The Role of Programmed Cell Death Receptor 1 in Lung Cancer

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Abstract. *Background/Aim: Lung cancer is the most common cancer worldwide. Cancer immunotherapy is the activation of the immune system against cancer. The latest method of immunotherapy involves immune checkpoint inhibitors. Increased levels of programmed death ligand 1 (PD-L1) expression were observed on non-small-cell lung cancer. The association between PD-L1 expression and clinicopathological characteristics in lung cancer is still unclear. Patients and Methods: This is a cross-sectional, observational study that evaluated a sample of 41 lung cancer patients diagnosed between March 2019 and December 2020. PD-L1 tumor expression is described as a percentage. Results: Patients were diagnosed with non-microcellular lung cancer and aged 37 to 87 years. Most patients were diagnosed with adenocarcinoma. According to the analysis, the average age of patients with negative PD-L1 tumors was 65.6 years, and of those with positive PD-L1 tumors was 63.6 years. The average value of the tumor proportion score for males was 26.97%, and for females 25.55%. Conclusion: No correlation was found between PD-L1 tumor expression and the age and sex of patients.*

Lung cancer is the most common cancer in the world. In 2018, there were 1.8 million new cases and was estimated to be responsible for 1.6 million deaths. It remains the first cause of death from cancer (1). Immunotherapy is a major breakthrough in thoracic oncology, and was first indicated as 2nd therapeutic line and then as 1st line. The concept of immunotherapy is an old concept and is defined as activation of the immune system against cancer

Immunotherapy drugs called immune checkpoint inhibitors work by blocking checkpoint proteins present on T cells, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1) proteins. PD-ligand 1 (PD-L1) is a transmembrane protein that binds to the PD-1 receptor. The use of these immune checkpoint inhibitors allows restoration of antitumor immunity, either in the lymphoid organs during presentation of tumor antigens (Ag) to dendritic cells or directly in the tumor microenvironment by enhancing the functionality of cytotoxic lymphocytes (2). Anti-CTLA4 (Ipilimumab) and anti-PD1 monoclonal antibodies (Nivolumab, Pembrolizumab) have been approved for the treatment of advanced or metastatic melanoma. Nivolumab and pembrolizumab, both PD-1 inhibitors, have been approved for the treatment of patients with advanced or metastatic melanoma and those with metastatic non-small cell lung cancer (3-5).

Although the results obtained with these new antibodies were impressive regarding the duration of tumor response, these effects often remain moderate, and the benefit is limited to a certain patient category. A major challenge is therefore the selection of patients who would benefit more from the administration of these new therapies. In parallel with the development of these new therapeutic agents, identification of reliable and standardized biomarkers to

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Figure 1. Distribution of patients by age groups.

predict tumor response is an important issue. The expression of PD-L1 in the tumor microenvironment could be a marker to predict the response to immunotherapies. However, the immunohistochemistry technique, antibody selection, and positivity threshold value remain to be precisely defined (6).

PD-L1 and PD-L2 are members of the B7 protein family. They appeared as the result of gene duplication and their sequences are 37% homologous. Unlike PD-L2 whose expression predominates on antigen presenting cells (APC), PD-L1 can be expressed on a variety of cells such as T, epithelial, and endothelial cells. The PD-L1 pathway regulates the function of T lymphocytes and prevents autoimmunity. In the case of infection, microbial antigens activate T lymphocytes that will increase the surface expression of PD-1. PD-L1 expression will also be induced in tissues through inflammatory signals, which will prevent

collateral tissue damage by inhibiting T lymphocytes. Excessive PD-1 expression, often induced by prolonged exposure to antigens, is associated with a hyporesponsiveness of T lymphocytes. In malignant tissues, PD-1 expression is amplified in tumor infiltrating lymphocytes (TIL) (10), and increased PD-L1 expression is common in many cancers, including NSCLC (12).

Patients and Methods

This is a cross-sectional, observational study that evaluated a sample of 41 lung cancer patients diagnosed and hospitalized at the Bucharest Oncology Institute, between March 2019 and December 2020.

Patients were informed of the purpose and conduct of the study and signed informed consent. PD-L1 tumor expression is described as a percentage and the anatomopathological type, frequency and

Figure 2. Representation of all tumor proportion score values according to age.

type of *EGFR* and *ALK* mutations are noted. The existence of a correlation between PD-L1 expression, represented by the tumor proportion score (TPS), and the age or sex of the patient was examined. Evaluation of PD-1 and PD-L1 expression was detected using qualitative immunohistochemistry. PD-L1 tumor expression was divided into groups according to TPS: PD-L1 negative tumor (TPS <1%); PD-L1 positive tumor (TPS ≥1%); Tumor with low PD-L1 expression (TPS ≥1% and TPS <50%); Tumor with high PD-L1 expression (TPS ≥50%).

Results

The sample studied consisted of 41 patients diagnosed with non-microcellular lung cancer, aged 37 to 87 years. By dividing the patients into different age groups, we noticed that the group of patients aged 60-69 years was the majority and represented 56.1% of the total. It was followed by the age group between 70 and 79 years (8 patients, 19.5%), then the age group 50-59 years (7 patients, 17.1%), and the age groups 30-39, 40-49, and 80-89 (1 patient in each group, 2.4%) (Figure 1). Of the patients included in the study, 73% were male (30 patients) and 27% female (11 patients) (Figure 1).

The highest value, 95% of tumor proportion score (TPS), was observed in a 59-year-old patient. The age of patients with negative expression ranged from 61 to 72 years. The mean TPS value of male patients differed from that of females (Figure 2).

The average value of TPS for males was 26.97%, and for females 25.55%. Moreover, adenocarcinomas accounted for 65.9% of the total, squamous cell carcinomas 22.0%, undifferentiated or poorly differentiated tumors 9.8%, and large cell carcinomas 2.4% (Figure 3).

Out of 41 patients tested, *EGFR* mutations were identified in 7.3% of patients (n=3), all of which were exon 19 deletions (E19del). Out of 27 patients diagnosed with adenocarcinoma, *ALK* abnormalities were identified in 7.4% of patients (n=2).

Table I. Data distribution normality test.

	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Age	0.131	41	0.073	0.919	41	0.007
TPS	0.302	41	0.000	0.759	41	0.000
Sex	0.457	41	0.000	0.554	41	0.000

TPS: Tumor proportion score; df: degrees of freedom; Sig: significance probability.

According to the analysis, the average age of patients with negative PD-L1 tumors was 65.6 years and that of those with positive PD-L1 tumors was 63.6 years. Increased PD-L1 expression has been observed in patients over 65 years of age.

Analysis of the distribution of patient data in the sample showed a distribution that did not meet the conditions of normality. Following the analysis, no correlation was found between PD-L1 tumor expression and the age or sex of the patients (Table I).

Because the distribution was not normal, correlation tests for nonparametric data, Spearman rho and Kendall tau, were used to verify the relationship between TPS value and age and between TPS value and patient sex (Figure 4).

Discussion

Various studies have examined the associations between PD-L1 tumor expression and clinical, histopathological, and immunological features of NSCLC. According to them, the histological type, the magnitude of tumor invasion, and TNM staging do not correlate with PD-L1 expression. However, there are conflicting results regarding smoking status, sex, and lymphatic invasion (17-18). In contrast, positive PD-L1 expression is associated with a low degree of differentiation (12), the presence of *EGFR* mutations (13), and *ALK* translocations (14), and in *EGFR*-positive patients, PD-L1 expression is considered to be an indicator of unfavourable prognosis (13).

The TPS value or classification according to TPS before the start of therapy is not associated with changes in PFS or overall survival in patients with unresectable NSCLC treated with chemoradiotherapy (CRT), thus PD-L1 expression does not affect CRT response (15).

In the case of resectable tumors, PD-L1 expression is constantly associated with decreased survival (16). The tumor microenvironment, compared to that of healthy lung tissue, is characterized by the presence of immunosuppressive factors even at the early stages.

Figure 3. Average tumor proportion score value for each sex.

Figure 4. Quantile-quantile (Q-Q) plot representation for tumor proportion score.

Immunotherapy strategies are very promising for the treatment of lung cancer: first, second line, maintenance, but also the combination with bevacizumab, or tyrosine kinase inhibitors (TKIs) in case of mutation in *EGFR* or of *ALK* rearrangement. Finally, the PD-1/PD-L1 checkpoint pathway is just one of the many mechanisms regulating the interaction between tumor cells and the immune system, and several other antibodies targeting other receptors, such as KIR, LAG3, OX40, TIM3, which regulate these mechanisms, are in development (17).

The results obtained in this study are consistent with those of other studies, which investigated the associations between PD-L1 expression and clinical variables. These investigations describe the lack of a significant difference between the mean age and PD-L1 expression (negative TPS, TPS $\geq 1\%$ and $<50\%$, TPS $\geq 50\%$), except for an association between age over 90 years and positive PD-L1 expression. However, according to meta-analyses, positive expression correlates with male sex (18, 19).

The clinical profile of patients with PD-1 expression differs from that of patients with no PD-L1 expression, as follows: a patient with PD-1 expression is more frequently male and smoker, and the histopathological type is adenocarcinoma. The profile of patients who expressed PD-L1 is more frequently female and non-smoker or former smoker, and well-differentiated adenocarcinoma. These patients can be carriers of *KRAS* and *EGFR* mutations (4).

It has been observed that PD-L1 expression in tumor tissues increases with the degree of malignancy, both in adenocarcinoma and squamous cell carcinoma, although in squamous cell carcinoma there is a significant difference only between well differentiated (G1) and moderately differentiated (G2) or poorly differentiated (G3) tumors.

Curative surgery and radiation therapy are not treatment options when the disease is at an advanced stage and until recently, these patients were offered chemotherapy (20). Based on existing data, PD-L1 has a limited prognostic value. However, given the contradictory results of many studies on the predictive value of PD-L1, further investigations are warranted, especially on larger samples.

The small size of the patient group and therefore the abnormal distribution of data and their unbalanced distribution by sex are the limitations of this study.

Conclusion

The results obtained in this study are consistent with those of other studies, which investigated the associations between PD-L1 expression and other variables. These investigations describe the lack of a significant difference between the mean age and tumor expression subgroups (negative TPS, TPS $\geq 1\%$ and $<50\%$, TPS $\geq 50\%$).

Conflicts of Interest

The Authors declare that they have no competing interests in relation to this study.

Authors' Contributions

AZ was responsible for writing the manuscript. AZ, BH, CM and DCB were responsible for reviewing and editing the manuscript. AZ, BH and RA made substantial contributions to the conception or design of the work. RA critically reviewed the manuscript. All Authors read and approved the final manuscript.

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