Clinicopathological characteristics and tumor infiltrating immune cells associations of PD-L1 tumor expression in non-small cell lung cancer patients

Caractéristiques clinicopathologiques et associations des cellules immunitaires infiltrant la tumeur avec l'expression tumorale du PD-L1 chez les patients atteints de cancer du poumon non à petites cellules

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Abstract

Aim: Our study aimed to perform on Moroccan patients non-small cell lung carcinoma (NSCLC) concerning the relationship between PD-L1 tumor expression, clinicopathological features and tumor infiltrating immune cells (ICs).

Methods: This is a retrospective study (2019 to 2021) conducted on samples from Moroccan patients with NSCLC at the Pathological Anatomy Laboratory of Ibn Rochd University Hospital in Casablanca. Eligible participants for our study had to meet the following predefined criteria: age ≥18 years, histologically confirmed NSCLC, no prior therapeutic interventions, availability of clinical and pathological data, and a usable tumor sample for determining PD-L1 status. Exclusion criteria applied to patients with other types of lung cancer and unusable tumor samples. The evaluation of tumor and immune expression of PD-L1 was performed using immunohistochemistry (IHC), with the 22C3 clone on the Dako Autostainer Link 48 platform. Tumor PD-L1 expression was categorized into 3 levels: TPS <1% (negative expression), TPS 1-49% (low expression), and TPS ≥50% (high expression). ICs infiltrating the tumor expressing PD-L1 were considered positive when more than 1% of positive ICs were present.

Results: Among the 316 analyzed samples, 56.6% showed a negative expression of PD-L1, 16.8% displayed a low expression of PD-L1, and 26.6% exhibited a strong expression. Regarding the histological type, among patients with TPS ≥ 50%, 25.8% had adenocarcinoma. Among patients with TPS ≥ 50%, 24.81% were smokers. PD-L1 was also strongly expressed in the lung (28.2%) and bronchi (26.5%). PD-L1 expression (TPS ≥ 50%) was observed in 35.29% of early-stage patients. Concerning tumor cells (TCs), 27.5% of tumors infiltrated by ICs had TPS ≥ 50%. Furthermore, coexpression of PD-L1 on both TCs and ICs infiltrating the tumor was found in 27.8% of tumors. Statistical analysis demonstrated a significant association between tumor PD-L1 expression and smoking status (P=0.019). However, no significant difference was observed between PD-L1 expression and the presence of ICs infiltrating the tumor (P=0.652), as well as the IHC expression of PD-L1 on ICs (P=0.259).

Conclusion: Our results demonstrate a significant association between PD-L1 expression and smoking status. However, no significant association was observed between PD-L1 expression and the presence of infiltrating ICs, nor with the IHC expression of PD-L1 on ICs. Our data underscore the importance of participating in the study of specific factors influencing PD-L1 expression in patients with NSCLC.

Key words: Non-small cell lung cancer, PD-L1, immunohistochemistry, tumor-infiltrating immune cells.

Résumé

Objectif: Le but de notre étude est d'évaluer chez des patients marocains atteints du cancer du poumon non à petites cellules (CPNPC), l'association entre l'expression tumorale du PD-L1, les caractéristiques clinicopathologiques et l'infiltration tumorale par les cellules immunitaires (CIs).

Méthodes: Il s'agit d'une étude rétrospective (2019 à 2021), menée sur des prélèvements de patients marocains atteints du CPNPC au sein du laboratoire d'Anatomie pathologique du Centre Hospitalier Universitaire Ibn Rochd de Casablanca. Les participants éligibles à notre étude doivent répondre aux critères préétablis suivants: âge ≥18 ans, présentant un CPNPC confirmé histologiquement, n'ayant subi aucune modalité thérapeutique préalable, disposant de données cliniques et pathologiques, et d'un échantillon tumoral exploitable pour la détermination du statut PD-L1. Les critères d'exclusion concernent les patients présentant d'autres types de cancer du poumon, un prélèvement tumoral inexploitable. L'évaluation de l'expression tumorale et immunitaire du PD-L1 a été réalisée par immunohistochimie (IHC), avec le clone 22C3 sur la plateforme DakoAutostainer Link 48. L'expression tumorale du PD-L1 a été classée en 3 niveaux : TPS <1% (expression négative), TPS 1-49 % (faible expression) et TPS ≥50 % (forte expression). Les CIs infiltrant la tumeur exprimant du PD-L1 ont été considérées positives lorsqu'il y a plus de 1 % des CIs positives.

Résultats: Parmi les 316 échantillons analysés, 56,6 % des échantillons présentaient une expression négative du PD-L1, 16,8 % une faible expression du PD-L1 et 26,6 % l'exprimaient fortement. En ce qui concerne le type histologique, parmi les patients présentant un TPS ≥ 50 %, 25,8 % avaient un adénocarcinome. Chez les patients présentant un TPS ≥ 50, 24,81 % étaient des fumeurs. Le PD-L1 était également fortement exprimé dans le poumon 28,2 % et dans les bronches 26,5 %. L'expression de PD-L1 (TPS ≥ 50 %) a été observée chez 35,29 % des patients au stade précoce. Concernant les cellules tumorales (CTs), 27,5% des tumeurs infiltrées par des CIs avaient un TPS ≥ 50%. De plus, une coexpression du PD-L1 à la fois sur les CTs et sur les CIs infiltrant la tumeur a été retrouvée dans 27,8 % des tumeurs. L'analyse statistique a montré une association significative entre l'expression tumorale du PD-L1 et le statut tabagique (P=0.019). En revanche, aucune différence significative n'a été observée entre l'expression du PD-L1 et la présence des CIs infiltrant la tumeur (P=0.652), de même que l'expression IHC du PD-L1 sur les CIs (P=0.259).

Conclusion: Nos résultats montrent une association significative entre l'expression du PD-L1 et le statut tabagique. Cependant, aucune association significative n'a été observée entre l'expression du PD-L1 et la présence des CIs infiltrant la tumeur, ni avec l'expression IHC du PD-L1 sur les CIs. Nos données soulignent l'importance de participer à l'étude des facteurs spécifiques influençant l'expression du PD-L1 chez les patients ayant un CPNPC.

Mots clés: Cancer du poumon non à petites cellules, PD-L1, cellules immunitaires infiltrant la tumeur.

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Introduction

Despite advances in the field of oncology diagnosis and therapeutic management. Lung cancer remains a major public health problem. In Morocco, lung cancer is the second most common cancer after breast cancer with a prevalence of 12.4% for both sexes and up to 22.3% in men followed by prostate and colorectal cancer (1).
Immunotherapy with immune checkpoint inhibitors, that comprising whether anti-PD-1 (Pembrolizumab) or anti-PD-L1(Atezolizumab), has turned out to be the standard treatment for the first and second-line therapy for patients who have locally advanced or metastatic non- small cell lung cancer (NSCLC). Nevertheless, the development of predictive biomarkers of response to immunotherapy is necessary for the clinical benefit of this treatment (2).
In order to predict the response to anti-PD1 and anti-PD-L1 immunotherapy, Tumor expression of PD-L1 is an important biomarker. Therefore, the evaluation of PD-L1 tumor expression in lung cancer is an important challenge and has been based principally on the result of PD-L1 immunohistochemical (IHC) staining (3).
In the NCCN guidelines, the FDA-approved 22C3 IHC assay for PD-L1 uses a threshold of 50% tumor proportion score (TPS) for monotherapy treatment and 1% TPS for combined chemotherapy and immunotherapy in NSCLC (4).
Clinically, immunohistochemical (IHC) expression of PD-L1 is the most considerable biomarker used for predicting the advantages of anti-PD-1/anti-PD-L1 therapies (5). High PD-L1 expression (TPS ≥ 50%) on tumor cells is correlated to a more favorable response to both either anti-PD-1 and anti-PD-L1 (6), proposing that PD-L1 IHC expression ought to be a clinically applicable predictive biomarker (7).
Immune cells (ICs) in the lung cancer tumor microenvironment primarily have T cells, macrophages, and monocytes (8). There are several works on NSCLC members that evaluated the correlation between PD-L1 expression and tumor-infiltrating immune cells which showed conflicting results. While other ones works on different varieties of malignancies in which to show PD-L1 expression is connected to significant infiltration of immune cells in the tumor microenvironment (MET) (9).
Our study aimed to perform on Moroccan patients non-small cell lung carcinoma (NSCLC) concerning the relationship between PD-L1 tumor expression, clinicopathological features and tumor infiltrating immune cells (ICs).

Methods

Ethical consideration

The protocol of our study was conducted in accordance with the principles of the Declaration of Helsinki (Version 2000) and was approved by the local ethics committee of our institution, The Ibn rochd university hospital of casablanca, Morocco (CHU Ibn rochd). Informed consent was obtained from all patients.

Samples

This is a retrospective observational study carried out on 316 patients, 299 of them had a biopsy and 17 surgical specimens, collected in the form of formalin-fixed, paraffin-embedded tissue blocks (FFPE).
The patient samples were retrieved from the archives of the Laboratory of Pathological Anatomy of the University Hospital Ibn Rochd of Casablanca. Thus, the characteristics of the patients including age, gender, histological type, Smoking status, site of sampling and stage of disease were obtained retrospectively (2019-2021) from the patient's medical records (Table 1). Eligible participants for our study had to meet the following predefined criteria: age ≥18 years, histologically confirmed NSCLC, no prior therapeutic interventions, availability of clinical and pathological data, and a usable tumor sample for determining PD-L1 status. Exclusion criteria applied to patients with other types of lung cancer and unusable tumor samples.

Characteristic details of the patients

The choice of various clinical and pathological criteria, such as age, sex, histological type, smoking status, sampling site, and disease stage, for the conduct of this study is based on the recommendations of the PATTERN group of thoracic pathologists, specialized in PD-L1 immunohistochemical testing for non-small cell lung cancers. In addition, we took into consideration previous studies, notably the one conducted by Yanqing Liu et al (Asian journal of surgery, 2022) (17, 10).

PD-L1 immunohistochemistry

Immunohistochemical staining for PD-L1 was performed using the Dako PD-L1 pharmDX kit (The 22C3 clone, Lot 11317345A) on the Dako Link 48 platform, on 3-5 micrometer FFPE sections on positively charged glass slides and slide reading was performed on a light microscope (Olympus BX43, Gr: x40) by the same pathologist (MK).
PD-L1 expression was assessed using the tumor proportion score (TPS) (16). which is defined as the percentage of PD-L1 positive tumor cells (TCs) to total TCs, Thus, PD-L1 expression was classified into three levels: Negative expression (TPS< 1%), low expression (TPS 1-49%) and high expression (TPS≥ 50%) (Figure 1). In addition, we also evaluated tumor-infiltrating immune cells (ICs) and were considered positive when there was more than 1% of positive ICs.

Classification of the tumor microenvironment

Based on PD-L1 expression on tumor-infiltrating TCs and ICs, we classified the tumor microenvironment into four categories (Table 3, Figure 3).
- Positive PD-L1 expression on tumor-infiltrating TCs and ICs (TCs +, ICs +).
- Positive PD-L1 expression on TCs and negative on tumor-infiltrating ICs (TCs +, ICs -).

- Negative PD-L1 expression on TCs and positive on tumor-infiltrating ICs (TCs -, ICs +).
- Negative PD-L1 expression on TCs and tumor-infiltrating ICs (TCs -, ICs -).

Statistical analysis

All statistical analyses were performed using SPSS Statistics V.21. The chi-square test was used to assess the association between PD-L1 expression and parameters such as age, sex, histological type, smoking status, site of sampling, stage of disease, presence of tumor infiltrating immune cells and IHC status of PD-L1 on tumor-infiltrating immune cells. (Table 2, Table 3). Statistically significant differences were considered P<0.05.

Results

Patient characteristics

The median age of the patients (N=316) is 61.5 years (24-96 years) of which 47.2% (N=149) is less than or equal to 61.5 years. The sex ratio is 3.33.
The characterization of the tumors revealed 3 histological types classified respectively as follows: 244 (77.2%) adenocarcinoma, 37 (11.7%) poorly differentiated carcinoma and 35 (11.1%) squamous cell carcinoma.
Regarding smoking status, 84.2% (N=266) of the subjects had history of smoking.
Concerning the site of the sampling, 56% were performed in the lung, 31% in the bronchi and 11.7% in the pleura.
Regarding the stage of the disease, 17 patients (5.4%) and 299 (94.6%) were diagnosed at early (I and II) and advanced (III and VI) stages respectively (Table 1).

Table 1. Clinicopathologic characteristics of patients with NSCLC

Variables	Number of patients	Percentage (%)
Age (years)		
Median [Rank]	61,5 [24-96]	
≤ 61,5	149	47.2
> 61,5	167	52.8
Gender		
Men	243	76.9
Women	73	23.1
Sex ratio	3.33	
Histological type		
Adenocarcinoma	244	**77.2**
Poorly differentiated carcinoma	37	11.7
Squamous cell carcinoma	35	11.1
Smoking status		
Current/ Former	266	84.2
Never	50	15.8
Site of sampling		
Lung	177	56
Bronchus	98	31
Pleura	37	11.7
Thorax	2	0.63
Mediastinum	1	0.32
Trachea	1	0.32
Stage of disease		
Early stage (I et II)	17	5,4
Advanced stage (III et IV)	299	94,6

Results of PD-L1 expression on tumor cells and its association with clinicopathological features.

PD-L1 expression on tumor cells was defined by partial or complete membrane staining (Figure 1).

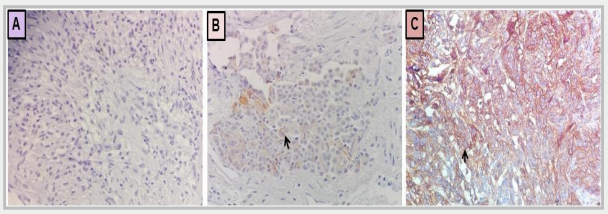

Figure 1. IHC expression of PD-L1 on tumor cells.
A: Negative expression;
B: Low expression (Black arrow: PD-L1-positive tumor cells) and
C: High expression (Black arrow: PD-L1-positive tumor cells).

Of all specimens (316), 56.6% had negative PD-L1 expression, 16.8% had low PD-L1 expression, and 26.6% expressed it strongly (Figure 2).

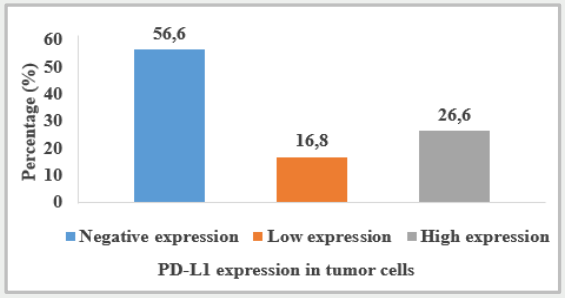

Figure 2. The percentage of different PD-L1 IHC expression levels on tumor cells.

Among patients with age less than or equal to 61.5 years, 51.7% had TPS < 1% and 30.2% had TPS ≥ 50%. Furthermore, among 149 patients aged 61.5 years or less, 45 (30.2%) strongly expressed PD-L1 (TPS≥ 50%).
The results of PD-L1 expression according to gender showed negative expression (TPS < 1%) in 58.4% of men and 50.7% of women. Whereas positive PD-L1 expression (TPS ≥ 50%) was found in 24.3% of men and 34.2% of women.
Regarding the expression of PD-L1 (TPS< 1% vs TPS ≥ 50%) according to histological type. We found the following results: adenocarcinoma (58.6% vs 25.8%), poorly differentiated carcinoma (45.9% vs 35.1%) and squamous cell carcinoma (54.3% vs 22.9%).
The results of PD-L1 expression (TPS< 1% vs TPS ≥ 50%) based on smoking status are depicted as follows: smoker (56.01% vs 24.81%) and non-smoker (60% vs 36%).
For the site of sampling, the results are as follows: lung (56.5 vs 28.2), bronchus (52% vs 26.5%) and pleura (73% vs 13.5%).
Finally, according to the stage of the disease, we found the following results: early stage (52.9 vs 35.29) and advanced stage (56.85 vs 26.1).
Of all the variables studied, only tumor expression of PD-L1 was significantly associated with the smoking status of patients recruited (P= 0.019). (Table 2).

Table 2. Association between tumor expression of PD-L1 and clinicopathological features.

Variables N (%)	Total (N=316)	PD-L1 expression N (%)			
		TPS <1%	TPS 1- 49%	TPS ≥50%	P value
Age (years)					**0.870[a]**
≤ 61.5	149(47.2)	77 (51.7)	27 (18.1)	45 (30.2)	
> 61.5	167(52.8)	102 (61.1)	26 (15.6)	39 (23.4)	
Gender					**0.882[a]**
Men Women	243(76.9)	142 (58.4)	42 (17.3)	59 (24.3)	
	73 (23.1)	37 (50.7)	11 (15.1)	25 (34.2)	
Histological type					**0.442[a]**
ADC	244(77.2)	143 (58.6)	38 (15.6)	63 (25.8)	
PDC	37 (11.7)	17 (45.9)	7 (18.9)	13 (35.1)	
SCC	35 (11.1)	19 (54.3)	8 (22.9)	8 (22.9)	
Smoking status					**0.019[a]**
Current/ Former	266 (84.2)	149 (56.01)	41 (15.41)	66 (24.81)	
Never	50 (15.80)	30 (60)	02 (04)	18 (36)	
Site of sampling					**0.173[a]**
Lung	177 (56)	100 (56.5)	27 (15.3)	50 (28.2)	
Bronchus	98 (31)	51 (52)	21 (21.4)	26 (26.5)	
Pleura	37 (11.7)	27 (73)	5 (13.5)	5 (13.5)	
Mediastinum	1 (0.32)	1 (100)	0	0	
Trachea	1 (0.32)	0	0	1 (100)	
Thorax	2 (0.63)	0	0	2 (100)	
Stage of disease					**0,594[a]**
Early stage (I and II)	17 (5,4)	9 (52,9)	2 (11,76)	6 (35,29)	
Advanced stage (III and IV)	299 (94,6)	170 (56,85)	51 (17,05)	78 (26,1)	

p[a] value obtained by chi-square test; **ADC**: Adenocarcinoma; **PDC**: Poorly differentiated carcinoma; **SCC**: Squamous cell carcinoma; **PD-L1**: programmed death ligand 1 and **TPS**: tumor proportion score.

Association between PD-L1 expression and tumor microenvironment status

In our study, we analyzed the tumor expression of PD-L1 in the tumor immune microenvironment in 316 samples of NSCLC patients.

Table 3 represents the results of PD-L1 expression in the tumor microenvironment. Thus, no statistically significant difference was observed between tumor PD-L1 expression and tumor microenvironment status.

Table 3. Association between tumor PD-L1 expression and tumor microenvironment status.

Variables N (%)	Total (N=316)	PD-L1 expression N (%)			
		TPS <1%	TPS 1- 49%	TPS ≥ 50%	P value
ICs infiltrating the tumor					**0.652[a]**
Presence	269 (85.1)	151 (56.1)	44 (16.4)	74 (27.5)	
Absent	47 (14.9)	28 (59.6)	9 (19.1)	10 (21.3)	
IHC status of PD-L1 on ICs					**0.259[a]**
Positive (≥ 1%)	187 (59.2)	99 (52.9)	33 (17.6)	55 (29.4)	
Negative (< 1%)	129 (40.8)	80 (62)	20 (15.5)	29 (22.5)	

P[a] value obtained by chi-square test. **ICs**: immune cells; **PD-L1**: programmed death ligand 1; **TPS**: tumor proportion score.

The cross expression of PD-L1 between tumor cells (TCs) and immune cells (ICs) is shown in Figure 3. Thus, 27.8% of tumors are double positive (TCs+, ICs+) and 25.31% are double negative (TCs-, ICs-).

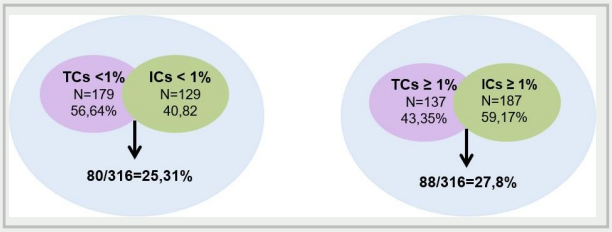

Figure 3. Intersection between PD- L1 positive (+) and negative (-) tumor cells (TCs) and immune cells (ICs) (IHC) in NSCLC patients.

Discussion

In the present study, we retrospectively analyzed the tumor and immune expression of PD-L1 by IHC (Clone 22C3) in 316 samples from Moroccan NSCLC patients. A positive expression of PD-L1 on TCs (TPS ≥ 1%) was observed in 43.4% of the tested samples of which 16.8% samples with low expression of PD-L1 and 26.6% samples with high expression (Figure 2).

The results of the Yu et al. (2018) study show a percentage of positive PD-L1 expression on tumor cells (TPS ≥ 1%) of 46.7% including (36.9% with low expression and 9.8% with high expression of PD-L1) (5).

Account to the study by Gen lin et al. (2017), the positive expression of PD-L1 on tumor cells (TPS ≥ 1%) is 35.3% including 24.7% of tumor cells weakly express PD-L1 (TPS 1-49%) and 10.6% of tumor cells strongly express it (TPS ≥ 50%) (7). Of note, both studies used the same clone, 22C3, as we did, whereas Yanqing et al (2022) used a different clone, SP263, and found a percentage of 24.9% of patients with positive PD-L1 expression (TPS ≥ 1%) (10). The 2 clones are comparable since phase 2 of the Blueprint PD-L1 Immunohistochemistry Comparability Project showed highly comparable staining by the 22C3, 28-8 and SP263 assays and consolidates the analytical evidence for interchangeability of the 22C3, 28-8, and SP263 assays (14). Thus, our results show that almost half of our patients (43.4%) could benefit from immunotherapy treatment in the first or second line according to most international current treatment guidelines (15).

In our study, the association between tumor expression of PD-L1 and clinicopathological characteristics of the studied population showed no significance according to age (P=0.338), gender (P=0.158) and histological diagnosis of the tumors (P= 0.283) (Table 2). These findings are confirmed by Gennen et al in 2020 respectively (P=0.834, P=0.513 and P=0.864 respectively) (11). but discordant with those of Lee et al (2019) and Yanqing et al (2022) who showed significant associations between tumor expression of PD-L1 and gender of patients (<0.001 and 0.017 respectively) (3 -10). Furthermore, prior research by Matthew et al. in 2018 and Jin et al. in 2019 revealed a significant correlation (<0.001) between tumor expression of PD-L1 and tissue type (lung, bronchus, pleura, lymph node, liver, bone, and brain), as well as the stage of the disease (early stage compared to advanced stage) (P=0.000 and P<0.001; respectively) (21,8). However, these findings were not replicated in our study (Sampling site: P=0.173; Disease stage: P=0.543) (Table 2).

Our cohort included 5.38% of stage I and II tumors that had been resected for curative purposes. Thus, we observed that tumor expression of PD-L1 is as frequent in the early stage (I and II) as in advanced stage (III and IV) respectively (47.05 vs 43.15) (Table 2). This may suggest that the expression of this ligand by the tumor is an early occurrence (12).

Unexpectedly, we found a significant association (P=0.019) between tumor expression of PD- L1 and smoking status. These findings align with the conclusions of the study conducted by Yanqing et al in 2022, which also revealed a significant association between PD-L1 expression and smoking status (P=0.003) (Table 2) (10).

In our study, we have highlighted that the tumor expression of PD-L1 does not seem to be influenced by clinicopathological characteristics. However, it is imperative to interpret our results considering several factors that could impact the final understanding of our conclusions, among which the type of analyzed sample plays a crucial role. Our study focused on tissue biopsies, a approach driven by the inoperability of patients at the time of diagnosis. It is worth noting that PD-L1 IHC tests may yield false-negative results due to the intra-tumoral heterogeneity of PD-L1 expression, a feature emphasized in the recommendations of the PATTERN group of thoracic pathologists (17).

Thus, among the 137 patients with positive PD-L1 expression, 83.94 had a history of smoking. It is plausible to consider that the tumor expression of PD-L1 stems from the inflammatory response induced by smoking, involving pro-inflammatory cytokines and T lymphocytes infiltrating the tumor, notably interferon-γ (IFN-γ), recognized for its role in inducing PD-L1 expression (18). Concurrently, 16.05 patients among the 137 with positive PD-L1 expression and no smoking history suggest that other mechanisms are at play, particularly EGFR mutations. These mutations could increase PD-L1 expression by activating several signaling pathways, including the MAPK, PI3K/AKT/mTOR, and JAK2/STAT1 pathways, or through hypoxia, thanks to the activation of hypoxia-inducible factors, especially (HIF-1alpha), or even via microRNAs, including miR-135 and miR-3127-5p, associated with an increase in PD-L1 expression (19, 20).

Regarding the association between tumor PD-L1 expression and tumor microenvironment status, 85.1% of the cases had tumor infiltration by immune cells out of which 27.5% had a TPS ≥ 50%. These findings show that the majority of our patients with an antitumor immune response may have immunosuppression-induced immunodepletion, hence the therapeutic interest of cross-point inhibitors immune controls to enhance to ongoing immune response (2).

In Gin Lin 2017 study, 83% of tumors were infiltrated by immune cells of which only 12.8% had TPS ≥ 50% (7). On the other hand, our results regarding PD-L1 expression by tumor- infiltrating immune cells compared with those found by Jin et al (2019) showed 59.2% vs 70% (8).

We note in our study a 27.8% co-expression of PD-L1 on tumor cells and tumor-infiltrating immune cells (Figure 3). Similar studies that have investigated the same parameters found a much lower rate (only 4% by Yu et al) (5). On the other side, it is remarkable that, in the KEYNOTE-001 study, 3 of 28 NSCLC patients with TPS <1% obtained thorough response after treatment with pembrolizumab, while 54.8% of NSCLC patients with TPS ≥ 50% had no response to pembrolizumab. Thus, PD-L1 expression assay based only on TCs can not the appropriate biomarker to pick out patients who is possible to respond to PD-1/PD-L1 inhibitors. PD-L1 Co-expression on TCs and ICs by IHC evaluation (SP142) has been shown to independently predict improved overall survival with atezolizumab. So, a-few studies demonstrated that PD-L1 expression in ICs was uncompromisingly associated with PD-1/PD- L1 inhibitor treatment outcomes (13).

Statistical analysis has shown no significant association between tumor expression of PD-L1 and the presence of tumor-infiltrating ICs (0.479) nor PD-L1 IHC status on tumor-infiltrating ICs (0.093) (Table 3). This is discordant with the results of the Jin et al (2019) study that showed a significant association between PD-L1 expression and the presence of tumor- infiltrating ICs (0.029) and PD-L1 IHC status on ICs (<0.001) (8). These data show may help the hypothesis that PD-L1 expression is mainly set by different mechanisms in TCs and tumor-infiltrating ICs (5).

This study has several limitations that should be taken into consideration. Firstly, it is important to note that our study is retrospective, covering data from 2019 to 2021. Retrospective studies are inherently limited by the availability of clinical patient data, such as their smoking history, comorbidities, and performance status (PS). Additionally, our study primarily focused on analyzing the expression of PD-L1 and its associations with certain clinical characteristics. However, it would also be relevant to explore the correlation between PD-L1 expression and other potential biomarkers for immunotherapy response, including tumor mutational burden (TMB). Finally, our study did not consider the molecular profile of patients, particularly mutations in EGFR, ALK, and Ros1.

Conclusion

In conclusion, 43.4% of the tested samples showed PD-L1 expression on TCs, whereas in patients with adenocarcinoma this rate can increase up to 77.2%. Furthermore, we found in patients with poorly differentiated carcinoma a ≥ 50% higher TPS compared to the other histological aspects studied and depending on the presence or absence of tumor-infiltrating ICs. Finally, our results show a significant association between PD-L1 expression and smoking status. However, no significant association was observed between PD-L1 expression and the presence of tumour-infiltrating ICs, nor with IHC expression of PD-L1 on ICs. Our data underscore the importance of participating in the study of specific factors influencing PD-L1 expression in patients with NSCLC.

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