CHEST

Accuracy of Cytologic vs Histologic Specimens for Assessment of Programmed Cell Death Ligand-1 Expression in Non-Small Cell Lung Cancer

A Systematic Review and Meta-Analysis

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BACKGROUND: Programmed cell death ligand-1 (PD-L1) expression on tumor cells, evaluated by immunohistochemistry, guides the use of immunotherapy in advanced non-small cell lung cancer (NSCLC).

RESEARCH QUESTION: What is the sensitivity and specificity of PD-L1 testing performed in cytologic vs paired histologic specimens in patients with NSCLC?

STUDY DESIGN AND METHODS: The MEDLINE, Embase, Web of Science, and Cochrane Library databases were searched through June 1, 2021. The primary outcome was pooled sensitivity and specificity of PD-L1 testing performed on cytologic specimens compared with the reference standard of histologic specimens, analyzed at the PD-L1 expression cutoffs (tumor proportion score) ≥ 1% and ≥ 50%. Pooled sensitivity and specificity, and associated 95% CIs, were estimated using bivariate generalized linear mixed models.

RESULTS: Twenty-six articles were included, encompassing a total of 1,064 pairs of histology specimens and cytology cell blocks, and 267 pairs of histology specimens and direct smears. Among these, 946 paired specimens were acquired without interval treatment between the collection of histology and cytology samples. The pooled sensitivity and specificity of cytology specimens compared with paired histology specimens at the PD-L1 expression cutoff ≥ 1% were 0.84 (95% CI, 0.77-0.89) and 0.88 (95% CI, 0.82-0.93), respectively, whereas the pooled sensitivity and specificity at cutoff ≥ 50% were 0.78 (95% CI, 0.69-0.86) and 0.94 (95% CI, 0.91-0.96), respectively. When only paired specimens acquired without interval treatment were considered, the pooled sensitivity and specificity of cytology specimens at PD-L1 expression cutoff ≥ 1% were 0.84 (95% CI, 0.76-0.90) and 0.89 (95% CI, 0.82-0.94), respectively, whereas the pooled sensitivity and specificity at cutoff ≥ 50% were 0.80 (95% CI, 0.71-0.89) and 0.94 (95% CI, 0.91-0.96), respectively.

INTERPRETATION: Cytologic specimens provide an accurate assessment of PD-L1 expression in most patients with NSCLC, at both ≥ 1% and ≥ 50% cutoffs, when compared with histologic specimens.

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KEY WORDS: cytology; diagnostic accuracy; immunotherapy; non-small cell lung carcinoma; PD-L1

Take-home Points

Study Question: What is the diagnostic accuracy of cytologic vs paired histologic specimens for assessment of programmed cell death ligand-1 (PD-L1) expression in patients with non-small cell lung cancer?

Results: The pooled sensitivity values of cytologic specimens at the clinically relevant PD-L1 expression cutoffs of ≥ 1% and ≥ 50% were 84% and 78%, respectively, whereas specificity values of cytologic specimens at these same cutoffs were 88% and 94%, respectively. When only paired specimens acquired in the absence of interval treatment were considered, the pooled sensitivity of cytologic specimens compared with paired histologic specimens at the ≥ 50% PD-L1 expression cutoff was 80%, with similar specificity.

Interpretation: Cytologic specimens provide an accurate assessment of PD-L1 expression in most patients with non-small cell lung cancer, at both ≥ 1% and ≥ 50% cutoffs, when compared with histologic specimens.

The introduction of immune checkpoint inhibitors (ICIs) into clinical practice has transformed the care of patients with non-small cell lung cancer (NSCLC). Programmed cell death protein-1 (PD-1) is a transmembrane receptor expressed on T cells. Interaction with programmed cell death ligand-1 (PD-L1), a transmembrane protein on tumor cells, inhibits the immune system's ability to destroy cancer cells. ICIs, whether targeting the PD-1 receptor or its ligand PD-L1, break this inhibitory system and allow T cells to attack cancer cells.[1,2]

Patients whose tumors demonstrate higher levels of PD-L1 expression derive greater clinical benefit from immunotherapy.[3-5] PD-L1 expression on tumor cells, assessed by immunohistochemistry (IHC), guides the use of immunotherapy in patients with advanced NSCLC. Currently, pembrolizumab (anti-PD-1) can be administered as a first-line single agent or combined with chemotherapy in patients with PD-L1 expression on at least 50% of tumor cells in a biopsy sample, what is known as tumor proportion score (TPS) ≥ 50%. Pembrolizumab combined with chemotherapy is approved for the front-line treatment of metastatic NSCLC with PD-L1 expression < 50%.[4,6,7]

The pivotal clinical trials of ICIs primarily examined PD-L1 expression using histologic samples. In the real-world setting however, 30% to 40% of patients with advanced NSCLC are diagnosed and/or staged with cytologic specimens exclusively.[8] Minimally invasive procedures have a prominent role in the investigation of patients with NSCLC, and guidelines recommend concomitant diagnosis and staging, whenever safely feasible.[9,10] The evaluation of PD-L1 expression has been formally validated in formalin-fixed, paraffin-embedded tissue specimens, but not in cytologic specimens.[8,11]

There has been significant interest in the feasibility of using cytologic cell blocks for evaluation of PD-L1 expression. A global survey conducted by the International Association for the Study of Lung Cancer Pathology Committee[12] in 2019 revealed that cell blocks and smears were used for PD-L1 testing by 75% and 11% of all participants, respectively. PD-L1 testing is feasible in cytologic samples,[13,14] but more limited data have examined the concordance of PD-L1 expression in paired cytologic and histologic specimens from patients with NSCLC.[15-17] The study by Sakata et al[16] in particular raised concerns about the risk of false-negative results when PD-L1 expression was assessed in endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) samples vs surgical resection specimens. Two previously published systematic reviews[18,19] have examined the percent agreement of

ABBREVIATIONS: EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration; FNA = fine needle aspiration; ICI = immune checkpoint inhibitor; IHC = immunohistochemistry; NSCLC = non-small cell lung cancer; PD-1 = programmed cell death protein-1; PD-L1 = programmed cell death ligand-1; QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies; TPS = tumor proportion score

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PD-L1 expression in cytologic vs histologic specimens, but no structured meta-analysis has explored the diagnostic accuracy of cytologic vs paired histologic specimens for assessment of PD-L1 expression. Herein, we aimed to systematically review and determine the sensitivity and specificity of PD-L1 testing performed in cytologic vs paired histologic specimens in patients with NSCLC.

Study Design and Methods

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement[20] and extension for Diagnostic Test Accuracy[21] and was registered in PROSPERO (No. CRD42020153279).

The literature search strategy was developed in collaboration with a McGill University medical librarian using Medical Subjects Headings terms related to NSCLC, PD-L1, and cytology (e-Table 1). The MEDLINE, Embase, Web of Science, and Cochrane Library databases were initially searched on October 7, 2019. Additional searches were performed on May 23, 2020, and June 1, 2021. No restriction was made based on language or date of publication; only human studies were included. Two reviewers (P. T. and F. A. or A. J. A.) independently screened all unique titles and abstracts, and subsequently evaluated full-text articles for inclusion. The reference lists of published systematic reviews were reviewed manually. All disagreements were resolved through discussion with a third reviewer (A. V. G).

Inclusion Criteria

Primary studies were included according to the following criteria: (1) patients with NSCLC for whom two separate cytologic and histologic specimens were evaluated for PD-L1 expression, (2) PD-L1 assessment by IHC with any antibody clone and/or platform, (3) specimens with $\geq$ 100 tumor cells, and (4) full-text article published in the indexed literature. Prospective and retrospective studies comparing the results of PD-L1 testing in paired human histologic and cytologic NSCLC specimens were considered. Two cutoffs of PD-L1 expression, namely $\geq$ 50% and $\geq$ 1% TPS, were used for comparison of paired specimens.

Histologic specimens included surgical resection specimens, surgical biopsies, transbronchial biopsies, endobronchial biopsies, pleural biopsies, and so-called core biopsies.[11] Cytologic specimens included transthoracic needle aspirations, EBUS-TBNAs, fine needle aspirations (FNAs), endoscopic brushings, BALs, and pleural or pericardial fluid samples. Paired specimens were defined as cytologic and histologic specimens acquired from the same patient, with or without interval treatment between acquisition of the two specimens.

Exclusion Criteria

Studies were excluded based on the following criteria: (1) PD-L1 testing in malignancy other than NSCLC, (2) specimens obtained outside the human body (eg, cytologic specimens obtained by FNA of the resected primary tumor), (3) specimens with $<$ 100 tumor cells for assessment of PD-L1 expression, (4) IHC method for assessment of PD-L1 expression not specified, and (5) insufficient information to calculate sensitivity and/or specificity using histologic specimens as the reference standard, even after repeated correspondence with authors. Non-peer reviewed literature was also excluded.

Quality Assessment

Two independent reviewers (P. T., F. A., or A. J. A.) evaluated the quality of included studies using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool for diagnostic accuracy studies.[22] The QUADAS-2 assessment covers both risk of bias and applicability concerns. Risk of bias is reviewed in four domains: patient selection, index test, reference standard, and flow and timing. Applicability concerns are evaluated in three domains including patient selection, index test, and reference standard. All disagreements were resolved through discussion.

Data Extraction

Each reviewer independently extracted the following data from each included article, using data extraction forms: author and year; study design (prospective or retrospective); antibody used for PD-L1 testing; type of platform used for PD-L1 testing (commercially available vs laboratory-developed test); type of cytologic specimens; cytologic specimen preparation method (cell block vs direct smear); fixative agent used for cytologic specimens; type of histologic specimens; number of paired specimens; time interval between acquisition of paired specimens; number of true-positive, false-positive, true-negative, and false-negative results using histologic specimens as the reference standard with the PD-L1 expression cutoffs $\geq$ 50% and $\geq$ 1%; and interval treatment between acquisition of paired specimens, if any. The occurrence of interval treatment was ascertained from the reported data, or through contact with authors. Studies for which the occurrence of

interval treatment could not be determined were excluded from the no interval treatment analyses. All extracted data were reviewed, and any disagreements were resolved by consensus. In cases where multiple antibody clones were used in a given study, we selected one antibody from each study for pooled analyses to avoid repeated data. The choice depended on the most common antibody used and/or the greatest number of paired samples.

Twenty-three authors were contacted by email (up to three email rounds) to request additional information, and to ensure no overlapping pairs between different publications. Twelve authors replied, and 11 authors were able to provide sufficient data for calculation of sensitivity and specificity using histologic specimens as the reference standard.

Analysis

The primary outcomes were pooled sensitivity and specificity of PD-L1 testing performed on cytologic specimens, compared with the reference standard of histologic specimens, analyzed at the PD-L1 expression cutoffs (TPS) $\geq$ 1% and $\geq$ 50%. Pooled sensitivity and specificity, and associated 95% CIs, were estimated using bivariate generalized linear mixed models,[23] including random effects at the level of the study for sensitivity and specificity, and allowing these to be correlated. Pooled positive predictive value and negative predictive value were also estimated using bivariate generalized linear mixed effects models.

The following prespecified subgroup analyses were performed: (1) diagnostic accuracy of cytologic vs histologic specimens in the absence of any interval treatment between collection of paired samples, (2) diagnostic accuracy of EBUS-TBNA samples vs paired histologic specimens for assessment of PD-L1 expression, (3) distinction of cell block and direct smear cytologic specimens vs paired histologic specimens for assessment of PD-L1 expression, and (4) diagnostic accuracy of cytologic vs histologic specimens according to PD-L1 antibody assay. Pooled sensitivity and specificity for subgroup analyses of cell block vs direct smears and according to antibody assay were estimated using bivariate generalized linear fixed-effects models because of convergence issues.

Heterogeneity was assessed using visual inspection of forest plots. All statistical analyses were carried out using the lme4 package in R.[24]

Declarations of Interest

This systematic review was funded in part by the Geoffrey Ogram Memorial Research Grant and Lung Cancer Canada.

Results

A total of 1,618 articles were identified from the initial search, with 23 articles selected for full-text review. Fifteen articles were subsequently added for full-text review from subsequent searches (Fig 1).

A total of 26 articles were included in the analysis, encompassing 1,064 pairs of histologic specimens and cytologic cell blocks, and 267 pairs of histologic specimens and direct smears. Among these, 946 paired specimens from 19 studies were acquired in the absence of any interval treatment (679 cell blocks and 267 direct smears). Three studies were prospective, whereas the remaining studies were retrospective. The most common antibody clone was 22C3. Four studies[15,25-27] reported results of PD-L1 testing using more than one antibody clone; the antibody selected for pooled analyses is specified (Table 1).[28-47]

The pooled sensitivity and specificity of cytologic specimens compared with paired histologic specimens at the PD-L1 expression cutoff $\geq$ 1% (n = 1,331) were 0.84 (95% CI, 0.77-0.89) and 0.88 (95% CI, 0.82-0.93), respectively, whereas the pooled sensitivity and specificity at the PD-L1 expression cutoff $\geq$ 50% were 0.78 (95% CI, 0.69-0.86) and 0.94 (95% CI, 0.91-0.96), respectively (e-Tables 2, 3; Fig 2). When only paired specimens acquired without interval treatment were considered, the pooled sensitivity and specificity of cytologic specimens at PD-L1 expression cutoff $\geq$ 1% were 0.84 (95% CI, 0.76-0.90) and 0.89 (95% CI, 0.82-0.94), respectively, whereas the pooled sensitivity and specificity at cutoff $\geq$ 50% were 0.80 (95% CI, 0.71-0.89) and 0.94 (95% CI, 0.91-0.96), respectively (e-Tables 4, 5; Fig 3). Individual study estimates of positive predictive values and negative predictive values, and pooled values, are detailed in e-Tables 2-5.

A total of 260 paired cytologic specimens were EBUS-TBNA samples, with 190 paired samples acquired in the absence of any interval treatment. The pooled sensitivity and specificity of cytologic specimens compared with paired histologic specimens at the PD-L1 expression cutoff $\geq$ 1% were 0.83 (95% CI, 0.73-0.92) and 0.84 (95% CI, 0.49-0.98), respectively, whereas the pooled sensitivity and specificity at the PD-L1 expression cutoff $\geq$ 50% were 0.78 (95% CI, 0.57-0.95) and 0.90

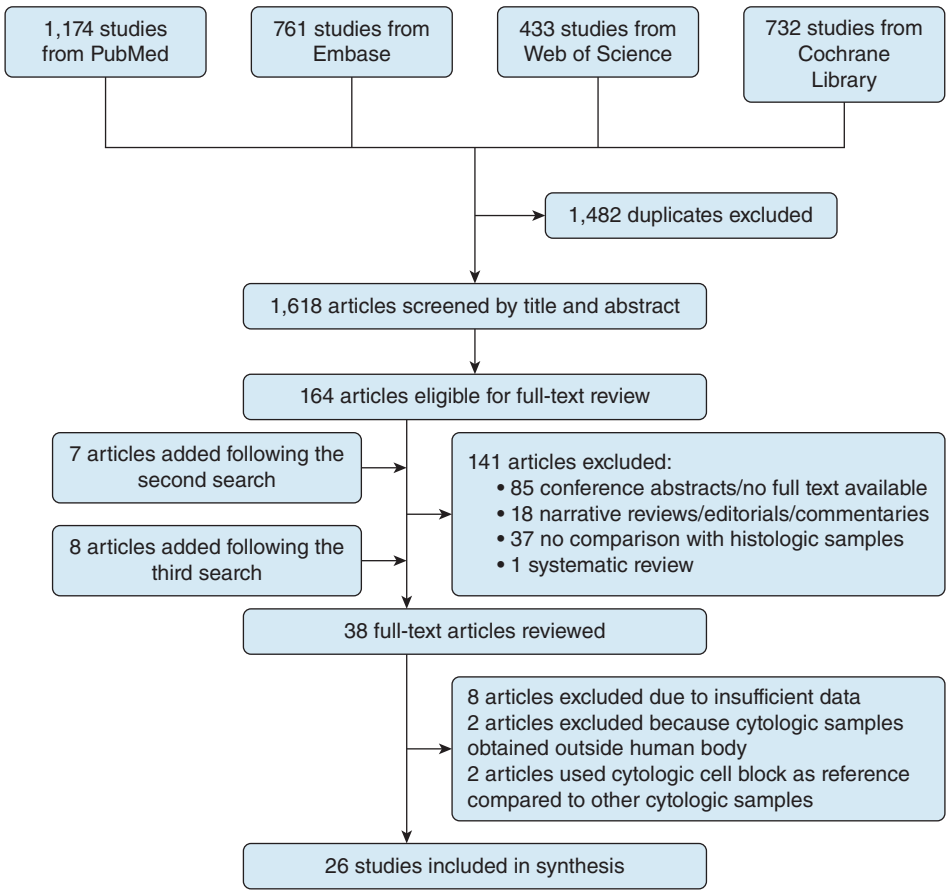

Figure 1 – *Preferred Reporting Items for Systematic Reviews and Meta-Analyses study flow diagram.*

(95% CI, 0.79-0.95), respectively (Fig 4). When only paired specimens acquired without interval treatment were considered, the pooled sensitivity and specificity of cytologic specimens at PD-L1 expression cutoff ≥ 1% were 0.81 (95% CI, 0.67-0.94) and 0.78 (95% CI, 0.29-0.97), respectively, whereas the pooled sensitivity and specificity at cutoff ≥ 50% were 0.86 (95% CI, 0.44-1.00) and 0.87 (95% CI, 0.69-0.95), respectively (e-Tables 6, 7).

The diagnostic accuracy of cytologic blocks, which constituted most paired cytologic specimens, was examined separately from direct smears. Cytologic cell blocks had a higher sensitivity than direct smears at both the 1% and 50% PD-L1 expression cutoffs, whereas pooled specificity was lower (e-Tables 8, 9). The most common antibody clone was 22C3 followed by SP263. The pooled analysis of paired specimens evaluated with the 22C3 antibody clone (15 studies) demonstrated the highest sensitivity, namely 0.85 (95% CI, 0.82-0.89) at the ≥ 1% cutoff and 0.80 (95% CI, 0.74-0.85) at the ≥ 50% cutoff. Further details are provided in e-Tables 10 and 11.

Quality assessment using QUADAS-2 found a high risk of bias in a number of studies. There were various sources of bias including possible unblinding in the interpretation of the index and reference tests, variable time intervals between acquisition of cytologic and histologic specimens (flow and timing), and possible selective enrollment of patients. A full quality assessment of the included studies is displayed in e-Table 12.

Discussion

ICIs have become an integral part of the management of patients with NSCLC, and their use continues to be guided by tumor cell PD-L1 expression.[7] In the current systematic review, the diagnostic accuracy of cytologic specimens for assessment of PD-L1 expression in patients with NSCLC was evaluated against paired histologic specimens. Pooled sensitivity of cytologic specimens at the clinically relevant PD-L1 expression cutoffs of ≥ 1% and ≥ 50% was 84% and 78%, respectively, whereas specificity of cytologic specimens at these same cutoffs was 88% and 94%, respectively. Importantly, in the subgroup of patients (n = 946) in whom paired samples were acquired in the absence of

TABLE 1] Study Characteristics

Study/Year	Study Type	Pairs	Antibody	Type of Cytology	Cytology Preparation	Fixation	Time Interval
Arriola et al[28]/2018	Retrospective	15	22C3	Mixed	Cell block	Formalin	Concurrent
Capizzi et al[29]/2018	Retrospective	49	SP263	FNA tumors or metastatic LN	Direct smear	Alcohol	0-90 d
Grosu et al[30]/2019	Retrospective	82	22C3	Pleural effusion	Cell block	Formalin	0-363 d
Hernandez et al[31]/2019	Retrospective	38	22C3	Mixed	Cell block	Formalin	N/A
Heymann et al[32]/2017	Retrospective	4	22C3	EBUS-TBNA 3, pleural/pericardial fluid 1	Cell block	Formalin	9 d to 4 mo
Ilie et al[33]/2018	Prospective	70	22C3	Bronchial washing, pleural fluid	Cell block	Formalin	Same day
Noll et al[34]/2018	Retrospective	38	22C3	Mixed	Cell block	Alcohol + formalin	Concurrent 30, separate 8 (1-66 d)
Pak and Roh[35]/2019	Retrospective	58	SP263	CT scan-guided TTNA	Cell block	Formalin	Median, 42 (7-93 d)
Sakata et al[16]/2018	Retrospective	47	22C3	EBUS-TBNA	Cell block	Formalin	4-329 d
Skov and Skov[15]/2017	Retrospective	69	22C3	Mixed	Cell block	Formalin	Within 6 wk
Smith et al[14]/2020	Retrospective	18	22C3	EBUS-TBNA	Cell block	Alcohol + formalin	0-180 d
Wang et al[36]/2018	Retrospective	20	22C3	Mixed	Cell block	Alcohol + formalin	Within 2 mo
Wang et al[37]/2019	Retrospective	34	22C3	EBUS-TBNA	Cell block	Alcohol + formalin	0-45 d
Yoshimura et al[38]/2019	Retrospective	68	E1L3N	EBUS-TBNA	Direct smear	None	Median, 25 (3-360 d)
Dong et al[25]/2020	Retrospective	112	28-8	Mixed	Cell block	Alcohol + formalin	1 wk to 1 mo
Hendry et al[39]/2020	Retrospective	29	SP263	Mixed	Cell block	Formalin	5-41 d
Song et al[40]/2020	Retrospective	29	SP263	Pleural effusion	Cell block	Formalin	N/A
Song et al[26]/2020	Retrospective	35	E1L3N	Mixed	Cell block	Formalin	5-13 mo
Zou et al[41]/2020	Retrospective	101	SP263	Pleural effusion	Cell block	Alcohol + formalin	0 d to 19.1 mo
Gagne et al[27]/2020	Retrospective	31	28-8	Mixed	Cell block	Alcohol + formalin	N/A
Ambrosini-Spaltro et al[42]/2021	Retrospective	13	22C3	FNA tumor, LN, metastatic site	Cell block	Formalin	0-7 mo
Daverio et al[43]/2020	Retrospective	60	SP263	TBNA, BAL	Cell block	Formalin	0 d

(Continued)

TABLE 1] (Continued)

Study/Year	Study Type	Pairs	Antibody	Type of Cytology	Cytology Preparation	Fixation	Time Interval
Jug et al[44]/2020	Retrospective	53	22C3	EBUS-TBNA	Cell block	Formalin	N/A
Lou et al[45]/2020	Retrospective	81	22C3	CT-FNA	Cell block	Formalin	1 mo (0-4 mo)
Martin-Deleon et al[46]/2021	Prospective	27	22C3	EBUS-TBNA	Cell block	Formalin	N/A
Ricci et al[47]/2020	Prospective	150	SP263	TTNA, EBUS, EUS	Direct smear	Other[a]	< 90 d

CT-FNA CT-guided fine needle aspiration; EBUS-TBNA = endobronchial ultrasound-guided transbronchial needle aspiration; EUS = endoscopic (or esophageal) ultrasound; FNA = fine needle aspiration; LN = lymph node; N/A = not applicable; TTNA = trans-thoracic needle aspiration.
[a]95% ethanol or isopropanol-based.

interval treatment, pooled sensitivity at the ≥ 50% cutoff was higher at 80%, whereas the estimated pooled specificity was similar. Overall, the present analysis provides reassuring results regarding the accuracy of cytologic specimens for assessment of PD-L1 expression in patients with NSCLC.

Cytologic specimens, including those acquired through minimally invasive needle techniques, play an important role in the diagnosis and staging of lung cancer. Minimally invasive needle techniques (eg, EBUS-TBNA) are first-line procedures in the investigation of patients with NSCLC with hilar and/or mediastinal involvement.[10] The samples acquired must provide sufficient tissue for diagnosis and NSCLC subtyping, and advanced molecular testing. The International Association for the Study of Lung Cancer Atlas[8] of PD-L1 IHC testing in lung cancer initially warned against the use of cytologic samples for determination of PD-L1 status because of lack of validation of the assays. In clinical practice, however, 30% to 40% of patients with advanced NSCLC are diagnosed and/or staged with cytologic specimens exclusively,[8] making their use for PD-L1 assessment unavoidable. This situation highlights the disconnect between the specimens acquired in routine practice, and the histologic samples mandated for participation in the pivotal clinical trials of immunotherapy.[11]

The feasibility of using cytologic samples for assessment of PD-L1 expression has been previously established. A multicenter study by Perrotta et al[13] reported that PD-L1 testing could be successfully performed in almost 95% of EBUS-TBNA samples.[48] In 2019, Gosney et al[18] systematically reviewed the success rate of using cytologic specimens to assess PD-L1 expression, and reported that 92% of 709 cytologic specimens (seven studies) were evaluable. A minimum of 100 viable tumor cells are required for PD-L1 testing.

Gosney et al[18] also examined the concordance of PD-L1 expression between matched cytologic and histologic specimens. Across nine studies identified, the overall percentage agreement for cytologic and histologic specimens (n = 428) was 88.3% (95% CI, 86.9-91.2) and 89.7% (95% CI, 86.5-92.4) for specimens with ≥ 1% and ≥ 50% of tumor cells expressing PD-L1, respectively.[19] Satturwar et al[19] reviewed the literature of PD-L1 immunostaining in lung cytologic samples, examining preanalytical (eg, fixation), analytical (eg, antibody clone, staining platforms), and postanalytical (eg, clinical outcomes) factors. Across 27 included studies, the concordance rate between cytologic and

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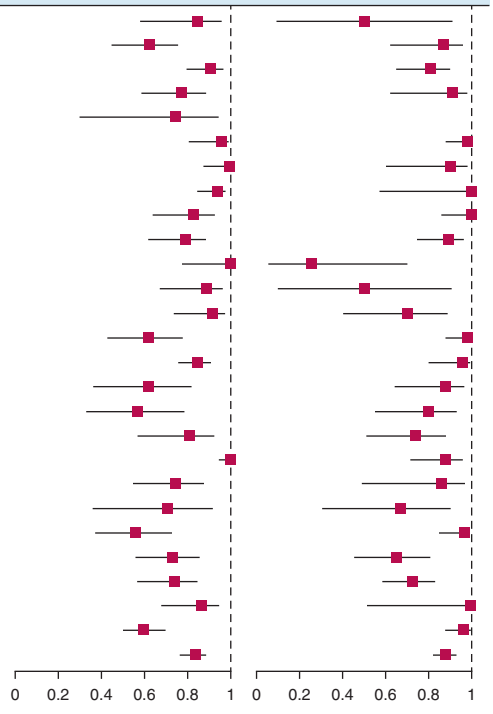
Author, year	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Arriola AGP, 2018	11	2	1	1	0.85 (0.58-0.96)	0.50 (0.09-0.91)
Capizzi E, 2018	21	13	13	2	0.62 (0.45-0.76)	0.87 (0.62-0.96)
Grosu HB, 2019	42	4	29	7	0.91 (0.80-0.97)	0.81 (0.65-0.90)
Hernandez A, 2019	21	6	10	1	0.78 (0.59-0.89)	0.91 (0.62-0.98)
Heymann JJ, 2017	3	1	0	0	0.75 (0.30-0.95)	N/A
Ilie M, 2018	25	1	43	1	0.96 (0.81-0.99)	0.98 (0.88-1.00)
Noll B, 2018	28	0	9	1	1.00 (0.88-1.00)	0.90 (0.60-0.98)
Pak MG, 2019	50	3	5	0	0.94 (0.85-0.98)	1.00 (0.57-1.00)
Sakata KK, 2018	20	4	23	0	0.83 (0.64-0.93)	1.00 (0.86-1.00)
Skov BG, 2018	26	7	32	4	0.79 (0.62-0.89)	0.89 (0.75-0.96)
Smith A, 2019	14	0	1	3	1.00 (0.78-1.00)	0.25 (0.05-0.70)
Wang H, 2018	16	2	1	1	0.89 (0.67-0.97)	0.50 (0.09-0.91)
Wang G, 2019	22	2	7	3	0.92 (0.74-0.98)	0.70 (0.40-0.89)
Yoshimura K, 2019	16	10	41	1	0.62 (0.43-0.78)	0.98 (0.88-1.00)
Dong Z, 2020	75	13	23	1	0.85 (0.76-0.91)	0.96 (0.80-0.99)
Hendry S, 2019	8	5	14	2	0.62 (0.36-0.82)	0.88 (0.64-0.97)
Song Z, 2020	8	6	12	3	0.57 (0.33-0.79)	0.80 (0.55-0.93)
Song SG, 2020	13	3	14	5	0.81 (0.57-0.93)	0.74 (0.51-0.88)
Zou Y, 2020	75	0	23	3	1.00 (0.95-1.00)	0.88 (0.71-0.96)
Gagne A, 2020	18	6	6	1	0.75 (0.55-0.88)	0.86 (0.49-0.97)
Ambrosini-Spaltro A, 2020	5	2	4	2	0.71 (0.36-0.92)	0.67 (0.30-0.90)
Daverio M, 2020	14	11	34	1	0.56 (0.37-0.73)	0.97 (0.85-0.99)
Jug R, 2020	22	8	15	8	0.73 (0.56-0.86)	0.65 (0.45-0.81)
Lou SK, 2020	25	9	34	13	0.74 (0.57-0.85)	0.72 (0.58-0.83)
Martin-Deleon R, 2021	20	3	4	0	0.87 (0.68-0.95)	1.00 (0.51-1.00)
Ricci C, 2020	56	37	55	2	0.60 (0.50-0.70)	0.96 (0.88-0.99)
Pooled	**654**	**158**	**453**	**66**	**0.84 (0.77-0.89)**	**0.88 (0.82-0.93)**



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Author, year	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Arriola AGP, 2018	4	0	11	0	1.00 (0.51-1.00)	1.00 (0.74-1.00)
Capizzi E, 2018	9	5	33	2	0.64 (0.39-0.84)	0.94 (0.81-0.98)
Grosu HB, 2019	14	3	59	6	0.82 (0.59-0.94)	0.91 (0.81-0.96)
Hernandez A, 2019	15	4	19	0	0.79 (0.57-0.91)	1.00 (0.83-1.00)
Heymann JJ, 2017	2	0	2	0	1.00 (0.34-1.00)	1.00 (0.34-1.00)
Ilie M, 2018	19	1	48	2	0.95 (0.76-0.99)	0.96 (0.87-0.99)
Noll B, 2018	13	3	21	1	0.81 (0.57-0.93)	0.95 (0.78-0.99)
Pak MG, 2019	13	4	40	1	0.76 (0.53-0.90)	0.98 (0.87-1.00)
Sakata KK, 2018	7	5	32	3	0.58 (0.32-0.81)	0.91 (0.78-0.97)
Skov BG, 2018	8	0	56	5	1.00 (0.68-1.00)	0.92 (0.82-0.96)
Smith A, 2019	8	0	6	4	1.00 (0.68-1.00)	0.60 (0.31-0.83)
Wang H, 2018	10	0	8	2	1.00 (0.72-1.00)	0.80 (0.49-0.94)
Wang G, 2019	13	1	18	2	0.93 (0.69-0.99)	0.90 (0.70-0.97)
Yoshimura K, 2019	2	4	59	3	0.33 (0.10-0.70)	0.95 (0.87-0.98)
Dong Z, 2020	16	15	74	7	0.52 (0.35-0.68)	0.91 (0.83-0.96)
Hendry S, 2019	3	0	26	0	1.00 (0.44-1.00)	1.00 (0.87-1.00)
Song Z, 2020	5	3	20	1	0.62 (0.31-0.86)	0.95 (0.77-0.99)
Song SG, 2020	7	4	22	2	0.64 (0.35-0.85)	0.92 (0.74-0.98)
Zou Y, 2020	28	1	60	12	0.97 (0.83-0.99)	0.83 (0.73-0.90)
Gagne A, 2020	12	5	11	3	0.71 (0.47-0.87)	0.79 (0.52-0.92)
Ambrosini-Spaltro A, 2020	2	1	8	2	0.67 (0.21-0.94)	0.80 (0.49-0.94)
Daverio M, 2020	4	6	50	0	0.40 (0.17-0.69)	1.00 (0.93-1.00)
Jug R, 2020	11	9	31	2	0.55 (0.34-0.74)	0.94 (0.80-0.98)
Lou SK, 2020	12	6	60	3	0.67 (0.44-0.84)	0.95 (0.87-0.98)
Martin-Deleon R, 2021	11	5	11	0	0.69 (0.44-0.86)	1.00 (0.74-1.00)
Ricci C, 2020	31	13	104	2	0.70 (0.56-0.82)	0.98 (0.93-0.99)
Pooled	**279**	**98**	**889**	**65**	**0.78 (0.69-0.86)**	**0.94 (0.91-0.96)**

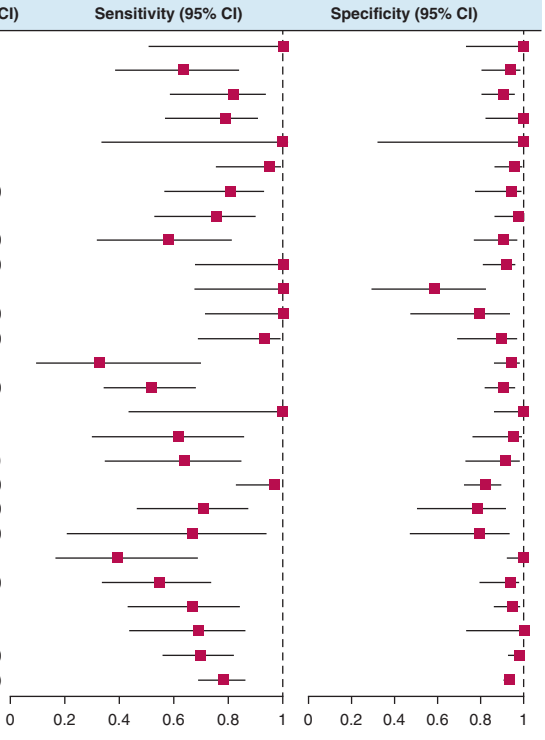


Figure 2 – *A, Sensitivity and specificity of cytologic specimens for assessment of programmed cell death ligand-1 (PD-L1) expression ≥ 1% vs paired histologic samples. B, Sensitivity and specificity of cytologic specimens for assessment of PD-L1 expression ≥ 50% vs paired histologic samples. FN = false negatives; FP = false positive; N/A = not available; TP = true positives; TN = true negatives.*

histologic specimens varied from 53% to 97.3%. Another recent analysis by Mansour et al,[49] which included 97 concurrent paired specimens from two Swedish cohorts, demonstrated good concordance of PD-L1 expression ≥ 1% and ≥ 50% cutoffs, in paired cytologic and biopsy specimens. Their review of 25 studies including about

B

Author, year	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Arriola AGP, 2018	4	0	11	0	1.00 (0.51-1.00)	1.00 (0.74-1.00)
Capizzi E, 2018	9	5	33	2	0.64 (0.39-0.84)	0.94 (0.81-0.98)
Grosu HB, 2019	14	3	59	6	0.82 (0.59-0.94)	0.91 (0.81-0.96)
Hernandez A, 2019	15	4	19	0	0.79 (0.57-0.91)	1.00 (0.83-1.00)
Heymann JJ, 2017	2	0	2	0	1.00 (0.34-1.00)	1.00 (0.34-1.00)
Ilie M, 2018	19	1	48	2	0.95 (0.76-0.99)	0.96 (0.87-0.99)
Noll B, 2018	13	3	21	1	0.81 (0.57-0.93)	0.95 (0.78-0.99)
Pak MG, 2019	13	4	40	1	0.76 (0.53-0.90)	0.98 (0.87-1.00)
Sakata KK, 2018	7	5	32	3	0.58 (0.32-0.81)	0.91 (0.78-0.97)
Skov BG, 2018	8	0	56	5	1.00 (0.68-1.00)	0.92 (0.82-0.96)
Smith A, 2019	8	0	6	4	1.00 (0.68-1.00)	0.60 (0.31-0.83)
Wang H, 2018	10	0	8	2	1.00 (0.72-1.00)	0.80 (0.49-0.94)
Wang G, 2019	13	1	18	2	0.93 (0.69-0.99)	0.90 (0.70-0.97)
Yoshimura K, 2019	2	4	59	3	0.33 (0.10-0.70)	0.95 (0.87-0.98)
Dong Z, 2020	16	15	74	7	0.52 (0.35-0.68)	0.91 (0.83-0.96)
Hendry S, 2019	3	0	26	0	1.00 (0.44-1.00)	1.00 (0.87-1.00)
Song Z, 2020	5	3	20	1	0.62 (0.31-0.86)	0.95 (0.77-0.99)
Song SG, 2020	7	4	22	2	0.64 (0.35-0.85)	0.92 (0.74-0.98)
Zou Y, 2020	28	1	60	12	0.97 (0.83-0.99)	0.83 (0.73-0.90)
Gagne A, 2020	12	5	11	3	0.71 (0.47-0.87)	0.79 (0.52-0.92)
Ambrosini-Spaltro A, 2020	2	1	8	2	0.67 (0.21-0.94)	0.80 (0.49-0.94)
Daverio M, 2020	4	6	50	0	0.40 (0.17-0.69)	1.00 (0.93-1.00)
Jug R, 2020	11	9	31	2	0.55 (0.34-0.74)	0.94 (0.80-0.98)
Lou SK, 2020	12	6	60	3	0.67 (0.44-0.84)	0.95 (0.87-0.98)
Martin-Deleon R, 2021	11	5	11	0	0.69 (0.44-0.86)	1.00 (0.74-1.00)
Ricci C, 2020	31	13	104	2	0.70 (0.56-0.82)	0.98 (0.93-0.99)
Pooled	**279**	**98**	**889**	**65**	**0.78 (0.69-0.86)**	**0.94 (0.91-0.96)**

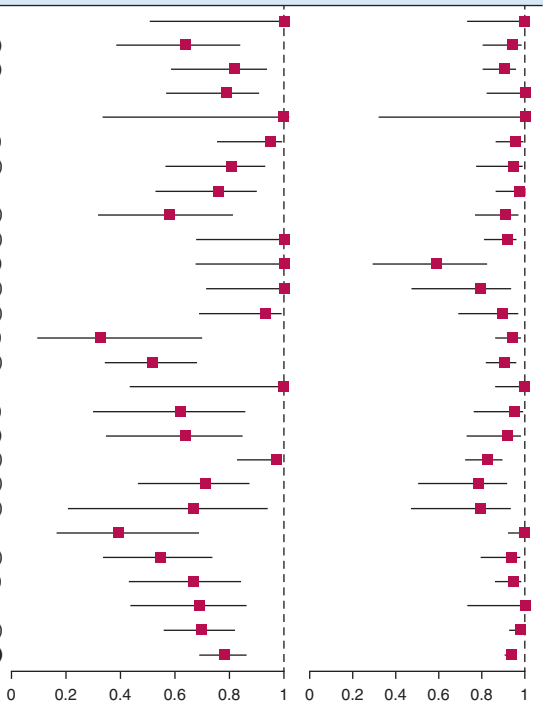


Figure 2 – *Continued*

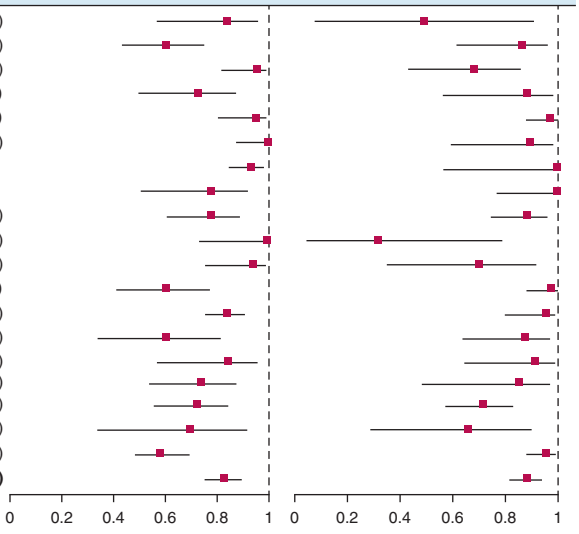
1,700 paired specimens yielded a median concordance of 81% to 85% at the 1% cutoff and 89% at the 50% PD-L1 expression cutoff. None of these published reviews performed a formal meta-analysis. To our knowledge, no prior review has systematically compiled and analyzed the accuracy of cytologic specimens vs paired histologic specimens for assessment of PD-L1 expression.

There are several strengths to this systematic review. The literature search was updated on two occasions, yielding a total of 1,331 paired histologic and cytologic specimens, of which 1,064 consisted of cytologic cell blocks. We repeatedly reached out to authors for clarifications, in particular as relates to interval treatment between acquisition of paired samples, and we are grateful for their collaboration. We also performed several planned and clinically relevant subgroup analyses.

The analysis does have certain limitations. Some studies were excluded because the necessary clarifications or additional data could not be obtained from the authors. Paired cytologic and biopsy specimens were from the same patient, but frequently not from the same site and collected at different times (with or without interval treatment). Concordance of PD-L1 expression in cytologic and histologic NSCLC specimens has been noted to be lower in samples obtained from different sites.[49] A retrospective study of 15,028 lung cancer cases, with 8,285 primary tumors and 6,743 unmatched metastatic lesions, suggested that metastatic lesions more frequently demonstrated high PD-L1 expression (TPS $\geq$ 50%) compared with primary lesions, particularly in nonsquamous histology.[50] In contrast, Argyropoulos et al[51] reported no difference in PD-L1 expression in matched primary and metastatic lung adenocarcinoma cases, but noted a significant correlation between higher TPS and high-grade tumor growth patterns. Whether histologic specimens are an appropriate reference standard against which paired cytologic samples should be compared is also open to some discussion. It has been suggested, for example, that the fanning motion of needles during tumor or nodal cytologic sampling may result in disruption and broader acquisition of tumor cells, compared with the more localized sampling of tissue biopsies.[52] Ultimately, the use of cytologic specimens for assessment of PD-L1 expression hinges on their ability to predict ICI treatment response. Limited studies to data have examined ICI treatment outcomes guided by cytologic specimens. We have previously reported on the real-world outcomes of patients with advanced NSCLC

A

Author, year	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Arriola AGP, 2018	11	2	1	1	0.85 (0.58-0.96)	0.50 (0.09-0.91)
Capizzi E, 2018	21	13	13	2	0.62 (0.45-0.76)	0.87 (0.62-0.96)
Grosu HB, 2019	26	1	11	5	0.96 (0.82-0.99)	0.69 (0.44-0.86)
Hernandez A, 2019	14	5	8	1	0.74 (0.51-0.88)	0.89 (0.57-0.98)
Ilie M, 2018	25	1	43	1	0.96 (0.81-0.99)	0.98 (0.88-1.00)
Noll B, 2018	27	0	9	1	1.00 (0.88-1.00)	0.90 (0.60-0.98)
Pak MG, 2019	50	3	5	0	0.94 (0.85-0.98)	1.00 (0.57-1.00)
Sakata KK, 2018	11	3	13	0	0.79 (0.52-0.92)	1.00 (0.77-1.00)
Skov BG, 2018	26	7	32	4	0.79 (0.62-0.89)	0.89 (0.75-0.96)
Smith A, 2019	11	0	1	2	1.00 (0.74-1.00)	0.33 (0.06-0.79)
Wang G, 2019	19	1	5	2	0.95 (0.76-0.99)	0.71 (0.36-0.92)
Yoshimura K, 2019	16	10	41	1	0.62 (0.43-0.78)	0.98 (0.88-1.00)
Dong Z, 2020	75	13	23	1	0.85 (0.76-0.91)	0.96 (0.80-0.99)
Hendry S, 2019	8	5	14	2	0.62 (0.36-0.82)	0.88 (0.64-0.97)
Song SG, 2020	11	2	11	1	0.85 (0.58-0.96)	0.92 (0.65-0.99)
Gagne A, 2020	18	6	6	1	0.75 (0.55-0.88)	0.86 (0.49-0.97)
Lou SK, 2020	25	9	34	13	0.74 (0.57-0.85)	0.72 (0.58-0.83)
Ambrosini-Spaltro A, 2020	5	2	4	2	0.71 (0.36-0.92)	0.67 (0.30-0.90)
Ricci C, 2020	56	37	55	2	0.60 (0.50-0.70)	0.96 (0.88-0.99)
Pooled	**455**	**120**	**329**	**42**	**0.84 (0.76-0.90)**	**0.89 (0.82-0.94)**



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Author, year	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Arriola AGP, 2018	4	0	11	0	1.00 (0.51-1.00)	1.00 (0.74-1.00)
Capizzi E, 2018	9	5	33	2	0.64 (0.39-0.84)	0.94 (0.81-0.98)
Grosu HB, 2019	8	1	33	1	0.89 (0.57-0.98)	0.97 (0.85-0.99)
Hernandez A, 2019	11	2	15	0	0.85 (0.58-0.96)	1.00 (0.80-1.00)
Ilie M, 2018	19	1	48	2	0.95 (0.76-0.99)	0.96 (0.87-0.99)
Noll B, 2018	12	3	21	1	0.80 (0.55-0.93)	0.95 (0.78-0.99)
Pak MG, 2019	13	4	40	1	0.76 (0.53-0.90)	0.98 (0.87-1.00)
Sakata KK, 2018	4	3	18	2	0.57 (0.25-0.84)	0.90 (0.70-0.97)
Skov BG, 2018	8	0	56	5	1.00 (0.68-1.00)	0.92 (0.82-0.96)
Smith A, 2019	6	0	4	4	1.00 (0.61-1.00)	0.50 (0.22-0.78)
Wang G, 2019	12	0	13	2	1.00 (0.76-1.00)	0.87 (0.62-0.96)
Yoshimura K, 2019	2	4	59	3	0.33 (0.10-0.70)	0.95 (0.87-0.98)
Dong Z, 2020	16	15	74	7	0.52 (0.35-0.68)	0.91 (0.83-0.96)
Hendry S, 2019	3	0	26	0	1.00 (0.44-1.00)	1.00 (0.87-1.00)
Song SG, 2020	7	1	15	2	0.88 (0.53-0.98)	0.88 (0.66-0.97)
Gagne A, 2020	12	5	11	3	0.71 (0.47-0.87)	0.79 (0.52-0.92)
Lou SK, 2020	12	6	60	3	0.67 (0.44-0.84)	0.95 (0.87-0.98)
Ambrosini-Spaltro A, 2020	2	1	8	2	0.67 (0.21-0.94)	0.80 (0.49-0.94)
Ricci C, 2020	31	13	104	2	0.70 (0.56-0.82)	0.98 (0.93-0.99)
Pooled	**191**	**64**	**649**	**42**	**0.80 (0.71-0.89)**	**0.94 (0.91-0.96)**

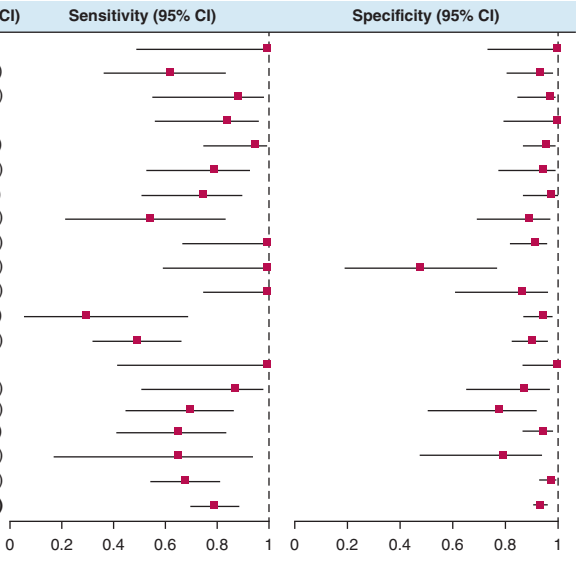


Figure 3 – *A, Sensitivity and specificity of cytologic specimens, acquired without interval treatment, for assessment of programmed cell death ligand-1 (PD-L1) expression ≥ 1% vs paired histologic samples. B, Sensitivity and specificity of cytologic specimens, acquired without interval treatment, for assessment of PD-L1 expression ≥ 50% vs paired histologic samples. FN = false negatives; FP = false positive; TP = true positives; TN = true negatives.*

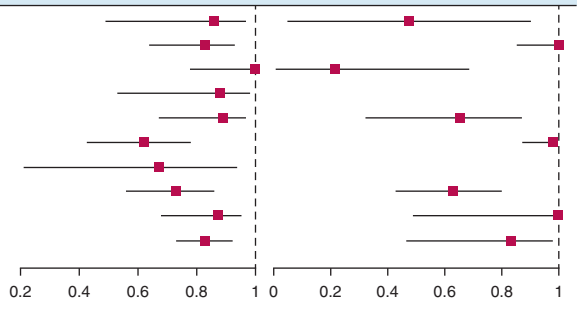
treated with anti-PD-1 therapy based on PD-L1 results in EBUS-TBNA vs histologic specimens, which were found to be comparable.[48]

Beyond the question of specimen type, we must be cognisant of the known technical and biological pitfalls of PD-L1 as a biomarker. Spatial and temporal heterogeneity of PD-L1 expression has been described,[53,54] and this may account for some of the variability in PD-L1 levels between paired samples. Additional factors include sample processing, antibody types and platforms, and cancer treatment.[19,55] The subgroup analyses performed provide insight into the impact of some of these factors. We examined the subgroup of paired samples acquired in the absence of interval treatment and the diagnostic accuracy of cytologic specimens was higher, particularly at the 50% PD-L1 expression cutoff.

A significant limitation of PD-L1 as a biomarker is the absence of a single, standardized analytical assay.

A

Author, year	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Hernandez A, 2019	6	1	1	1	0.86 (0.49-0.97)	0.50 (0.09-0.91)
Sakata KK, 2018	20	4	23	0	0.83 (0.64-0.93)	1.00 (0.86-1.00)
Smith A, 2019	14	0	1	3	1.00 (0.78-1.00)	0.25 (0.05-0.70)
Wang H, 2018	7	1	0	0	0.88 (0.53-0.98)	NA
Wang G, 2019	16	2	6	3	0.89 (0.67-0.97)	0.67 (0.35-0.88)
Yoshimura K, 2019	16	10	41	1	0.62 (0.43-0.78)	0.98 (0.88-1.00)
Heymann JJ, 2017	2	1	0	0	0.67 (0.21-0.94)	NA
Jug R, 2020	22	8	15	8	0.73 (0.56-0.86)	0.65 (0.45-0.81)
Martin-Deleon R, 2021	20	3	4	0	0.87 (0.68-0.95)	1.00 (0.51-1.00)
Pooled	**123**	**30**	**91**	**16**	**0.83 (0.73-0.92)**	**0.84 (0.49-0.98)**



B

Author, year	TP	FN	TN	FP	Sensitivity (95% CI)	Specificity (95% CI)
Hernandez A, 2019	4	1	4	0	0.80 (0.38-0.96)	1.00 (0.51-1.00)
Sakata KK, 2018	7	5	32	3	0.58 (0.32-0.81)	0.91 (0.78-0.97)
Smith A, 2019	8	0	6	4	1.00 (0.68-1.00)	0.60 (0.31-0.83)
Wang H, 2018	5	0	2	1	1.00 (0.57-1.00)	0.67 (0.21-0.94)
Wang G, 2019	10	1	14	2	0.91 (0.62-0.98)	0.88 (0.64-0.97)
Yoshimura K, 2019	2	4	59	3	0.33 (0.10-0.70)	0.95 (0.87-0.98)
Heymann JJ, 2017	1	0	2	0	1.00 (0.21-1.00)	1.00 (0.34-1.00)
Jug R, 2020	11	9	31	2	0.55 (0.34-0.74)	0.94 (0.80-0.98)
Martin-Deleon R, 2021	11	5	11	0	0.69 (0.44-0.86)	1.00 (0.74-1.00)
Pooled	**59**	**25**	**161**	**15**	**0.78 (0.57-0.95)**	**0.90 (0.79-0.95)**

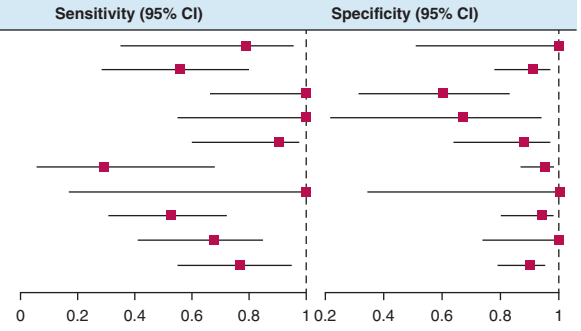


Figure 4 – *A, Sensitivity and specificity of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) specimens (acquired with or without interval treatment) for assessment of programmed cell death ligand-1 (PD-L1) expression ≥ 1% vs paired histologic samples. B, Sensitivity and specificity of EBUS-TBNA specimens (acquired with or without interval treatment) for assessment of PD-L1 expression ≥ 50% vs paired histologic samples. FN = false negatives; FP = false positive; NA = not available; TP = true positives; TN = true negatives.*

There are multiple antibodies and IHC platforms currently available on the market; a high concordance rate was observed among the 28-8, 22C3, and SP263 assays in the Blueprint phase 1 project.[56,57] In this analysis, 15 of 26 included studies assessed PD-L1 expression using the 22C3 assay, with the highest sensitivity and negative predictive value demonstrated in this subgroup (e-Tables 10, 11). Centers may also choose to set up laboratory-developed tests, which have been shown equivalent to commercial platforms.[58] Beyond technical variations, close communication with pathology colleagues is essential to ensure optimal pathways for processing of cytologic samples are established.

The use of alcohol-based fixatives for processing samples destined for PD-L1 testing has previously raised concerns. Formalin fixation was used in 16 of 26 included studies, whereas a combination of alcohol-based fixatives and formalin was mostly used otherwise. The review by Gosney et al[59] demonstrated no significant difference in PD-L1 expression when comparing EBUS-TBNA samples prepared using a methanol-based fixative (CytoLyt; Hologic, Inc) vs formalin. The vast majority of included studies used cytologic cell block samples, with subgroup analyses confirming their diagnostic accuracy for assessment of PD-L1 expression (e-Tables 8, 9). In contrast, only three studies examined the accuracy of direct smears, with a lower pooled sensitivity; therefore, caution is warranted.

Finally, we examined the diagnostic accuracy of EBUS-TBNA specimens specifically, given the importance of minimally invasive needle techniques in the efficient and accurate diagnosis and staging of lung cancer.[10,60] The feasibility of using EBUS-TBNA for *EGFR* and *ALK* testing is well established, as demonstrated in a previous meta-analysis.[61] The current analysis provides reassuring results regarding the sensitivity of EBUS-TBNA samples for assessment of PD-L1 positivity, particularly at the ≥ 50% expression level, and in the absence of interval treatment between acquisition of the paired samples (e-Table 7). Pulmonologists performing EBUS-TBNA must continue to ensure sufficient tissue is acquired for molecular testing, beyond the three or more needle passes typically obtained for diagnostic purposes,[62,63] and can be reassured that the cytologic

specimens they obtain will accurately reflect PD-L1 status in their patients.

Interpretation

Cytologic specimens provide an accurate assessment of PD-L1 expression in most patients with NSCLC, at both the ≥ 1% and ≥ 50% cutoffs, when compared with paired histologic specimens. Clinical trials of immunotherapy should include cytologic specimens for assessment of PD-L1 expression, to more closely reflect the tissue samples being routinely acquired in clinical practice.

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Additional information: The e-Tables are available online under "Supplementary Data."

References

1. Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. *Trends Mol Med*. 2015;21(1):24-33.
2. Ichikawa M, Chen L. Role of B7-H1 and B7-H4 molecules in down-regulating effector phase of T-cell immunity: novel cancer escaping mechanisms. *Front Biosci*. 2005;10:2856-2860.
3. Brody R, Zhang Y, Ballas M, et al. PD-L1 expression in advanced NSCLC: insights into risk stratification and treatment selection from a systematic literature review. *Lung Cancer*. 2017;112:200-215.
4. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med*. 2016;375(19):1823-1833.
5. Reck M, Rodriguez-Abreu D, Robinson AG, 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 Clin Oncol*. 2019;37(7):537-546.
6. 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(22):2078-2092.
7. National Comprehensive Cancer Network, Non-small cell lung cancer (version 1.2023). National Comprehensive Cancer Network website. Accessed February 7, 2023. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf
8. Tsao MS, Kerr KM, Dacic S, Yatabe Y, Hirsch F. *IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer*. International Association for the Study of Lung Cancer; 2017.
9. Ost DE, Jim Yeung SC, Tanoue LT, Gould MK. Clinical and organizational factors in the initial evaluation of patients with lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2013;143(5 suppl):e121S-e141S.
10. Silvestri GA, Gonzalez AV, Jantz MA, et al. Methods for staging non-small cell lung cancer: diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2013;143(5 suppl):e211S-e250S.
11. Beattie J, Yarmus L, Wahidi M, et al. The immune landscape of non-small-cell lung cancer. Utility of cytologic and histologic samples obtained through minimally invasive pulmonary procedures. *Am J Respir Crit Care Med*. 2018;198(1):24-38.
12. Mino-Kenudson M, Le Stang N, Daigneault JB, et al. The International Association for the Study of Lung Cancer Global Survey on Programmed Death-Ligand 1 Testing for NSCLC. *J Thorac Oncol*. 2021;16(4):686-696.
13. Perrotta F, Nankivell M, Adizie JB, et al. Endobronchial ultrasound-guided transbronchial needle aspiration for PD-L1 testing in non-small cell lung cancer. *Chest*. 2020;158(3):1230-1239.
14. Smith A, Wang H, Zerbo A, et al. Programmed Death Ligand 1 testing of endobronchial ultrasound-guided transbronchial needle aspiration samples acquired for the diagnosis and staging of non-small cell lung cancer. *J Bronchology Interv Pulmonol*. 2020;27(1):50-57.
15. Skov BG, Skov T. Paired comparison of PD-L1 expression on cytologic and histologic specimens from malignancies in the lung assessed with PD-L1 IHC 28-8pharmDx and PD-L1 IHC 22C3pharmDx. *Appl Immunohistochem Mol Morphol*. 2017;25(7):453-459.
16. Sakata KK, Midthun DE, Mullon JJ, et al. Comparison of Programmed Death Ligand-1 immunohistochemical staining between endobronchial ultrasound transbronchial needle aspiration and resected lung cancer specimens. *Chest*. 2018;154(4):827-837.
17. Torous VF, Rangachari D, Gallant BP, Shea M, Costa DB, VanderLaan PA. PD-L1 testing using the clone 22C3 pharmDx kit for selection of patients with non-small cell lung cancer to receive immune checkpoint inhibitor therapy: are cytology cell blocks a viable option? *J Am Soc Cytopathol*. 2018;7(3):133-141.
18. Gosney JR, Boothman AM, Ratcliffe M, Kerr KM. Cytology for PD-L1 testing: a systematic review. *Lung Cancer*. 2020;141:101-106.
19. Satturwar S, Girolami I, Munari E, Ciompi F, Eccher A, Pantanowitz L. Program death ligand-1 immunocytochemistry in lung cancer cytological samples: a systematic review. *Diagn Cytopathol*. 2022;50(6):313-323.
20. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
21. McInnes MDF, Moher D, Thombs BD, et al. Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: the PRISMA-DTA statement. *JAMA*. 2018;319(4):388-396.
22. Whiting PF, Rutjes AW, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy

studies. *Ann Intern Med*. 2011;155(8):529-536.

23. Chu H, Cole SR. Bivariate meta-analysis of sensitivity and specificity with sparse data: a generalized linear mixed model approach. *J Clin Epidemiol*. 2006;59(12):1331-1332.
24. Doebler P, Holling H. Meta-analysis of diagnostic accuracy with mada. Accessed April 22, 2020. https://cran.r-project.org/web/packages/mada/vignettes/mada.pdf
25. Dong Z, Liu Y, Jiang T, et al. Cell block as a surrogate for Programmed Death-Ligand 1 staining testing in patients of non-small cell lung cancer. *J Cancer*. 2020;11(3):551-558.
26. Song SG, Lee J, Koh J, Kim S, Chung DH, Jeon YK. Utility of PD-L1 immunocytochemistry using body-fluid cell blocks in patients with non-small-cell lung cancer. *Diagn Cytopathol*. 2020;48(4):291-299.
27. Gagne A, Orain M, Ionescu D, Tsao MS, Joubert D, Joubert P. Comprehensive assessment of PD-L1 immunohistochemistry on paired tissue and cytology specimens from non-small cell lung cancer. *Lung Cancer*. 2020;146:276-284.
28. Arriola AGP, Bashover E, Joseph C, Staerkel G, Wang WL, Roy-Chowdhuri S. The usefulness of various cytologic specimen preparations for PD-L1 immunostaining in non-small cell lung carcinoma. *J Am Soc Cytopathol*. 2018;7(6):324-332.
29. Capizzi E, Ricci C, Giunchi F, et al. Validation of the immunohistochemical expression of programmed death ligand 1 (PD-L1) on cytological smears in advanced non small cell lung cancer. *Lung Cancer*. 2018;126:9-14.
30. Grosu HB, Arriola A, Stewart J, et al. PD-L1 detection in histology specimens and matched pleural fluid cell blocks of patients with NSCLC. *Respirology*. 2019;24(12):1198-1203.
31. Hernandez A, Brandler TC, Zhou F, Moreira AL, Schatz-Siemers N, Simsir A. Assessment of Programmed Death-Ligand 1 (PD-L1) immunohistochemical expression on cytology specimens in non-small cell lung carcinoma. *Am J Clin Pathol*. 2019;151(4):403-415.
32. Heymann JJ, Bulman WA, Swinarski D, et al. PD-L1 expression in non-small cell lung carcinoma: comparison among cytology, small biopsy, and surgical resection specimens. *Cancer Cytopathol*. 2017;125(12):896-907.
33. Ilie M, Juco J, Huang L, Hofman V, Khambata-Ford S, Hofman P. Use of the 22C3 anti-programmed death-ligand 1 antibody to determine programmed death-ligand 1 expression in cytology samples obtained from non-small cell lung cancer patients. *Cancer Cytopathol*. 2018;126(4):264-274.
34. Noll B, Wang WL, Gong Y, et al. Programmed death ligand 1 testing in non-small cell lung carcinoma cytology cell block and aspirate smear preparations. *Cancer Cytopathol*. 2018;126(5):342-352.
35. Pak MG, Roh MS. Cell-blocks are suitable material for programmed cell death ligand-1 immunohistochemistry: comparison of cell-blocks and matched surgical resection specimens in lung cancer. *Cytopathology*. 2019;30(6):578-585.
36. Wang H, Agulnik J, Kasymjanova G, et al. Cytology cell blocks are suitable for immunohistochemical testing for PD-L1 in lung cancer. *Ann Oncol*. 2018;29(6):1417-1422.
37. Wang G, Ionescu DN, Lee CH, et al. PD-L1 testing on the EBUS-FNA cytology specimens of non-small cell lung cancer. *Lung Cancer*. 2019;136:1-5.
38. Yoshimura K, Inoue Y, Karayama M, et al. Heterogeneity analysis of PD-L1 expression and copy number status in EBUS-TBNA biopsy specimens of non-small cell lung cancer: comparative assessment of primary and metastatic sites. *Lung Cancer*. 2019;134:202-209.
39. Hendry S, Byrne DJ, Christie M, et al. Adequate tumour cellularity is essential for accurate PD-L1 immunohistochemistry assessment on cytology cell-block specimens. *Cytopathology*. 2020;31(2):90-95.
40. Song Z, Cheng G, Zhang Y. PD-L1 expression in malignant pleural effusion samples and its correlation with oncogene mutations in non-small cell lung cancer. *J Thorac Dis*. 2020;12(4):1385-1392.
41. Zou Y, Xu L, Tang Q, et al. Cytology cell blocks from malignant pleural effusion are good candidates for PD-L1 detection in advanced NSCLC compared with matched histology samples. *BMC Cancer*. 2020;20(1):344.
42. Ambrosini-Spaltro A, Dubini A, Pieri F, Ravaglia C, Delmonte A, Poletti V. PD-L1 expression in NSCLC: role of cell blocks and concordance between samples. *Diagn Cytopathol*. 2021;49(2):303-310.
43. Daverio M, Patrucco F, Gavelli F, et al. Comparative analysis of programmed death ligand 1 expression in paired cytologic and histologic specimens of non-small cell lung cancer. *Cancer Cytopathol*. 2020;128(8):580-588.
44. Jug R, Giovacchini CX, Liu B, et al. EBUS-FNA cytologic-histologic correlation of PD-L1 immunohistochemistry in non-small cell lung cancer. *J Am Soc Cytopathol*. 2020;9(6):485-493.
45. Lou SK, Ko HM, Kinoshita T, et al. Implementation of PD-L1 22C3 IHC pharmDx™ in cell block preparations of lung cancer: concordance with surgical resections and technical validation of CytoLyt(R) prefixation. *Acta Cytol*. 2020;64(6):577-587.
46. Martin-Deleon R, Teixido C, Lucena CM, et al. EBUS-TBNA cytological samples for comprehensive molecular testing in non-small cell lung cancer. *Cancers (Basel)*. 2021;13(9):2084.
47. Ricci C, Capizzi E, Giunchi F, et al. Reliability of programmed death ligand 1 (PD-L1) tumor proportion score (TPS) on cytological smears in advanced non-small cell lung cancer: a prospective validation study. *Ther Adv Med Oncol*. 2020;12:1758835920954802.
48. Tajarernmuang P, Ofiara L, Beaudoin S, Wang H, Benedetti A, Gonzalez AV. Real-world outcomes of patients with advanced non-small cell lung cancer treated with anti-PD1 therapy on the basis of PD-L1 results in EBUS-TBNA vs histological specimens. *Chest*. 2021;160(2):743-753.
49. Mansour MSI, Lindquist KE, Seidal T, et al. PD-L1 testing in cytological non-small cell lung cancer specimens: a comparison with biopsies and review of the literature. *Acta Cytol*. 2021;65(6):501-509.
50. Moutafi MK, Tao W, Huang R, et al. Comparison of programmed death-ligand 1 protein expression between primary and metastatic lesions in patients with lung cancer. *J Immunother Cancer*. 2021;9(4):e002230.
51. Argyropoulos K, Basu A, Park K, Zhou F, Moreira AL, Narula N. Correlation of Programmed Death-Ligand 1 expression with lung adenocarcinoma histologic and molecular subgroups in primary and metastatic sites. *Mod Pathol*. 2023;36(9):100245.
52. A. Saqi, D. Jain, L. Bubendorf, K. Kerr and A. Moreira, PD-L1 in cytology specimens. Accessed April 30, 2023. https://www.ilcn.org/pd-l1-in-cytology-specimens/
53. Ben Dori S, Aizic A, Sabo E, Hershkovitz D. Spatial heterogeneity of PD-L1 expression and the risk for misclassification of PD-L1 immunohistochemistry in non-small cell lung cancer. *Lung Cancer*. 2020;147:91-98.
54. Evans M, O'Sullivan B, Smith M, Taniere P. Predictive markers for anti-PD-1/PD-L1 therapy in non-small cell lung cancer-where are we? *Transl Lung Cancer Res*. 2018;7(6):682-690.
55. Tsao MS, Kerr KM, Kockx M, et al. PD-L1 immunohistochemistry comparability study in real-life clinical samples: results of Blueprint Phase 2 Project. *J Thorac Oncol*. 2018;13(9):1302-1311.
56. Buttner R, Gosney JR, Skov BG, et al. Programmed Death-Ligand 1 immunohistochemistry testing: a review of analytical assays and clinical implementation in non-small-cell lung cancer. *J Clin Oncol*. 2017;35(34):3867-3876.
57. Hirsch FR, McElhinny A, Stanforth D, et al. PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. *J Thorac Oncol*. 2017;12(2):208-222.
58. Torlakovic E, Lim HJ, Adam J, et al. "Interchangeability" of PD-L1 immunohistochemistry assays: a meta-analysis of diagnostic accuracy. *Mod Pathol*. 2020;33(1):4-17.
59. Gosney JR, Haragan A, Chadwick C, et al. Programmed death ligand 1 expression in EBUS aspirates of non-small cell lung cancer: is interpretation affected by type of fixation? *Cancer Cytopathol*. 2020;128(2):100-106.

60. Navani N, Nankivell M, Lawrence DR, et al. Lung cancer diagnosis and staging with endobronchial ultrasound-guided transbronchial needle aspiration compared with conventional approaches: an open-label, pragmatic, randomised controlled trial. *Lancet Respir Med*. 2015;3(4):282-289.

61. Labarca G, Folch E, Jantz M, Mehta HJ, Majid A, Fernandez-Bussy S. Adequacy of samples obtained by endobronchial ultrasound with transbronchial needle aspiration for molecular analysis in patients with non-small cell lung cancer. Systematic review and meta-analysis. *Ann Am Thorac Soc*. 2018;15(10):1205-1216.

62. Wahidi MM, Herth F, Yasufuku K, et al. Technical aspects of endobronchial ultrasound-guided transbronchial needle aspiration: CHEST Guideline and Expert Panel Report. *Chest*. 2016;149(3):816-835.

63. Roy-Chowdhuri S, Dacic S, Ghofrani M, et al. Collection and handling of thoracic small biopsy and cytology specimens for ancillary studies: Guideline from the College of American Pathologists in Collaboration With the American College of Chest Physicians, Association for Molecular Pathology. *American Society of Cytopathology, American Thoracic Society, Pulmonary Pathology Society, Papanicolaou Society of Cytopathology, Society of Interventional Radiology, and Society of Thoracic Radiology*. 2020;144:933-958.