latter. Therefore, in the selection of patients suitable for first-line treatment (tumor proportion score of at least 50%), multiple biopsy specimens may be needed to avoid the occurrence of false detection results and allow clinicians to make a more confident decision with regard to patient selection for therapy. Munari *et al.* reported in 2017 that caution must be taken when using biopsy specimens to assess immunotreatment-eligible patients with advanced NSCLC, requiring at least 4 biopsy specimens to minimize the risk of error (15). Then in 2018, Munari *et al.* compared the detection value of PD-L1 expression in 39 biopsy specimens and surgical resection specimens and found that four and three biopsy specimens had the highest accuracy in detecting PD-L1 expression when the threshold value was 1% and 50% respectively (32).

For each PD-1/PD-L1 inhibitor, a specific PD-L1 qualitative IHC assay was developed to assess PD-L1 expression levels on malignant NSCLC tumor cells and/or immune cells. They are not necessary for treatment but may support clinical decision-making. In order to analyze the influence of common PD-L1 monoclonal antibodies on the detection of PD-L1 expression in biopsies specimens and surgical resection specimens, we excluded two clinically infrequently used PD-L1 monoclonal antibodies and performed subgroup analysis on the antibodies used in the remaining studies. The results showed that compared to the SP263 IHC and the 22C3 IHC assays, the SP242 IHC assay resulted in an underestimation of PD-L1 expression in tumor cells. The findings are not surprising since each assay was developed and optimized as predictive biomarker for a specific antibody. The National Comprehensive Cancer Network analyzed 90 resected NSCLC tumor specimens stained using four PD-L1 IHC assays (28-8, 22C3, SP142, and E1L3N). Their results also showed that the SP142 assay detected lower PD-L1 expression levels on tumor and immune cells than the other assays (33). The overall concordance among the four assays was excellent (k=0.813), and was even higher if SP142 was excluded (k=0.971) (33). In addition, Hendry *et al.* obtained similar results as ours by comparing the effects of different PD-L1 monoclonal antibodies on IHC results (34). Judging from their studies, all assays detected tumor cells but to a different extent, probably confounded by pathologist variability in the overall scores for tumor cells across the assays. Since the purpose of our study is not to explore the differences among these antibodies, more clinical evidence is needed to prove this hypothesis.

Different types of biopsy specimens may have different IHC test results, so this study conducted a subgroup analysis on the types of biopsy specimens. Based on the experimental indicators, this present meta-analysis compared the surgical resection specimens with the histological specimens (9). However, not all patients with NSCLC have access to histological specimens; In more than 30% of patients, only cytological specimens obtained by fine needle aspiration (FNA) are available. In clinical practice, cytological specimens serve as the only diagnostic material available for PD-L1 expression in approximately 16% of patients. Meta-analysis indicated that histological specimens had a lower detection values than cytological specimens. However, Noll *et al.* reached a conclusion contrary to ours by comparing the value of detecting PD-L1 expression in tumor cells of cytological samples and histological samples from 41 NSCLC patients. They believed that the cytological

4308

Wang et al. Concordance of PD-L1 expression between biopsy and resection specimens

samples used for detecting PD-L1 expression in NSCLC patients were highly consistent with histological samples and could be used to evaluate PD-L1 expression (35).

To best of our knowledge, this is the first study comparing the detection value of biopsy specimens and surgical resection specimens for PD-L1 expression in patients with NSCLC. By expanding the influencing factors and results of PD-L1 expression detection in tumor cells, a comprehensive subgroup analysis was conducted to carefully analyze the potential confounding factors, and it was confirmed that the PD-L1 qualitative IHC assays and the type of biopsy specimens were the possible factors leading to the difference in PD-L1 expression between biopsy specimens and surgical resection specimens in NSCLC patients.

Our meta-analysis had several limitations that must be considered. One major limitation of this meta-analysis is that all included researches were retrospective cohort studies which have a certain inevitable bias. We evaluated the quality of 12 original studies based on the QUADAS-2 evaluation standard and judged most included studies at high quality. Although the funnel plot and bias test of major outcome did not indicate evident publication bias among included studies, it is necessary to wait for more results from RCT to draw a valid conclusion.

Conclusions

Current evidence suggests that caution must be taken when using biopsy specimens from patients with advanced NSCLC to evaluate PD-L1 status eligible for immunotherapy, additional biopsy specimens sampling may be needed in order to minimize the risk of tumor misclassification. In addition, PD-L1 qualitative IHC assays and the type of biopsy specimens related to PD-L1 expression detection.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the PRISMA reporting checklist. Available at https://dx.doi.org/10.21037/jtd-21-543

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/jtd-21-543). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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