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Programmed Death Ligand 1 (PD-L1) Expression and its Association with Clinicopathologic Profile in Patients with Non-Small Cell Lung Cancer in a Philippine Tertiary Medical Center

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ABSTRACT

Introduction. The current management of advanced non-small cell lung cancer (NSCLC) includes the characterization of Programmed Death Ligand-1 (PD-L1) expression for potential immune checkpoint inhibitor treatment. There is currently no available data regarding the patterns of PD-L1 expression in NSCLC, as well as their association with clinicopathologic profile in Filipino patients.

Methodology. Clinicopathologic characteristics of 187 consecutive NSCLC clinical samples with PD-L1 testing using the clone 22C3 pharmaDx kit were collected. The presence of stromal tumor-infiltrating lymphocytes (TILs) were assessed in hematoxylin and eosin-stained slides. PD-L1 expression on tumor cells (TC) and stromal TILs were evaluated.

Results. Of the 187 cases, there were 112 males and 75 females. The mean age at diagnosis was 66.4 years old (32-92 years old). It is composed of 131 cases of adenocarcinoma, 15 squamous cell carcinoma, 4 adenosquamous carcinoma, 32 non-small cell carcinoma, not otherwise specified, 3 poorly differentiated malignancy, 1 large cell carcinoma, and 1 mucinous carcinoma. Specimen types included 17 pleural fluid cell blocks, 60 tumor cell block samples, and 110 tissue biopsies. Tumor cell PD-L1 expression was identified in 59.1% of the 110 tissue biopsies. PD-L1 TPS for histologic specimens are as follows: TPS ≥50%, TPS 1-49%, and TPS <1% were observed in 23.6%, 35.5%, and 40.9% in our lung cancer cohort, respectively. Of the 77 cytology specimens, 50.6% presented with TC PD-L1 expression. TPS for this subgroup include: 49.4% with no PD-L1 expression, 35.1% with low PD-L1 expression, and 15.6% showing high PD-L1 expression. PD-L1 expression on TC did not correlate with age, sex, or histology for both specimen type subgroups. Stromal tumor-infiltrating lymphocytes were noted in 74.5% of tissue biopsies. Tumor cell block samples did not demonstrate stromal TILs. For tissue biopsies, female gender and TPS 1-49% were more likely to have <50% PD-L1 expression on TILs.

Conclusion. Overall TC PD-L1 expression was observed in more than half (55.6%) of NSCLC patients in our cohort. The prognostic value of PD-L1 and clinical response to immune checkpoint inhibitors in the Filipino population needs to be further investigated.

Key words: non-small cell lung cancer, lung cancer, PD-L1, Philippines

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INTRODUCTION

Worldwide and local statistics show that lung cancer remains to be the most frequently diagnosed of the cancer types, and also the most common cause of cancer mortality in both sexes.[1,2] In addition to traditional platinum-based chemotherapy, use of targeted and immune therapies have increased in the past years and yielded favorable outcomes. Molecular diagnostic tests including PD-L1 immunohistochemistry are utilized to identify possible targets of treatment.[3,4]

The World Health Organization has classified LC based on biology, treatment and prognosis into: 1) non-small cell lung cancer (NSCLC) and 2) small cell lung cancer. NSCLC is the more common (80%) of the variants, and it is further subdivided into two major types: 1) nonsquamous (including adenocarcinoma, large cell carcinoma and other cell types) and 2) squamous cell carcinoma. Majority of patients with NSCLC are diagnosed during the advanced

course of the disease, with about a third to half of patients diagnosed using cytology specimens alone.[3-5]

T cells play a vital role in tumor cell recognition and eradication. However, one of the major mechanisms by which a variety of solid tumors (melanoma and carcinoma of the lung, pancreas, breast, colon, stomach, ovary, kidney, and urothelium) evade the host immune response is through "adaptive immune resistance" by overexpression of Programmed Death Ligand-1 (PD-L1). In normal tissue, the Programmed Death-1 (PD-1)/ PD-L1 pathway controls the immune response. PD-1 (also known as CD279) is a surface receptor expressed on activated T cells, B cells, natural killer cells, activated monocytes and dendritic cells and is usually expressed in high numbers by tumor-inflitrating lymphocytes (TILs). PD-L1 (also known as B7-H1 or CD274) and Programmed Death Ligand-2 (PD-L2) are the two identified ligands of PD-1. Of the two ligands, PD-L1, which is expressed by a variety of cell types (placenta, endothelial cells, pancreatic islet cells, muscle, hepatocytes, epithelium, mesenchymal stem cells, B cells, T cells, dendritic cells, macrophages and mast cells), mainly moderates the immunosuppressive effects of the pathway. Contact of PD-L1 and its receptor PD-1 leads to apoptosis and suppresses T cell function, differentiation and survival. Hence, increased tumor cell (TC) expression of PD-L1 results in T cell tolerance by downregulation of the host immune response and avoidance of immune recognition which facilitates cancer proliferation.[6-14] The prognostic impact of PD-L1 expression in lung cancer is quite contradicting; however, several studies demonstrated that PD-L1 expression in NSCLC is associated with a poorer relapse-free and overall survival, the association being clearer in stage I tumors.[9,15-17]

Lung cancer treatment include immune checkpoint mechanisms involving the PD1-PD-L1 axis. PD-L1 expression on tumor cells has been previously reported to predict response to PD-1/PD-L1 inhibitors. Patterns of PD-L1 expression of lung cancer patients in many Asian (China, Japan and Korea) as well as some Western (Australia, Germany, Italy and USA) countries have been reported.[15] In a large clinical trial with 1143 lung cancer patients, prevalence and pattern of PD-L1 expression are as follows: 23.2% of patients with tumor proportion score (TPS) ≥50, 37.6% with TPS 1-49% and 39.2% had TPS <1%.[18] Data on the PD-L1 expression status of patients with lung cancer in the Philippines are not available at present, and since PD-L1 expression studies among ethnic groups are still relatively limited, further epidemiologic studies will be beneficial.

PD-L1 expression, as evaluated by immunohistochemistry (IHC), is a predictive biomarker for response to PD-1/PD-L1 monoclonal antibodies (mAbs) or immune checkpoint inhibitors/blockers (ICIs or ICBs). Pharmacologic action of the mAbs is based on PD-1 or PD-L1 inhibition. The goal of treatment is to restore the host immune responses in order to recognize and eliminate tumor cells. Nivolumab and Pembrolizumab inhibit PD-1 receptors, while Atezolizumab and Durvalumab inhibit PD-L1.[3] Each monoclonal antibody is paired with their own PD-L1 antibody: Nivolumab (Bristol-Myers Squibb) with 28-8 rabbit antibody; Pembrolizumab (Merck & Co., Inc.) with 22C3 mouse antibody; Atezolizumab (Genentech) with SP142 rabbit antibody; Durvalumab (AstraZeneca) with SP263 rabbit antibody; and Avelumab (EMD Serono, Inc. and Pfizer) with 73-10 rabbit antibody.[19] Studies have shown a good concordance in TC scoring among the five different assays except for the SP142 clone, which showed lower rates of PD-L1 expression on tumor cells.[20-24] Although these assays were validated using resection or tissue specimens, and pre-analytic variability in cytology specimens is greater than surgically resected specimens, it is suggested that cell block material is as good as their histologic counterpart in PD-L1 biomarker analysis.[15,19,24] Moreover, data by Stoy et al., showed 91% success rate in PD-L1 IHC using cell blocks obtained using bronchoscopy.[25]

Pembrolizumab has been approved by the U.S. Food and Drug Administration (FDA) with the PD-L1 IHC 22C3 pharmDx as "companion diagnostic assay" in which only the patients who tested positive for the marker can be treated with the mAb. Initial approval involved stratification of PD-L1 expression in high (≥50%) and low (1-49%). Monotherapy with Pembrolizumab is preferred as the first line therapy option for metastatic NSCLC with PD-L1 Tumor Proportion Score (TPS) ≥50%, and as second line treatment in metastatic NSCLC patients with PD-L1 TPS ≥1%, provided that these tumors lack *EGFR* mutations, *ALK* translocations, *ROS1*, *METex14* skipping, *RET* or *BRAF* variants. Pembrolizumab had a better safety profile; and treatment resulted in longer overall survival and progression free survival in patients with previously untreated and previously treated, PD-L1 positive, advanced NSCLC as compared with traditional platinum-based chemotherapy. The National Comprehensive Cancer Network (NCCN) NSCLC panel also recommends the combination of Pembrolizumab plus chemotherapy as a first line treatment option in patients with metastatic NSCLC and negative for the specific molecular variants or mutations previously mentioned.[3,11,25-29] Nivolumab was FDA-approved as second line treatment for metastatic NSCLC with the PD-L1 IHC 28-8 only as a "complementary diagnostic assay," which only guides the therapy but is not required for patients to receive the drug.[3,11,19,26] The US FDA initially approved Atezolizumab in combination with traditional chemotherapeutic drugs, as another first-line treatment option for metastatic NSCLC patients with no *EGFR* or *ALK* aberrations, regardless of histology or level of PD-L1 expression.[30,31] Months later, Atezolizumab monotherapy has been FDA-approved as another first line therapy intervention for patients with metastatic NSCLC with PD-L1 TPS ≥50% or PD-L1 stained tumor-infiltrating immune cells (IC) covering ≥10% of the tumor area, and wild-type with respect to *EGFR* mutations and *ALK* translocations. The Ventana SP-142 PD-L1 assay (Ventana Medical Systems, Inc.) was simultaneously approved as a companion diagnostic device in selecting patients eligible for treatment with Atezolizumab. Among the mAbs, however, therapeutic options that include Pembrolizumab and Atezolizumab are preferred, and have received a category 1 recommendation from the NCCN NSCLC panel based on tolerability and experience with these regimens.[3] Despite all these advances in therapy, data on the prognostic effect of PD-L1 expression on tumor cells are conflicting.[16,17,32] In fact, an Egyptian study concluded

that the evaluation of both PD-L1 staining on tumor cells and CD8 TILs density, instead of PD-L1 alone, have a more relevant impact on prognosis.[6]

Lastly, interaction between tumor cells and TILs are described by the concept of immunoediting which involves an elimination phase, equilibrium phase, and escape phase. The innate and adaptive immune cells of the host identify and destroy the tumor cells during the elimination phase. In the equilibrium phase, cancer cells that survive the first phase enter the state of dormancy and escape immune surveillance. Once these tumor cells induce an immunosuppressive state, the host immune response fails to restrict their growth (escape phase) and this results in a clinically apparent disease. The presence of TILs, specifically CD8+ lymphocytes, in the tumor microenvironment has been shown by various studies to be associated with positive clinical outcome in many solid tumors including NSCLC and is also associated with increased responsiveness to PD-1 inhibition in NSCLC. The presence of TILs has also been proven to significantly affect the prognostic yield of TNM classification in both colorectal and breast cancer. PD-L1 expression on TILs is postulated to be driven by adaptive mechanisms wherein inflammation-mediated release of cytokines, particularly interferon-γ (IFN-γ), by activated TILs lead to increase in their PD-L1 expression. PD-L1 expression on TILs has been associated with favorable patient outcome or survival.[6,17,33-35,39]

The study aims to determine the prevalence and pattern of PD-L1 expression on tumor cells of patients with NSCLC in a tertiary medical center in the Philippines, and identify their correlation with the presence of TILs as well as with PD-L1 expression on TILs. It also intends to identify the clinicopathologic features of lung cancer patients associated with PD-L1 expression on both tumor cells and TILs.

Operational definitions

PD-L1 expression on TILs (with 50% as cutoff):
0 – No membrane and/or cytoplasmic staining (at any intensity) using PD-L1 IHC on all mononuclear inflammatory cells within tumor nests and adjacent supporting stroma

less than 50% – membrane and/or cytoplasmic staining (at any intensity) of less than 50% of mononuclear inflammatory cells within tumor nests and adjacent supporting stroma

50% or more – membrane and/or cytoplasmic staining (at any intensity) of 50% or more of mononuclear inflammatory cells within tumor nests and adjacent supporting stroma

METHODOLOGY

Study design, samples and data gathering

This cross sectional study includes 187 consecutive LC clinical samples with PD-L1 testing using the clone 22C3 pharmaDx kit in St. Luke's Medical Center, Global City (SLMC-GC) from January 2017 to June 2018. It includes biopsy and cytology specimens from the lungs and pleura from patients with biopsy-confirmed NSCLC who submitted their tissue samples for PD-L1 testing in SLMC-GC. Only samples with at least 100 viable tumor cells were included in the study since this is the minimum quantity of cells deemed acceptable for PD-L1 IHC testing. Small cell lung cancer specimens were excluded from the study. Data on clinicopathologic features including age, sex, and tumor characteristics (specimen, biopsy type and histologic subtype) were collected from the histopathology reports in the hospital database. The samples were categorized as primary or metastatic NSCLC based on histomorphologic features and/or clinical data or immunohistochemistry results, if available. Institutional review board approval of the research protocol for the study was obtained through the St. Luke's Medical Center Research and Biotechnology group.

Immunohistochemistry and assessment of stromal tumor-infiltrating lymphocytes

Both hematoxylin and eosin (H&E) slides and PD-L1 slides were prepared from 4-μm thick tissue sections of the formalin-fixed, paraffin-embedded (FFPE) tissue blocks of samples from NSCLC patients that were submitted for PD-L1 testing. The PD-L1 IHC 22C3 pharmDx assay employs the Monoclonal Mouse Anti-PD-L1 Clone 22C3 and is visualized utilizing the EnVision FLEX visualization system on the Dako Autostainer Link 48 system. The PD-L1 IHC staining were performed in batches, and the assay

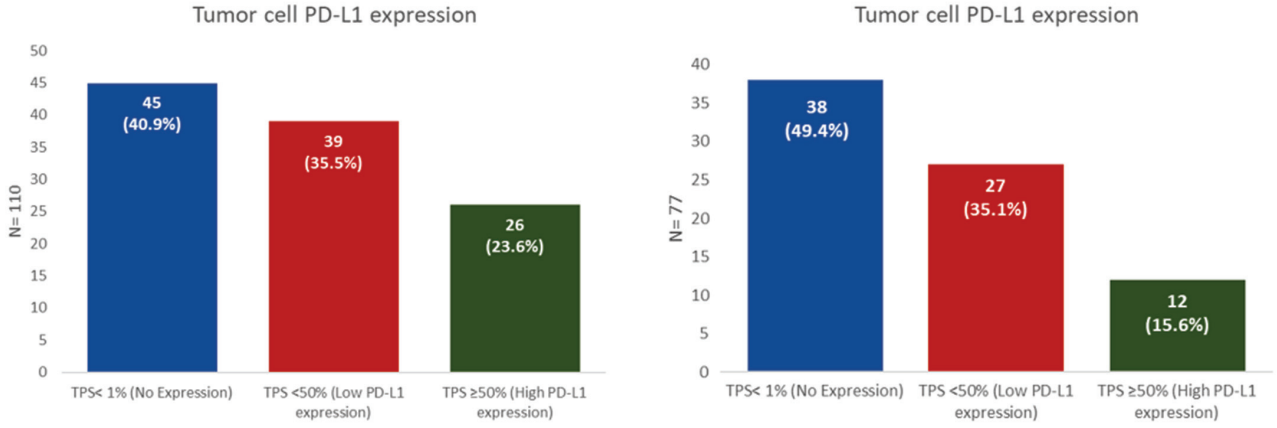

Figure 1. (A) Prevalence of low, high, and no PD-L1 expression on tumor cells in biopsy/resection specimens; **(B)** Prevalence of low, high, and no PD-L1 expression on tumor cells in cytology specimens.

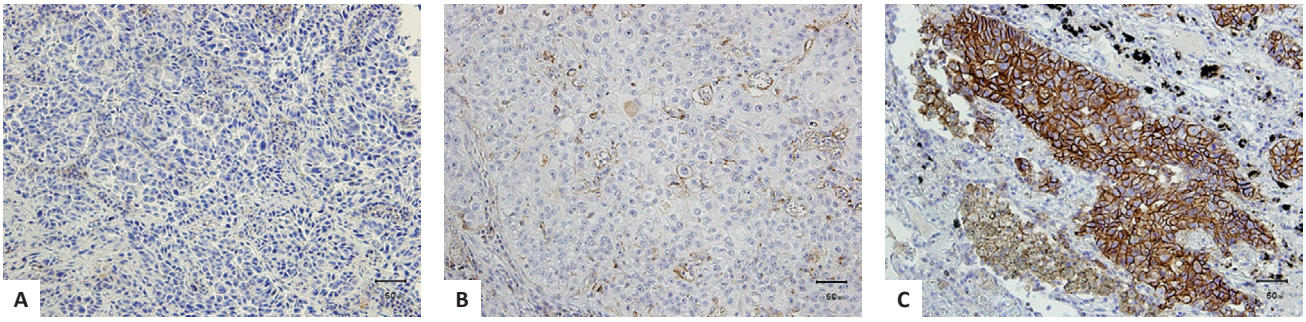

Figure 2. Immunohistochemistry images (10x). **(A)** TPS <1% (no PD-L1 expression); **(B)** TPS 1-49% (low PD-L1 expression); and **(C)** TPS ≥50% (high PD-L1 expression).

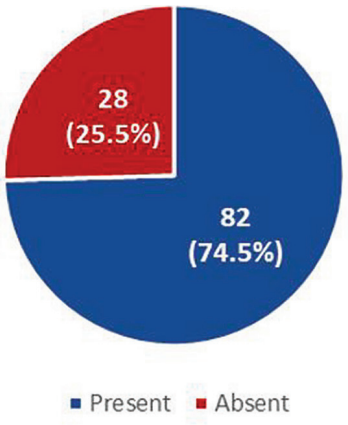

Figure 3. Prevalence of tumor-infiltrating lymphocytes in biopsy/resection specimens.

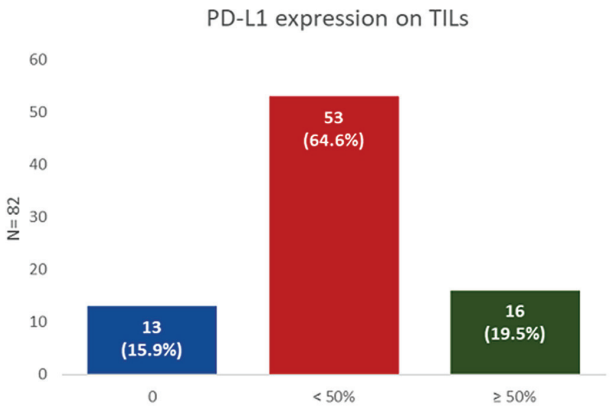

Figure 4. Prevalence of PD-L1 expression on tumor-infiltrating lymphocytes in biopsy/resection samples.

was used according to the manufacturer's instructions. Manufacturer- provided positive and negative cell line slides were included per batch run. Additional positive and negative controls (primary antibody was replaced by the buffer) using tonsil and skeletal muscle tissues were also included for every test slide. The cases underwent routine assessment of PD-L1 expression by tumor proportion scoring by two board-certified anatomic pathologists who have been previously trained in PD-L1 interpretation. Slides were filed after release of the official PD-L1 report which included the percentage of PD-L1 expression on tumor cells and presence or absence of tumor-infiltrating lymphocytes.

The above-mentioned pathologists independently reviewed the H&E and PD-L1 slides for the presence of stromal TILs, using the method previously described for breast cancers.[36] PD-L1 expression of stromal TILs was scored thereafter.

PD-L1 positive TC are viable tumor cells that exhibit membranous staining at any level. As per standard recommendation, PD-L1 expression on TC is determined by the tumor proportion score (TPS), which is the percentage of viable tumor cells displaying partial or complete membranous staining at any intensity. PD-L1 expression on TC was classified into: TPS <1% (No PD-L1 expression; Figure 2A), TPS 1 to 49% (Low PD-L1 expression; Figure 2B) and TPS ≥50% (High PD-L1 expression; Figure 2C). On the other hand, as there is no standardized method for assessment of PD-L1 expression on TILs yet, we arbitrarily assigned 50% as the cutoff, and PD-L1 expression on TILs were subdivided into: 0 (Figure 5A), less than 50% (Figure 5B) and 50% or more (Figure 5C). In cases of disagreement, the slides were reviewed by the two pathologists (JLA and DCA) to reach a consensus. There is substantial agreement between the two pathologists with a Kappa coefficient of 0.663, p value of less than 0.001, and 95% CI (0.543, 0.753). This is based on the interpretation of Kappa values by Landis and Koch scale, with Kappa value <0.0 having poor agreement, and 1 having almost perfect agreement.[40]

Data Analysis

An interobserver reliability analysis using the Cohen's Kappa (κ) was performed to determine the reproducibility of scoring for PD-L1 expression on TILs by the participating pathologists. Interpretation was based on the methodology by Landis and Koch.[40] Descriptive statistics were presented as frequencies, proportions, and tables. Numerical data were described using mean, standard deviation, median, and minimum and maximum values. Relationship between the clinicopathologic characteristics and PD-L1 expression was assessed using logistic regression analysis. Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM, Armonk, New York). A p-value of <0.05 was considered statistically significant.

RESULTS

Overall

A total of 187 subjects were included in the study, the clinicopathologic characteristics of whom were summarized

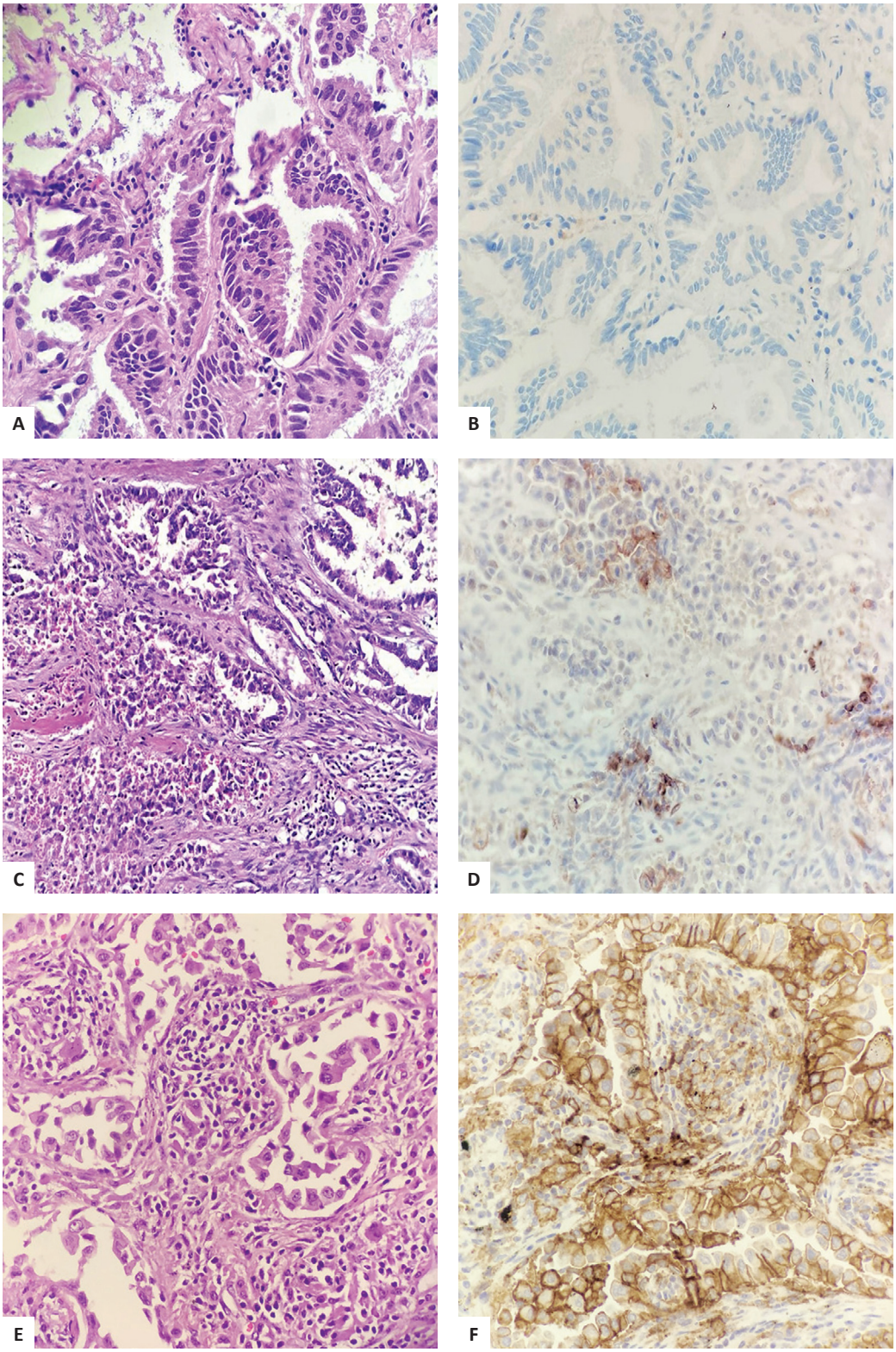

Figure 5. Representative H&E and IHC images (10x). **(A, B)** No PD-L1 expression on TILs; **(C, D)** <50% PD-L1 expression on TILs; and **(E, F)** ≥50% PD-L1 expression on TILs.

in Table 1. The mean age at presentation was 66.4 years old (± 10.7), with a minimum age of 32 years and a maximum age of 92. One hundred twelve (60.0%) were males and 75 (40.0%) were females. Majority (86.6%) of the specimen were obtained from the lungs and 25 (13.4%) were from pleural tissue or effusion. One hundred ten (58.8%) biopsy/resection and 77 (41.2%) cell block samples were evaluated in this study. Sixty (77.92%) of cell block samples were from the lungs, while 17 (22.08%) were from pleural effusion. The most common histopathologic diagnosis was adenocarcinoma (70.1%) followed by NSCLC (17.1%), and squamous cell carcinoma (8.0%). The overall prevalence of tumor cell PD-L1 expression in NSCLC patients in this study was 55.6% (104).

Tumor biopsy/resection specimens

The clinicopathologic profile of the 110 patients with biopsy/resection specimen were displayed in Table 2. The mean age was 65.48 years old (±10.66). Patients were 32 to 92 years of age. Forty-five (40.9%) were females and 65 (59.1%) males. The most common histopathologic diagnosis was adenocarcinoma (70%) followed by NSCLC, NOS (16.4%), and squamous cell carcinoma (10.9%). The prevalence of tumor cell PD-L1 expression in lung cancer patients with biopsy/resection samples in this study was 59.1%. Figure 1A shows that among the 110 subjects with biopsy/resection specimens, 26 (23.6%) had high PD-L1 expression (Figure 2C), while 39 (35.5%) had low PD-L1 expression (Figure 2B).

As displayed in Figure 3, tumor-infiltrating lymphocytes were noted in 82 (74.5%) biopsy/resection samples, and PD-L1 expression on TILs was noted in 69 (84.1%) of these patients. Figure 4 exhibits that 53 (64.6%) of 82 subjects with biopsy/resection specimen demonstrated less than 50% PD-L1 expression (Figures 5C and 5D), 16 (19.5%) showed ≥ 50% PD-L1 expression (Figures 5E and 5F), and the remaining 13 (15.9%) did not express PD-L1 (Figures 5A and 5B).

Table 3 shows that in biopsy/resection samples, tumor cell PD-L1 expression was noted in 28 (37.8%) of the 45 females and 37 (56.9%) of 65 males. Sixty-one (59.2%) of the 103 specimens from the lungs, and 4 (57.1%) of 7 pleural tissue specimens had PD-L1 expression on tumor cells. Tumor cell PD-L1 expression were noted in 45 (58.4%) of 77 biopsy/resection specimens diagnosed as adenocarcinoma, 12 (66.7%) of 18 cases diagnosed as NSCLC, NOS, 6 (50.0%) of 12 cases diagnosed as squamous cell carcinoma, and all cases with the diagnosis of adenosquamous carcinoma and poorly differentiated malignancy. Fifty-one (62.2%) biopsy/resection samples with tumor-infiltrating lymphocytes showed PD-L1 expression on tumor cells. There is no statistically significant association between the histopathologic features and PD-L1 expression on tumor cells in lung cancer patients with biopsy/resection specimens.

Tumor-infiltrating lymphocytes were noted in 33 (73.3%) of 45 females and 49 (75.4%) of 65 males with biopsy/resection samples. Seventy-seven (74.8%) 103 lung specimens and 5 (71.4%) of 7 pleural tissue specimens demonstrated tumor-infiltrating lymphocytes. TILs were noted in all biopsy/resection specimens diagnosed with squamous cell carcinoma, adenosquamous carcinoma, poorly differentiated malignancy, and large cell carcinoma. TILs were also noted in 55 (72.4%) adenocarcinoma cases, and in 12 (66.7%) of biopsy/resection samples with a diagnosis of NSCLC, NOS. No TILs were demonstrated in the sample diagnosed with mucinous carcinoma.

PD-L1 expression on TILs was displayed by 31 (90.0%) of 33 females and 39 (79.6%) of 49 males. Sixty-five (84.4%) of 77 lung resection specimens and 4 (80.0%) of 5 pleural tissue specimens exhibited PD-L1 expression on TILs. Majority (84.1%) of the 82 biopsy/resection samples showed tumor-infiltrating lymphocyte PD-L1 expression. All cases of large cell carcinoma and poorly differentiated malignancy demonstrated tumor-infiltrating lymphocyte PD-L1 expression. PD-L1 expression on TILs was also noted in 91.7% of biopsy/resection samples diagnosed with squamous cell carcinoma, 83.6% of adenocarcinoma cases, and 83.3% of NSCLC, NOS cases.

Logistic regression analysis showed no association between tumor location and tumor histology, and PD-L1 expression on tumor-infiltrating lymphocytes. There is an association between sex and PD-L1 expression on TILs, wherein females are 8.25 times more likely to have <50.0%

Table 1. Clinicopathologic characteristics of the lung cancer cohort (overall)

Age (years)		
Mean	66.4 ± 10.7	
Median	67.0	
Minimum	32.0	
Maximum	92.0	
	Frequency	**Percent**
Sex (n=187)		
Female	75	40.0
Male	112	60.0
Tumor Location (n=187)		
Lung	162	86.6
Pleural tissue/pleural fluid	25	13.4
Type of Biopsy (n=187)		
Biopsy/resection	110	58.8
Cell Block	77	41.2
Tumor Histology (n=187)		
Adenocarcinoma	131	70.1
Adenosquamous carcinoma	4	2.1
Squamous cell carcinoma	15	8.0
NSCLC, NOS	32	17.1
Poorly differentiated malignancy	3	1.6
Large cell carcinoma	1	0.5
Mucinous carcinoma	1	0.5
Total Tumor Cell PD-L1 Expression (n=187)		
Present	104	55.6
Absent	83	44.4

Table 2. Clinicopathologic characteristics of lung cancer patients with lung or pleural biopsy/lung resection and pleural fluid/lung cytology specimens

	Biopsy/resection		**Cytology**	
Age (years)				
Mean	65.48 ± 10.66		67.65 ± 10.70	
Median	66.5		68.0	
Minimum	32.0		36.0	
Maximum	92.0		90.0	
	Frequency	**Percent**	**Frequency**	**Percent**
Sex				
Female	45	40.9	30	39.0
Male	65	59.1	47	61.0
Tumor Location				
Lung	103	93.6	59	76.6
Pleural tissue/pleural fluid	7	6.4	18	23.4
Tumor Histology				
Adenocarcinoma	77	70.0	54	70.1
Adenosquamous carcinoma	1	0.9	3	3.9
Squamous cell carcinoma	12	10.9	3	3.9
NSCLC, NOS	18	16.4	14	18.2
Poorly differentiated malignancy	1	0.9	2	2.6
Large cell carcinoma	1	0.9	0	0.0
Mucinous carcinoma	0	0	1	1.3
Total Tumor Cell PD-L1 Expression				
Present	65	59.1	39	50.6
Absent	45	40.9	38	49.4

Table 3. Clinicopathologic characteristics and total tumor cell PD-L1 expression of lung cancer patients with biopsy/ resection specimens

Characteristics	Total Tumor Cell PD-L1 Expression		Total
	Presence n (%)	Absence n (%)	
Sex			
Female	28 (37.8)	17 (62.2)	45
Male	37 (56.9)	28 (43.1)	65
Tumor Location			
Lung	61 (59.2)	42 (40.8)	103
Pleural tissue	4 (57.1)	3 (42.9)	7
Tumor Histology			
Adenocarcinoma	45 (8.4)	32 (41.6)	77
Adenosquamous carcinoma	1 (100.0)	0	1
Squamous cell carcinoma	6 (50.0)	6 (50.0)	12
NSCLC	12 (66.7)	6 (33.3)	18
Poorly differentiated malignancy	1 (100.0)	0	1
Large cell carcinoma	0	1 (100.0)	1
Mucinous carcinoma	0	0	0
Tumor-Infiltrating Lymphocytes			
Presence	51 (62.2)	31 (37.8)	82
Absence	14 (50.0)	14 (50.0)	28

Table 4. Clinicopathologic characteristics and total tumor cell PD-L1 expression of lung cancer patients with cytology specimens

Characteristics	Total Tumor Cell PD-L1 Expression		Total
	Presence n (%)	Absence n (%)	
Sex			
Female	14 (46.7)	16 (53.3)	30
Male	25 (53.2)	22 (46.8)	47
Tumor Location			
Lung	29 (49.2)	30 (50.8)	59
Pleural tissue	10 (55.6)	8 (44.4)	18
Tumor Histology			
Adenocarcinoma	27 (50.0)	27 (50.0)	54
Adenosquamous carcinoma	2 (66.7)	1 (33.3)	3
Squamous cell carcinoma	3 (100.0)	0	3
NSCLC	6 (42.9)	8 (57.1)	14
Poorly differentiated malignancy	1 (50.0)	1 (50.0)	2
Large cell carcinoma	0	0	0
Mucinous carcinoma	0	1 (100.0)	1
Tumor-Infiltrating Lymphocytes			
Presence	0	0	0
Absence	0	0	0

TIL PD-L1 expression than males. An association was also noted between the percent PD-L1 expression on TILs and PD-L1 expression on tumor cells. Biopsy/resection samples with low tumor cell PD-L1 expression were 12.4 times more likely to have <50.0% PD-L1 expression than samples with high tumor cell PD-L1 expression.

Cytology specimens

A total of 77 subjects with cell block specimens were included in this study, the clinicopathologic features of whom were listed in Table 2. The mean age is 67.65 years old (± 10.70) with a range of 36 to 90 years old. 30 (39.0%) were females and 47 (61.0%) males. The most common histopathologic diagnosis was adenocarcinoma (70.1%) followed by NSCLC, NOS (18.2%), and squamous cell carcinoma and adenosquamous carcinoma (3.9%). In this study, the prevalence of tumor cell PD-L1 expression for lung cancer patients with cytology specimen was 50.6%. Figure 1B shows that of the 77 cytology samples evaluated, 12 (15.6%) had high PD-L1 expression and 27 (35.1%) had low PD-L1 expression.

Tumor-infiltrating lymphocytes were not appreciated in lung cancer patients with cell block samples.

In patients with cell block samples (Table 4), tumor cell PD-L1 expression was noted in 14 (46.7%) of the 30 females and 25 (53.2%) of 47 males. Twenty-nine (49.2%) of the 59 specimens from the lungs, and 10 (55.6%) of 18 pleural fluid specimens had PD-L1 expression on tumor cells. Tumor cell PD-L1 expression were noted in all 3 cytology samples with a diagnosis of squamous cell carcinoma, 27 (50.0%) of 54 adenocarcinoma cases, 6 (42.9%) of 14 NSCLC, NOS cases, 2 (66.7%) of 3 adenosquamous carcinoma cases, and 1 (50%) of 2 cases diagnosed with poorly differentiated malignancy. PD-L1 expression was not demonstrated by the cytology specimen with a diagnosis of mucinous carcinoma. There is no association between clinicopathologic characteristics and PD-L1 expression on tumor cells among the subjects.

DISCUSSION

Over the last few years, various novel therapeutic options, including immune checkpoint inhibitors (ICIs), were introduced for NSCLC. Current NCCN guidelines incorporate immune checkpoint inhibitors, either as monotherapy or in combination with traditional chemotherapeutic drugs, for the treatment of NSCLC. Immune checkpoint blockade leads to reactivation of immune response against tumor cells.[13] The NCCN NSCLC panel also recommends molecular diagnostic tests for *EGFR* mutations, *ALK* translocation, *ROS1* fusion, *BRAF* variants and PD-L1 immunohistochemistry for advanced and metastatic cases. ICIs target the PD-1/ PD-L1 pathway, a dominant pathway that contributes to immune evasion by certain tumors. Assessment of PD-L1 protein expression by IHC is the best predictive marker for response to immunotherapy with ICIs.[3,16]

Several IHC assays using different antibody clones have been developed to assess for PD-L1 protein expression for eventual targeted therapy. These assays follow different staining protocols, and scoring algorithms and cutoffs are not standardized. Except for the antibody clone SP142 in which scoring is based on either TC PD-L1 expression or the fraction of tumor area of the tumor occupied by PD-L1 expressing tumor-infiltrating IC, scoring of PD-L1 expression for most assays involve the evaluation of TC PD-L1 expression alone.[21,22,41] Among the ICIs available in the market, regimens which include Pembrolizumab and Atezolizumab received a category 1 recommendation for use in NSCLC treatment by the NCCN.[3] PD-L1 IHC 22C3 pharmDx has been approved by the U.S. FDA as "companion diagnostic assay" for patient selection for Pembrolizumab therapy. The drug is recommended for patients with TC PD-L1 expression of at least 1%.[3,11,26-30] This recent recommendation and subsequent change in scoring of TC PD-L1 expression.[42] occurred along the course of this study. Across studies, the proportion of PD-L1 expressing tumor cells in lung cancer is wide-ranging. This might be attributed to the difference in

sample size, subject population, and antibody panel used, tumor heterogeneity and decay of antigenicity in archived specimens.[7,15,18,26]

Our study aimed to determine the prevalence of PD-L1 expression in NSCLC in a tertiary hospital in the Philippines and determine their association with pathologic features. The overall prevalence of PD-L1 expression on tumor cells in NSCLC in our study population is 55.6%. Tumor cell PD-L1 expression was appreciated in 59.1% of histologic specimens. Of these, 40.9% of samples had no TC PD-L1 expression, 35.5% displayed low TC PD-L1 expression and 23.6% showed high TC PD-L1 expression. On the other hand, 50.6% of cytology samples demonstrated PD-L1 expression on tumor cells. Among these cases, 49.4%, 35.1% and 15.6% showed no, low and high PD-L1 expression, respectively. As in most previous studies,[7,16-17,33] we found no significant association between clinicopathologic characteristics and PD-L1 expression on the tumor cells in NSCLC in both specimen types.

Lung cancers as well as some other solid tumors exhibiting TILs, particularly CD8+ lymphocytes, in the tumor stroma are found to have better survival, and are associated with increased responsiveness to ICI. In addition, past studies have observed that NSCLC tumors demonstrating PD-L1 expression on TILs have better prognosis as compared to tumors with no PD-L1 expression on stromal TILs.[6,17,33-35,41] Therefore, information on the presence of TILs as well as their PD-L1 expression status adds up to the prognostic value of the test. Evaluation for the presence of TILs based on morphology in H&E-stained slides following the criteria established for breast cancer[37] was also done in the study.

Zhao et al., demonstrated that the median positive rate of PD-L1 expression on tumor-infiltrating immune cells is 36.37%. The group also described the different mechanisms implicated in PD-L1 expression in tumor cells and tumor-infiltrating immune cells.[34]

In our study, 82 (74.5%) of 110 biopsy/resection samples displayed stromal TILs. PD-L1 expression on TILs was noted in 84.1% of these 82 cases. TILs in 53 (64.6%) specimens showed less than 50% PD-L1 expression, and 16 (19.5%) samples exhibited ≥50% PD-L1 expression. A few studies discovered a correlation between tumor cell PD-L1 expression and the presence of stromal tumor-infiltrating lymphocytes.[30,37] As with PD-L1 expression in tumor cells, PD-L1-expression in tumor-infiltrating lymphocytes is also assumed to weaken the host immune response against tumor cells.[9]

Except for the finding that female subjects with biopsy/resection specimen are more likely to have less than 50% PD-L1 expression on TILs, there was no association between TILs and PD-L1 expression on TILs, and clinicopathologic features in both specimen type subgroups. The interpretation might be limited by the lack of clinical data i.e., smoking history and clinical stage upon procedure. We also found that histologic samples with low TC PD-L1 expression were more likely to have <50% PD-L1 expression on TILs. Further investigation should be carried out to explore the association between low PD-L1 expression on TILs and gender, as well as its correlation with low PD-L1 expression in tumor cells. Phases 1 and 2 of the Blueprint PD-L1 immunohistochemistry comparability project concluded that variability of PD-L1 staining in immune cells is greater than that of TC staining. Also, in contrast with the strong reliability in PD-L1 scoring of TCs among pathologists, evaluation of PD-L1 expression on ICs has poor reliability.[23,24] He et al., concluded that PD-1, instead of PD-L1 expression, was correlated with TILs.[17,32]

The present study is limited by its retrospective approach, lack of clinical data (smoking history, staging and clinical response) and lack of information regarding driver mutation status, the substantial number of cytology and biopsy specimens, and the non-uniformity of the pre-analytic variables involving the samples submitted for testing. In actuality, since it is a less invasive method of specimen collection, at least a third of lung malignancies are diagnosed based on cytology alone. However attractive it may seem, greater pre-analytic variability involving preparation techniques, fixatives, preservatives and stains, affects cytology specimens. Nonetheless, although they provide a limited tumor material and some tumors may display heterogeneity, good agreement has been observed between cytologic and histologic specimens.[5,19] With sufficient tumor sample, PD-L1 testing will greatly impact patient management and assist the oncologists in their treatment decisions for patients with lung cancer. This is the first local study to describe PD-L1 expression on both TC and TILs in NSCLC and its association with histopathologic features. The prognostic value of PD-L1 expression and clinical response to ICIs in the Filipino population remains to be explored.

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STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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