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Case report

Clinical characteristics and PD-L1 expression in primary lung squamous cell carcinoma: A case series

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ABSTRACT

Background: Squamous cell lung carcinoma(SCLC), accounts for 20% of lung cancer(LC). The binding of programmed cell death 1(PD-1) to its ligand PD-L1 is a key checkpoint regulator of immune response, and overexpression of the latter leads to immune surveillance escape. This might represent an important oncogenic mechanism, as well as a predictor for immunotherapy treatment success in SCLC.
Methods: A retrospective series of 24 patients with SCLC was included(2009–2013). These patients presented with a single pulmonary lesion and no history of previous cancer. Expression of PD-L1 was evaluated on tumoral biopsies with immunohistochemistry. PD-L1 tumor proportion score(TPS) was considered high when ≥50%. Clinical characteristics regarding diagnosis were reviewed and recorded. Data were analysed in STATA v.14®.
Results: Twenty four patients were included in this series. Mean age was 67 + 14 years, and 62.5% were men. Smoking status was positive in 54%. Cancer stage IV was present in 54%. PD-L1 was positive in 13(54%). (+)PD-L1 was more frequent in smokers than in non-smokers(11 vs 2)($p = 0.001$), as well as in COPD patients($p = 0.006$). General overall survival was 21.8% at 5 years. Overall survival at one year in PD-L1(+) was 30.7% and 72.7% for PD-L1(-) patients. Survival median for PD-L1(+) patients was 10.5mo, as well as for the whole series.
Conclusion: Patients with primary SCLC who have a high PD-L1 TPS, had a worse overall survival than their counterparts. PD-L1 expression in SCLC in a Colombian sample lies between the one found in the literature.

1. Introduction

Lung cancer (LC) is the leading cause of cancer worldwide, and cancer-related deaths [1]. During 2018, more than 2 million cases were diagnosed, equivalent to 11.6% of all cancer diagnoses and 18.4% of all cancer-related deaths [1]. Most deaths from LC are attributed to smoking [2].

Approximately 20% of all lung cancer cases are a type of non-small cell lung cancer (NSCLC) called squamous cell lung carcinoma (SCLC) [3] that develops from squamous cells in the airway and is usually related to a history of smoking. Other factors like environmental pollution and genetic variants have also been associated with SCLC [4]. As such, not all smokers will develop lung cancer and some lung neoplasms might develop in non-smoking patients [5]. Furthermore, SCLC patients usually have a shorter survival when compared with non-squamous NSCLC patients [6].

Malignant cells escaping immune surveillance is a hallmark in cancer pathogenesis [7]. Different evidence sources have demonstrated that immune checkpoint molecules like PD1/PD-L1 and CTLA4 are critical in keeping self-antigen tolerance under homeostatic conditions, but are commonly used by different tumors to evade immune surveillance [8–10].

Monoclonal antibodies are currently successfully used to inhibit the interaction of the PD-1 receptor with the PD-L1 protein [11]. The inhibition of the PD-1/PD-L1 interaction has been reported to have a significant antitumor efficacy in different types of neoplasms besides lung cancer, including renal cell carcinoma, colorectal cancer and melanoma [12–15]. However, there are very few reports on PD-L1 expression,

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specially for SCLC, in Latin America, where smoking rates vary by country but are overall still quite high [16]. This test was only recently implemented in Colombia along with the introduction of pembrolizumab, and they have changed the clinical course and survival of NSCLC patients.

In this study we describe PD-L1 expression in a case series of patients with SCLC, a very selected sample of NSCLC, in which checkpoint inhibitors are progressively taking a more important role for targeted treatment. We aim to describe if Colombian patients with squamous cell carcinoma of the lung express PD-L1 in a similar proportion as reported in the literature.

2. Material and methods

This is a descriptive case series study of patients with primary SCLC diagnosed between 2009 and 2013 and confirmed by immunohistochemistry, without history of previous cancer. PD-L1 expression was studied in tumoral tissue using the PD-L1 IHC 22C3 pharmDx immunohistochemical assay (Agilent, Santa Clara, CA, USA) in the platform Autostainer Link 48 by DAKO®. Expression level of PD-L1 was assessed with Tumor Proportion Score (TPS). We considered TPS≥50% as high expression, TPS = 1–49% as low expression, and TPS = 0% as negative. Because of our small sample we decided to divide our patients in two groups regarding PD-L1 expression, a group with a high PD-L1 expression (TPS≥50%) and a group with a low expression or negative expression of PD-L1 (TPS<50%). Tumor Infiltrating Lymphocytes (TILs) were assessed by immunohistochemistry using a semiquantitative analysis in which 0 was negative, 1+ was low levels, 2+ was intermediate levels and 3+ was high levels. Clinical characteristics at diagnosis, including smoking history, history of chronic obstructive pulmonary disease (COPD), tumor size, radiologic findings and staging were reviewed form the clinical records.

Data were analysed in STATA v.14®. For the descriptive statistical analysis we established proportions for qualitative variables. For quantitative variables, central tendency measures with their respective measures of dispersion were calculated according to distribution assessed with the Shapiro-Wilk test. Chi-squared test was used for qualitative variables. The *t* Student test was used for quantitative variables according to data distribution. Estimation of survival was calculated with the Kaplan-Meier estimator.

3. Results

3.1. General characteristics

We included 24 patients in the study. Their mean age at diagnosis was 67 ± 14 years, and 63% were men. Thirteen patients (54%) had a history of smoking and seven (29.2%) presented with a history of chronic obstructive pulmonary disease (COPD). Thirteen patients (54%) had hemoptysis and 6 (25%) presented with pleural effusion. Twenty-two patients (92%) had a mass in the CT-scan, the other two had nodulary lesions <30 mm, and one-third presented with cavitary lung lesions. High expression of PD-L1 was present in 54% (n = 13), and had no significant association with age (p = 0.251) or gender (p = 0.675), but was related with history of smoking (p = 0,001) and history of COPD (p = 0,006). In patients with high expression of PD-L1, tumor infiltrating lymphocytes (TILs) were present in low levels in 61% of cases and intermediate levels in 38%. Other general demographic characteristics are shown in Table 1.

3.2. Overall survival

Overall survival (OS) for the whole cohort was 21,8% at 5 years (95% confidence interval [CI], 7.5 to 40) with a median OS (mOS) of 10.5 months. OS at one year for PD-L1 high expression patients was 30,7%, compared with 72,7% for PD-L1 low expression/negative patients. At five years, only PD-L1 low expression/negative patients remained alive, with a 52% OS (Log-rank test $p = 0.0041$). (Fig. 1).

Table 1
Clinical-pathological description of the patients according to their expression of PD-L1.

Characteristics	General (n = 24)	PD-L1/TPS (n = 24)		p value
		Negative (n = 11)	Positive (n = 13)	
Age				
30–39	2 (8.33)	2 (18.18)	0 (0)	0,251
50–59	4 (16.67)	1 (9.09)	3 (23.08)	
60–69	6 (25)	4 (36.36)	2 (15.38)	
70–79	6 (25)	3 (27.27)	3 (23.08)	
80–89	6 (25)	1 (9.09)	5 (38.46)	
Gender				
Female	9 (37.5)	5 (45.45)	4 (30.77)	0,675
Male	15 (62.5)	6 (54.55)	9 (69.23)	
Clinical characteristics				
History of smoking	13 (54.17)	2 (18.18)	11 (84.62)	0,001
History of COPD	7 (29.17)	0 (0)	7 (53.85)	0,006
Hemoptysis	11 (45.83)	3 (27.27)	8 (61.54)	0,093
Imaging findings				
Lesion size > 30mm	22 (91.67)	10 (90.91)	12 (92.31)	1,0
Lesion size < 30 mm	2 (8.33)	1 (9.09)	1 (7.69)	
Cavitated lesion	8 (33.33)	2 (18.18)	6 (46.15)	0,211
Pleural effusion	6 (25)	1 (9.09)	5 (38.46)	0,166
TNM				
IIA	1 (4.17)	0 (0)	1 (7.69)	0,526
IIB	1 (4.17)	1 (9.09)	0 (0)	
IIIA	6 (25)	4 (36.36)	2 (15.38)	
IIIB	3 (12.50)	1 (9.09)	2 (15.38)	
IV	13 (54.17)	5 (45.45)	8 (61.54)	
TILs				
Low	18 (75)	10 (90.91)	8 (61.54)	0,166
Intermediate	6 (25)	1 (9.09)	5 (38.46)	
Treatment				
Chemotherapy	4 (16.67)	1 (9.09)	3 (23.08)	0,57
Surgery	1 (4.17)	0 (0)	1 (7.69)	
Chemotherapy, radiotherapy, and surgery	3 (12.5)	3 (27.27)	0 (0)	
Chemotherapy and radiotherapy	4 (16.67)	2 (18.18)	2 (15.38)	
Radiotherapy and surgery	2 (8.33)	1 (9.09)	1 (7.69)	
Chemotherapy and surgery	4 (16.67)	2 (18.18)	2 (15.38)	
Palliative care	6 (25)	2 (18.18)	4 (30.77)	

4. Discussion

In our SCLC series, the prevalence of high-expressing PD-L1 patients was 54% and was associated with history of smoking ($p = 0.001$) and

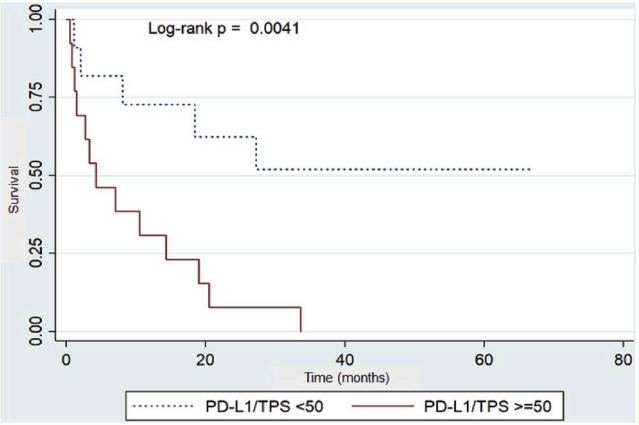

Fig. 1. Survival analysis according to PD-L1 status.

history of COPD ($p = 0.006$). Patients who expressed high levels of PD-L1 had a worse prognosis compared with patients with low expression/negative TPS. This finding is consistent with the process of immune evasion due to T-cell exhaustion secondary to PD-1 stimulation on T-cell membranes when binding to PD-L1 from tumor cells. This behavior is also shown in other types of solid tumors like gastric cancer, ovarian cancer, melanoma and renal cell cancer [17,18].

The prevalence of PD-L1 expression has been found to be variable in different studies. In general, for NSCLC, percentages of patients with PD-L1 TPS ≥50% and TPS ≥1%, respectively are estimated as followed: 22%/52% in Europe; 22%/53% in Asia Pacific; 21%/47% in the Americas, and 24%/55% in other countries [19]. A study in Poland found a prevalence of PD-L1 expression of 11% in SCLC [11]; in Slovenia is as high as 52% in tumor cells of SCLC [20]; 44% in Japan [21]; 30% in Taiwan [22]; 58% in China [23]; 21% in Denmark [24] and 40% in patients screened for enrollment in KEYNOTE-001, -010 and −024 [25]. In this study, despite having a small sample of SCLC, prevalence of PD-L1 expression lies between what has been found in the aforementioned studies (between 11% and 58%).

In our series, in patients with high expression of PD-L1, tumor infiltrating lymphocytes (TILs) were present in low levels in 61% of cases and intermediate in 38%. Some studies in melanoma have shown that TILs that are PD-1 positive have downregulation of effector cytokines compared with PD-1 negative TILs and peripheral blood lymphocytes [26].

Lung cancer has been considered poorly immunogenic; thus, TILs were not routinely assessed in tumor samples [27]. But recent evidence supports that the inhibition of PD-1/PD-L1 pathway is a strong weapon against solid tumors, and some current studies showed very good responses in selected patients with NSCLC. In the last decade, the KEYNOTE trials [28–30] results have taken lung cancer treatment into the era of immunotherapy for patients presenting with PD-L1 TPS≥50%, including both non-squamous and squamous NSCLC [31,32]. Pembrolizumab has recently been approved as first-line treatment for SCLC in Colombia.

Sixty percent of the world's new cancer cases are diagnosed in mid-to-low-income countries, this high cancer burden explains why access to new cancer drugs is needed and regulatory government authorities and pharmaceutical companies must cooperate for ideal cancer treatment [33]. Finally, even though this study has a small sample that is highly selected, this report shows clinical and immunohistochemical characteristics of this SCLC population in a tertiary care hospital where patients are referred for oncologic diagnosis and treatment. These data are of great importance for epidemiologic purposes.

5. Conclusion

Patients with primary SCLC with high expression of PD-L1 exhibited poor survival in our case series. PD-L1 expression assessment can determine a patient's eligibility to be treated with PD-L1/or PD1 inhibitors, for which this test must be evaluated in all patients eligible for personalized treatment.

Ethics approval and consent to participate

This a case series report was prepared in accordance with the ethical standards of the institutional ethics committee and with the 1964 Helsinki Declaration. We have approval letter of Ethics Committee in biomedical research IRB 280–2014 of the Fundación Valle del Lili to publish this manuscript.

Consent for publication

Written informed consent was obtained from patients for publication of this case series and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

All authors have significantly contributed to the paper: LFT: Conception and design, literature review, manuscript writing and correction, final approval of manuscript. JER: Literature review, manuscript writing and correction, final approval of manuscript. VZR: Literature review, manuscript writing and correction, final approval of manuscript. LFS: Conception and design, literature review, manuscript writing and correction, final approval of manuscript.

Declaration of competing interest

The authors declare that they have no competing interests. This manuscript has not been published and is not under consideration for publication elsewhere. Additionally, all of the authors have approved the contents of this paper and have agreed to the journal's submission policies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmcr.2020.101114.

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